# ECOLOGY AND ECOPHYSIOLOGY OF BURYING BEETLES IN A FRAGMENTED EASTERN DECIDUOUS FOREST

by

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*To my friends and family for their love and support.*

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# LIST OF ABBREVIATIONS

ABB – American burying beetle (*Nicrophorus americanus*)

RMR – Resting metabolic rate

PMR – Photographic mark-recapture

SIA – Stable isotope analysis

SIMMs – Stable isotope mixing model

CSIA – Compound-specific stable isotope analysis

IRMS – Isotope ratio mass spectrometry

KUD – Kernel Utilization Density

$MR_{CP}$ – Metabolic rate of carcass preparation

$VCO_2$ – Mass-specific rate of $CO_2$ production

FMS – Field metabolic system

$F_eCO_2$ – Fractional concentration of $CO_2$ leaving the animal chamber

$F_iCO_2$ – Fractional concentration of $CO_2$ entering the animal chamber

# ABSTRACT

Animal species that consume carrion provide an essential ecosystem service by recycling the resource's nutrients into the ecosystem. Carrion is an unpredictable and ephemeral resource that is variable across a landscape and is an important resource to many taxa. Furthermore, the colonization of small vertebrate carcasses by different species influences competition and coexistence dynamics, which in turn influence species dominance. The American burying beetle, *Nicrophorus americanus* (ABB) has recently experienced a dramatic decline in abundance and geographic range. An essential requirement of the ABBs life cycle is the availability of small vertebrate carcasses for reproduction. We know little about the preferred carrion base necessary to support a healthy ABB population. However, we know that reproduction is costly in buying beetles, and physiological trade-offs associated with resource use likely influences metabolic activity, fecundity, and survivorship. Furthermore, successful monitoring of wildlife populations requires reliable estimates of abundance, dispersal, and population demographics. This is often problematic within ABB populations because they are elusive, nocturnal, often occur at low population densities, and are a species of conservation concern. These factors constitute a management and conservation challenge in ecology and conservation biology. Therefore, identifying and evaluating the resources used for reproduction, along with life history trade-offs associated with resource use, in addition to species abundance within a habitat are key requirements for this species' conservation and management. We used stable isotope analysis of carbon and nitrogen to determine the carrion base used by burying beetles in situ. Additionally, we evaluated resting metabolic rate and the energetics of prehatching parental care using flow through respirometry. Finally, we investigated the utility of using photographs with an individual identification machine learning software program paired with program MARK to estimate population abundances of burying beetles.

Between populations, ABBs are not specializing on either avian or mammalian carrion but are using both natural and provisioned carrion for reproduction. Furthermore, among co-occurring burying beetle species, we observed large niche overlap in both populations. Periods of sexual development and prehatching parental care were periods of elevated metabolic activity, which provides insight into life-history tradeoffs associated with resource quality. Carcass size did not significantly influence the metabolic rate of parents, however, the number of days needed to

prepare a small carcass was significantly shorter compared to large carcass preservation. Furthermore, beetle pairs on larger carcasses accumulated significantly larger metabolic cost over the course of parental care. Additionally, using digital images of naturally occurring spot patterns on beetles' elytra, we tested the feasibility and the application of photographic mark-recapture (PMR) using machine learning software. We demonstrated the utility of using PMR in estimating population abundance for *Nicrophorus* spp. based on elytral spot patterns. Future research is needed to fully quantify reproductive resource use over time, and how it influences ABB abundance in extant and reintroduced populations. For successful management and reintroduction of ABBs, managers must consider the resources used for reproduction, the composition and availability of appropriately sized potential reproductive carrion, they should limit intra-/interspecific competition for carrion resources and need accurate data on species abundance.

# CHAPTER 1. BURYING BEETLE ECOLOGY AND CONSERVATION

## 1.1 Introduction

Beetles from the genus *Nicrophorus* (Coleoptera: Silphidae: Nicrophorinae), bury and prepare small vertebrate carcasses as food for their young. This resource is necessary for reproduction; however, carrion is a short-lived resource that is unreliable temporally and spatially and the resource is sought out by many taxa (Scott 1998). Carrion provides a critical resource for a subset of animal species that preform a vital service to the ecosystem by utilizing dead animal remains and recycling its nutrients. In most ecosystems, carcasses represent a relatively small part of the total available detritus for decomposers, however, its role in community interactions, community distributions, and nutrient cycling is uneven when compared to plant detritus because carrion is more nutrient rich, and is utilized by organisms at a much faster pace than plant detritus (Barton et al. 2013). These characteristics make carrion resources critical components of detritus in ecosystems. In addition, organisms that colonize carrion, such as microbes, flies, and burying beetles, play a vital role in coexistence dynamics through competitive interactions, which in turn effects species dominance (Scott 1998, Barton et al. 2013).

In burying beetles, the "small carrion" niche is differentiated by spatial and temporal patterns of activity and by the body size of beetles, which dictates preference in carcass size (Scott 1998). Additionally, seasonal patterns of reproductive activity vary among burying beetle communities, and emergence times as well as patterns of sexual maturity differ. In North American communities, *Nicrophorus sayi*, becomes reproductively active in early spring. In late spring, populations of burying beetles emerge to dominate the ecosystem until late summer. These species include *Nicrophorus orbicollis* and *Nicrophorus defodiens* (Anderson 1981, Scott 1998). Of the rarer species, *Nicrophorus americanus* emerges in late spring while *Nicrophorus pustulatus* first appears in early May but is reproductively active in the late summer (Anderson 1981). Additionally, *Nicrophorus tomentosus*, is reproductively active from late summer into the fall.

Temperature and competition between species for carrion resources appear to be a driving force in shaping seasonal and temporal activity patterns among burying beetles. North American burying beetles are active earlier and longer in southern locations compared to more northern populations. However, in southern locations, late summer species begin to breed about two months

later and thus avoid substantial overlap with the summer breeders (Trumbo 1990). Even though there are longer seasons in southern locations, burying beetle communities are less rich in these habitats (Scott 1998). It has been proposed that the geographical range of some burying beetle species is determined by competition with larger beetles within the genus (Scott 1998, Bedick et al. 1999). However, in areas where species co-occur, it appears that temperature may be influencing competitive interactions. In a field experiment where carcasses were placed out over consecutive nights, *N. orbicollis* found the highest proportion of experimental carcasses on relatively warm nights, but *N. defodiens* was able to find and bury carcasses at lower temperatures. Thus, cool nights are thought to be serving as a temporal refuge for coexistence between the species. This is further backed up by the fact that in more southern locations, where cool nights are absent, *N. defodiens* are not found as they cannot compete with the larger *N. orbicollis* (Wilson et al. 1984).

Most burying beetle communities are characterized by a broad overlap in habitat use (Anderson 1981, Lomolino et al. 1995), and ecological opportunities such as available carrion type, success in locating carcasses, competition with other species, and breeding on a carcass can influence niche variation within and among species and may differ among habitats and over time. It is well documented that different burying beetle species exhibit preference for carcass size (Scott 1998); however, it is unclear if individual species have resource preferences beyond carcass mass (i.e. preference for mammals or birds). Additionally, more information is needed to determine how much niche variation occurs in burying beetle communities that share a home range. Some studies have attempted to provide insight into niche variation in burying beetle communities by conducting dietary analysis of carrion using stable isotopes. Studies in burying beetles have focused on resource preference in a salmon fed watershed (Hocking et al. 2006), niche variation associated with marine and terrestrial carrion (Hocking et al. 2007; Hocking et al. 2009), and niche variation in relation to body size (Ikeda et al. 2006). These studies confirm that differences in available carrion and variation in burying beetle body size allow for additional niche variation, however this has not been evaluated in populations where more than two species co-occur, and up to this point, little effort has been made to evaluate carcass preference in *N. americanus*, a necessary component for ongoing conservation efforts.

The American burying beetle was first listed as endangered in 1989 by the US Fish and Wildlife Service (Federal Register 1989). As recently as the 1920's, *N. americanus* was considered

common across most of the eastern half of North America and was known from 35 states and three Canadian provinces (Anderson 1982). However, the species now occurs in 5-10% of its former range. Extant populations are restricted to Block Island, Rhode Island, and the western periphery of the historic range (western Arkansas, eastern Oklahoma, central and southern Nebraska, southeastern Kansas, and southcentral South Dakota; Sikes and Raithel 2002). The species has been the subject of intense study and speculation since its decline was announced in 1980, as it is difficult to imagine a scenario that would lead to the dramatic range collapse and endangerment of *N. americanus*, which is physically dominant within its guild, to the brink of extinction and leave its eight sympatric congeners untouched (Lomolino et al. 1995, Sikes and Raithel 2002).

Reasons for the decline of the species are not well understood but several hypotheses have been proposed and may include pesticide use such as exposure to DDT, artificial lighting, disease, and habitat alteration (decreased carrion abundance, habitat change, or increased competition from vertebrates and other carrion beetle species; USFWS 1997, Lomolino et al. 1995, Szalanski et al. 2000). However, it is likely that multiple effects interacted to influence the decline of N. americanus (Sikes and Raithel 2002). The most plausible explanation for the decline of this species incorporates hypotheses including the reduction of optimally sized carrion, increased vertebrate scavenger competition for carrion, and increased congener competition (USFWS 1997, Lomolino et al. 1995). *Nicrophorus americanus* is the largest species of *Nicrophorus* in North America and requires carcasses of 100-200g to maximize its fecundity, whereas all other *Nicrophorus* species can breed on much smaller carcasses (Scott 1998). At least one bird species in the ideal weight range and the historical geographic range of *N. americanus*, the Passenger pigeon (*Ectopistes migratorius*) is extinct, and additional ground nesting birds of the ideal weight and size have declined throughout their ranges during the last century (Sikes and Raithel 2002), however this hypothesis has yet to be tested. Furthermore, although passenger pigeons were of ideal weight and shared much of its historic range with *N. americanus*, it is unlikely that they would have been a consistently reliable carrion source for *N. americanus* across the entire home range due to migratory and feeding patterns of the species (J.C. Creighton *personal communication*).

With habitat alteration, one would expect both an increase in vertebrate scavenger pressure, and a decrease in potential carrion of ideal weight and size (Gibbs and Stanton 2001; Creighton et al. 2009); the competition between *N. americanus* and sympatric congeners for sub-optimally sized carcasses would also be expected to increase (Lomolino and Creighton 1996, Szalanski et al. 2000).

*Nicrophorus orbicollis*, the sister species to *N. americanus*, appears to be less successful in using large carcasses (> 100 g) but evidence suggests they may outcompete *N. americanus* on smaller and medium sized carcasses (Scott 1998). However, to what extent *N. americanus* is losing potential breeding carcasses to congeners throughout its range remains unstudied.

It should be noted that several investigations important to our understanding of the decline of N. *americanus* remain to be done. Studies that contrast multiple portions of the historical center of *N. americanus'* range with the eastern and western extant populations with regards to habitat fragment size, available carrion, vertebrate predation, scavenger pressure, and congener competition are of upmost importance for future management and conservation of the species (Sikes and Raithel 2002). Burying beetles must coordinate reproduction with the location of a critical resource that is unpredictable in space and time. They make up an essential component of the detrital food web, and their unique behavior and ecology help shape the small carrion niche throughout the Northern Hemisphere. Research on burying beetles can provide key insights into carrion use and nutrient cycling within many ecosystems.

## 1.2 Journal Selections and Justification

The following chapters are formatted differently as per the requirements by the selected journal. I organized Chapter 2, entitled "Stable Isotope Ecology in Insects: A Review", to the requirements of Ecological Entomology because of the journal's focus on insect studies and methodologies and their connection to insect ecology. For Chapter 3, entitled "Evaluation of the Vertebrate Carrion Resources Used by the American Burying Beetle (*Nicrophorus americanus*)", I used the formatting for Biological Conservation because the focus of the journal is advancement of the science and practice of conservation and conservation management. For Chapter 4, entitled "Evaluating Resting Metabolic Rate and the Effect of Resource Size on Carcass Preparation Energetics in a Burying Beetle", I used the formatting for the Journal of Experimental Biology because the focus of the journal is comparative physiology regarding the form and function of living organisms. Finally, Chapter 5, titled "Estimating Population Abundance of Burying Beetles Using Photo-Identification and Mark-Recapture Methods", I present using the guidelines for Environmental Entomology because of its focus on interactions of insects with biological and physical aspects of their environment.

## 1.3 References


Anderson, R.S. (1981) Resource partitioning in the carrion beetle (Coleoptera:Silphidae) fauna of southern Ontario: ecological and evolutionary considerations. *Canadian Journal of Zoology,* **60**, 1314-1325.

Anderson, R.S. (1982). On the decreasing abundance of *Nicrophorus americanus* Olivier (Coleoptera: Silphidae) in eastern North America. *Coleopterist Bulletin,* **36**, 362-365.

Barton, P.S., Cunningham, S.A., Lindenmayer, D.B., and Manning, A.D. (2013) The role of carrion in maintaining biodiversity and ecological processes in terrestrial ecosystems. *Oecologia.* **171**, 761-772.

Bedick, J.C., Ratcliffe, B.C., Hoback, W.W., and Higley, L.G. (1999) Distribution, ecology, and population dynamics of the American burying beetle [*Nicrophorus americanus* Olivier (Coleoptera, Silphidae)] in south-central Nebraska, USA. *Journal of Insect Conservation,* **3**, 171-181.

Creighton, J.C., Bastarache, R., Lomolino, M.V. and Belk, M.C. (2009) Effect of forest removal on the abundance of the endangered American burying beetle, *Nicrophorus americanus* (Coleoptera: Silphidae). *Journal of Insect Conservation,* **13**, 37-43.

Federal Register. (1989). Endangered and threatened wildlife and plants: determination of endangered status for the American burying beetle. Federal Register 50, CFR Part 17, 54, 133, 29652-29655, July 13, 1989.

Gibbs, J.P. and Stanton, E.J. (2001) Habitat fragmentation and arthropod community change: carrion beetles, phoretic mites, and flies. *Ecological Applications,* **11**, 79-85.

Hocking, M.D., Ring, R.A. and Reimchen, T.E. (2006) Burying beetle *Nicrophorus investigator* reproduction on Pacific salmon carcasses. *Ecological Entomology*, **31**, 5-12.

Hocking, M.D., Darimont, C.T., Christie, K.S., and Reimchen, T.E. (2007) Niche variation in burying beetles (*Nicrophorus* spp.) associated with marine and terrestrial carrion. *Canadian Journal of Zoology,* **85**, 437-442.

Hocking, M.D., Ring, R.A. and Reimchen, T.E. (2009) The ecology of terrestrial invertebrates on Pacific salmon carcasses. *Ecological Research,* **24**, 1091-1100.

Ikeda, H., Kubota, K., Kagaya, T. and Abe, T. (2006). Niche differentiation of burying beetles (Coleoptera: Silphidae: Nicrophorinae) in carcass use in relation to body size: estimation from stable isotope analysis. *Applied Entomology and Zoology,* **41**, 561-564.

Lomolino, M.V., Creighton, J.C., Schnell, G.D., and Certain, D.L. (1995) Ecology and conservation of the endangered American burying beetle (*Nicrophorus americanus*). *Conservation Biology,* **9**, 605-614.

Lomolino, M.B. and Creighton, J.C. (1996) Habitat selection, breeding success and conservation of the endangered American burying beetle *Nicrophorus americanus. Biological Conservation*, **77**, 235-241.

Scott, M.P. (1998) The ecology and behavior of burying beetles. *Annual Review of Entomology*, **43**, 595-618.

Sikes, D.S., and Raithel, C.J. (2002) A review of hypotheses of decline of the endangered American burying beetle (Silphidae: *Nicrophorus americanus* Olivier). *Journal of Insect Conservation*, **6**, 103-113.

Szalanski, A.L., Sikes, D.S., Bischof, R., and Fritz, M. (2000) Population genetics and phylogenetics of the endangered American burying beetle, *Nicrophorus americanus* (Coleoptera:Silphidae). *Annals of the Entomological Society of America,* **93**, 589-594.

Trumbo, S.T. (1990) Reproductive success, phenology and biogeography of burying beetles (Silphidae, *Nicrophorus*). *American Midland Naturalist*, **124**, 1-11.

U.S. Fish and Wildlife Service. 1997. *American burying beetle fact sheet.* U.S. Fish and Wildlife Service, Newton Corner, Massachusetts.

Wilson, D.S., Knollenberg, W.G., and Fudge, J. (1984) Species packing and temperature dependent competition among burying beetles (Silphidae, *Nicrophorus*). *Ecological Entomology*, **9**, 205-216.

# CHAPTER 2. STABLE ISOTOPE ECOLOGY IN INSECTS: A REVIEW

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## 2.1 Abstract

1. The use of stable isotope analysis (SIA) in ecological research has dramatically increased in recent years largely because it allows researchers to investigate ecological questions that have been previously difficult to address.
2. Ecological applications of SIA include estimating fundamental niche space and overlap, evaluating trophic or species level interactions, and investigating food web structure. Increasingly, researchers have been incorporating SIA in studies of animal migration, disease transmission, diet composition, nutrient assimilation, and body condition to list a few.
3. Compared to other taxa, studies using SIA to evaluate the ecology of terrestrial insects are lacking. This poor representation of stable isotope studies in publications likely stems from a lack of familiarity of entomologists with this technique.
4. An improved understanding of SIA, as well as the advantages and disadvantages specifically related to insect research, will benefit the field of entomology. Additionally, insect model systems provide unique opportunities for entomologists to incorporate SIA in their research to advance our knowledge of insect biology and the stable isotope ecology of insects.
5. We provide background information on stable isotopes, explain sources of isotopic variation, describe the processes of how isotopes are differentially routed and incorporated into an individual's tissues, explain the principles that influence isotopic fractionation and discrimination, highlight different methods and advancements in SIA, review innovative stable isotope studies, and provide an overview of common mistakes, considerations, and future directions entomologists can explore.

## 2.2 Introduction

### 2.2.1 Stable Isotopes in Entomology

While the number of stable isotope studies published in ecological journals continues to increase rapidly, studies of terrestrial insects has lagged behind other taxonomic groups (Fig. 1). This under-representation may reflect a lack of understanding among entomologists regarding applications of this technique. Previous reviews encouraging the use of stable isotope analysis (SIA) in entomological studies focused on applications to a specific taxon (i.e. termites [Tayasu, 1998], ants [Feldhaar et al., 2010], collembolans [Semenina & Tiunov, 2011]), or narrowly focused on aspects of arthropod physiology and trophic ecology [Hood-Nowotny & Knols, 2007; Hyodo, 2015]). However, SIA has a much broader range of potential applications to entomology. Moreover, insect model systems provide an excellent opportunity to advance the field of stable isotope ecology.

Since the publication of previous reviews of SIA in entomological studies (Tayasu, 1998; Feldhaar et al., 2010; Semenina & Tiunov, 2011; Hood-Nowotny & Knols, 2007; Hyodo, 2015), there have been important analytical and conceptual developments in the larger realm of stable isotope ecology (Ben-David & Flaherty, 2012; Hyodo, 2015). Our goals for this review are to introduce entomologists to these advances in stable isotope ecology and discuss how other animal ecologists use these techniques to explore ecological questions and concepts. We expand on the previous entomological reviews and introduce potential questions that SIA can address beyond specific study systems and trophic ecology. We discuss common mistakes and the advantages and disadvantages of SIA specifically related to entomological research. Finally, we discuss future research needs and opportunities for the application in entomology.

### 2.2.2 Stable Isotope Ecology Basics

In nature, isotopes differ from the most common form of an element by possessing additional neutrons in the nucleus. Commonly used isotopes in ecological studies include hydrogen, oxygen, carbon, and nitrogen. Isotopes have the same physical and chemical properties as the most common form of the element, but have different physiochemical reaction rates (i.e., reaction rate and bond strength due to differences in vibrational energy). This physical difference leads to variation in the isotope composition of organic compounds because of slight differences in atomic

mass (Fry, 2006; Sulzman, 2007). These heavier isotopes are extremely rare compared to the most common form (Sharp, 2007). We express stable isotope ratios in most ecological applications using the following equation,

$$\delta X = \left[ \left( {}^{R_{\text{Sample}}}\!/_{R_{\text{Standard}}} \right) - 1 \right] \times 1000(‰) \quad (1)$$

where $R_{sample}$ and $R_{standard}$ are the corresponding ratios of heavy to light isotopes (e.g. $^{13}C/^{12}C$) in the sample and the standard, respectively. We express stable isotope ratios in delta (δ) notation in parts per thousand (‰).

How dietary isotopes incorporate into an individual organism depends on the metabolic pathways employed during assimilation. The metabolic pathways are characterized by a preference for a specific carbon isotope (usually the lighter $^{12}C$) during anabolism and catabolism, resulting in the reduction of $^{13}C$ between the resources consumed and the organism. In general, the partitioning into biomass during anabolism is less than partitioning during catabolism, and turnover rates in metabolically active tissues are faster than rates in less active tissues (Tieszen *et al.*, 1983; Freude & Blaser, 2016).

Most SIA studies are considered natural abundance studies because they exploit the natural variations in stable isotope signatures within ecosystems. Variation in the natural abundance of isotope ratios create spatial and temporal patterns that allow us to estimate habitat use (Schell *et al.*, 1989; Koch *et al.*, 1995; Post, 2002), track the flow of nutrients (Collier *et al.*, 2002; Post, 2002; Fischer *et al.*, 2003), evaluate nutritional status (Hobson *et al.*, 1993; Gannes *et al.*, 1998; Polischuk *et al.*, 2001), determine nutrient assimilation into tissue (Tiezen *et al.*, 1983, Tieszen & Farge, 1993; Martínez del Rio & Carleton, 2012), evaluate trophic relationships (Hobson *et al.*, 1994; Pethybridge *et al.*, 2018; Santi-Júnior *et al.*, 2018), estimate animal diets (Ben-David *et al.*, 1997a; 1997b; Stewart *et al.*, 2003; Newsom *et al.*, 2007; Divine *et al.*, 2017; Gómez *et al.*, 2018), assess species interactions (Caut *et al.*, 2006; Sagouis *et al.*, 2015) and determine animal movements (Macneale *et al.*, 2005; Vander Zanden *et al.*, 2015; Santi-Júnior *et al.*, 2018). In contrast, SIA studies that use artificially enriched isotopes as tracers are referred to as enrichment studies (see McDermott *et al.*, 2019).

### 2.2.3 Sources of Variation in Stable Isotopes

Naturally occurring and artificially enriched stable isotopes, primarily carbon and nitrogen, to study animals since the late 1970s. Isotope signatures of organisms reflect the ratios of heavy to light isotopes of the resources consumed and the physiological processes (i.e. enzymatic reactions) used when assimilating resources in body tissues and discarding unused products (Peterson & Fry, 1987; Vander Zanden *et al.*, 1999; Post, 2002; Bearhop *et al.*, 2002; Ben-David & Flaherty, 2012). Isotopes of carbon and nitrogen behave differently from each other. Differences in $\delta^{13}C$ among plants is determined by isotopic fractionation of carbon during photosynthesis ($C_3$, $C_4$, or CAM plants) or by other physiological factors that allow for differentiation of plant use by herbivores (DeNiro & Epstein, 1978; 1981; Peterson & Fry, 1987; Post, 2002). These differences transfer up the food web into the tissues of predators.

Compared to carbon ($\delta^{13}C$), the processes that generate variation in nitrogen ($\delta^{15}N$) are understudied. Stable isotope ecologists use nitrogen isotopes to evaluate ecological processes, relying on the existence of isotopic differences and patterns, however, they do not fully understand the processes and mechanisms that create them (Karasov & Martínez del Rio, 2007). Primary producers differ in $\delta^{15}N$, in part, because: (1) $\delta^{15}N$ values vary widely in soils, (2) shallow-rooted plants tend to be depleted in $^{15}N$ relative to deep-rooted plants, (3) nitrogen-fixing plants tend to be depleted (more negative) in $^{15}N$ by approximately 2-4‰ relative to non-nitrogen-fixing plants, and (4) compared to terrestrial plants, marine phytoplankton tends to be enriched (more positive) by about 4‰ (Kelly, 2000; Karasov & Martínez del Rio, 2007). Variation in $\delta^{15}N$ values generated by these cases is broad (approximately 26‰), which allows stable isotope ecologists to track the contribution of different plants into consumers (Karasov & Martínez del Rio, 2007). However, the isotopic variation among individuals and species is often too large to identify the relative contribution of various primary producers to food chains using nitrogen isotope ratios alone. Therefore, ecologists often use values for $^{15}N$ in combination with other isotopes such as $^{13}C$ (Karasov & Martínez del Rio, 2007). Traditionally, the primary use of nitrogen isotope ratios has been to estimate a species trophic level because nitrogen isotope values are usually enriched in consumers when compared to isotope levels in their diets (Peterson & Fry, 1987; Post 2002; Ben-David & Flaherty, 2012).

Isotopic ratios are naturally variable with respect to photosynthetic pathways, trophic position, latitude, elevation, precipitation, light availability, soil characteristics, marine vs.

terrestrial resource use, and climate (West *et al.,* 2006; Flaherty & Ben-David, 2010). This, coupled with the tendency of animals to incorporate ratios of stable isotopes into their tissues with predictable modifications, allows for the investigation of animal diets, body composition, nutrient flow, metabolic rates, species interactions, and trophic relationships (West *et al.,* 2006; Caut *et al.,* 2009; Ben-David & Flaherty, 2012). Additional studies focused on factors that affect isotopic signatures such as incorporation into animal tissues (Fry & Arnold, 1982; Tieszen *et al.,* 1983; Carleton & Martínez del Rio, 2005), routing of different dietary constituents (proteins, lipids, and carbohydrates) to different tissues ('isotopic routing'; Schwarcz, 1991; Bearhop *et al.,* 2002), and isotopic discrimination between the diet source and consumer tissues (Caut *et al.,* 2009; Robins *et al.,* 2010; Ben-David *et al.,* 2012).

### 2.2.4 Stable Isotope Incorporation

The incorporation of stable isotope signatures into a consumer's tissue is complex and depends on the physiological condition (Carleton & Martínez del Rio, 2005), animal size (Lorrain *et al.,* 2004; Carleton & Martínez del Rio, 2005), age (Reich *et al.,* 2008), feeding behaviour (Lee *et al.,* 2005), tissues sampled (Tieszen *et al.,* 1983; Kurle *et al.,* 2014), composition of the diet (Pearson *et al.,* 2003; Kurle *et al.,* 2014), and assimilation efficiency of dietary items (Martínez del Rio & Wolf, 2005; Martínez del Rio & Carleton, 2012; Ben-David & Flaherty, 2012). Consideration of incorporation rates of isotopes into an animal's tissue and the cellular turnover rates within the tissue are critical because they determine the timeline for evaluating the animal's diet (Newsome *et al.,* 2007; Martínez del Rio *et al.,* 2009). For example, an insect's exoskeleton is deposited in a discrete interval and reflects the isotopic composition of resources incorporated during development (Schimmelmann, 2011), whereas reproductive and fatty tissues have a shorter cellular turnover rate reflecting diet over days (e.g., five days in *Harmonia axyridis* Pallas and *Coccinella septempunctata* Linnaeus, Coleoptera: Coccinellidae; Gratton & Forbes, 2006).

### 2.2.5 Stable Isotope Routing

Isotopic routing occurs because there is variation in the distribution of isotopically distinct dietary components to various tissues (Schwarcz, 1991). Internal routing of dietary components can result in a nonuniform distribution of the isotopes acquired from dietary resources. For

example, a carbon isotope attached to an essential amino acid is preserved as the molecule is assembled to form proteins (Podlesak & McWilliams, 2006) whereas –NH (amino groups) of many amino acids undergo transamination before protein synthesis (Schwarcz, 1991). This is problematic with omnivores that obtain carbohydrates or lipids from different dietary sources than protein (Martínez del Rio *et al.*, 2009) and is important when estimating trophic enrichment (Caut *et al.*, 2010). This is because the preservation of some isotopes and not others can lead to incorrect calculations of the proportions of nutrients resulting in over- or under-estimates of their importance in the diet (Schwarcz, 1991).

When reconstructing an animal's diet, different tissues may provide different information (Voigt *et al.,* 2008; Martínez del Rio *et al.*, 2009) because they reflect varying time scales of assimilation and isotopic incorporation into tissue (i.e., turnover rates). Although specific tissue types can confound results if the tissue does not reflect the proper timeline (Podlesak & McWilliams, 2006; Martínez del Rio *et al.*, 2009), sampling different tissue types in a single individual allows researchers to explore how organisms use resources over different temporal scales (Martínez del Rio *et al.*, 2009).

Insect life cycles, hemimetabolous or holometabolous, influence tissue development and affect the temporal scale of isotope incorporation (Webb *et al.*, 1998; Hood-Nowotny *et al.*, 2006). Turnover rates result from tissue growth and catabolic turnover (Fry & Arnold, 1982) and they vary for insect tissues at each life stage (Webb *et al.*, 1998; Gratton & Forbes, 2006). The temporal scales over which isotope values vary depends on the species in question (Gratton & Forbes, 2006). For vertebrates, isotopic signatures of blood, muscle, skin, hair, feathers, and bones represent time scales from days to months or years (Kelly, 2000; Post 2002; Martínez del Rio & Wolf, 2005; Ben-David & Flaherty, 2012; Ben-David *et al.*, 2012), whereas signatures in whole body invertebrate samples can experience rapid change (5-10 days; Ostrom *et al.,* 1996; Gratton & Forbes, 2006). Less metabolically active tissues (e.g., exoskeleton) reflect dietary intake over longer periods (e.g., months or specific life stages; Gratton & Forbes, 2006; Schimmelman, 2011; Quinby *et al* 2020) and remain understudied.

### 2.2.6 Diet-Tissue Discrimination

Isotopic discrimination (enrichment, trophic shift, which is noted by Δ, is the difference between the consumer's isotopic ratios and the isotopic ratios of its prey; Tayasu, 1998; Feldhaar

*et al.,* 2010) results from selective assimilation of heavy to light isotopes from consumed resources (McCutchan *et al.,* 2003; Ben-David & Flaherty, 2012). Before investigating animal diets, nutrient flows, trophic relations, or species interactions, researchers must consider and incorporate diet-tissue trophic discrimination factors because they vary among species, tissues within species, and across diets (Newsome *et al.,* 2012; Brauns *et al.,* 2018). Determination of discrimination factors for all dietary sources should be confirmed experimentally with controlled feeding trials, however, this is not always possible (Martínez del Rio *et al.,* 2009; Wolf *et al.,* 2009). When discrimination factors that are species-specific have not been previously determined, it is common to use a 1‰ discrimination for $\delta^{13}C$ and 3‰ for $\delta^{15}N$ (DeNiro & Epstein, 1978; 1981; Martínez del Rio *et al.,* 2009) or to use discrimination factors from the literature (Post, 2002; Trapp *et al.,* 2017). Discrimination factors have been experimentally determined for some invertebrates (Scrimgeour *et al.,* 1995; Haubert *et al.,* 2005; deVries *et al.,* 2015, Quinby *et al.,* 2020) but are lacking for most insects.

## 2.3 Quantifying Nutrient Flows, Trophic Relations, and Insect Diets Using Natural Abundance Stable Isotopes

### 2.3.1 Stable Isotope Mixing Models

Stable-isotope mixing models (SIMMs) are an important tool when determining the relative contributions of different dietary sources to the overall bulk diet mixture of individuals (Fry, 2006). Before assessing trophic relationships and insect diets, isotope ratios of all potential foods should be quantified with verification that their signatures are unique before incorporation into separate analyses (Rosing *et al.,* 1998). For large sample sizes consisting of many different potential dietary sources that are normally distributed, we recommend using a multivariate analysis of variance with the dietary sources as grouping variables and the stable isotopes of interest as dependent variables (Blüthgen *et al.,* 2003; Stewart *et al.,* 2003). When sample sizes are small, we suggest using the K nearest-neighbor randomization test described by Rosing *et al.,* (1998) because it has high power with comparatively low displacement for dietary sources (Ben-David & Flaherty, 2012). It is important to incorporate any known or unknown priors into SIMMs before analysis as they may strongly influence the model's output (Ward *et al.,* 2010; Phillips *et al.,* 2014, Derbridge *et al.,* 2015). Accounting for uncertainty in the mean and variance of multiple sources, fractionation, isotope signatures, and including prior source information into your chosen SIMM

can reduce deviations in source contribution estimations (Ward *et al.*, 2010; Phillips *et al.*, 2014). Furthermore, accounting for prior unknowns in SIMM inputs can change the magnitude, variability, and ranking orders of estimated source compositions to the overall dietary mixture (Moore & Semmens, 2008; Phillips *et al.*, 2014).

To account for these variations, standard linear SIMMs assume that the relative contribution of a dietary source to a mixture or specific tissue is the same for each element (e.g., C, N). However, this is not always realistic because some dietary sources are rich or poor in one element (e.g., N), which can lead to a proportionate change in that source's contribution to the mixture for that element (Phillips & Koch, 2002). Concentration-dependent SIMMs account for large variations in source elemental concentrations and assume the dietary source's contribution to the mixture is directly proportional to the mass of the consumed resource multiplied by the concentration of elements in the source (Phillips & Koch, 2002; Phillips *et al.*, 2014). Thus, they better identify known proportions of food sources that vary widely in source elemental concentrations when compared to standard models (Hopkins & Ferguson, 2012; Philips *et al.*, 2014; Hopkins *et al.*, 2017). Phillips and Koch (2002) highlight that isotopic routing or the use of internal nutrient stores under nutritional stress can only be reliably detected and quantified once elemental concentration variation in potential food sources are accounted for in an appropriate SIMM. Therefore, whenever elemental concentrations of dietary sources vary greatly we recommend using concentration-weighted linear mixing models. Elemental concentrations for $\delta^{13}C$ and $\delta^{15}N$ are often provided in outputs from commercial analysis laboratories.

The earliest SIMMs were arranged as a linear algebra equation that allowed for n +1 sources. These initial SIMMS were limited to systems involving a single consumer (or the mean of multiple consumers) resulting in a single solution (Parnell *et al.*, 2012). Advances in SIMMs resulted from a rigorous Bayesian statistical framework that allowed researchers to incorporate a larger numbers of sources, differences in concentration dependences, system uncertainties, , discrimination factors, as well as other variables (Phillips *et al.*, 2014). These models include MixSIR (Semmens & Moore, 2008), SIAR (Parnell & Jackson, 2008), IsotopeR (Hopkins & Ferguson, 2012), MixSIAR (Stock & Semmens, 2013), FRUITS (Fernandes *et al.*, 2014) and SISUS (Erhardt *et al.*, 2014). An additional benefit of these SIMMs is that they are open source, most are available in R, and can be updated and individualized for specific systems as knowledge advances.

### 2.3.2 Lipid Extraction Versus Concentration-Dependent Mixing Models

Variation in lipids among tissue types sampled or organisms consumed can introduce considerable bias into SIA that use $\delta^{13}C$ (Post *et al.,* 2007). Compared to carbohydrates and proteins, lipids are depleted in $^{13}C$ (DeNiro & Epstein, 1977; Griffiths, 1991). The lipid effect on $\delta^{13}C$ has been investigated (Phillips & Koch, 2002; Post *et al.,* 2007; Logan *et al.,* 2008; Tarroux *et al.,* 2010), but the effect of lipid removal on $\delta^{15}N$ remains understudied (Bodin *et al.,* 2007). An increasing number of studies have evaluated the effects of lipid removal on stable isotope ratios (Sotiropoulos *et al.,* 2004; Sweeting *et al.,* 2006; Bodin *et al.,* 2007; Yurkowski *et al.,* 2015) by extracting lipids chemically or using mathematical normalization methods (Post *et al.,* 2007; Tarroux *et al.,* 2010; Yurkowski *et al.,* 2015).

Logan *et al.,* (2008) evaluated the effects of different correction approaches (lipid extraction versus concentration-dependent corrections) on carbon and nitrogen isotopes in fishes and aquatic invertebrates. They determined that for almost all species and tissue types $\delta^{13}C$ values increased significantly following lipid extraction. In contrast, for only a few freshwater and marine species lipid extraction affected $\delta^{15}N$ but only in muscle and whole-body samples (Logan *et al.,* 2008). Using C:N as a representation of lipid content, models predicted lipid-corrected $\delta^{13}C$ more closely with factors specific to the tissue type and species, indicating that tissue- and species-specific models on C:N are a dependable alternative to lipid extraction methods (Logan *et al.,* 2008). Another step in determining if lipids bias the interpretation of diet reconstruction results is testing the sensitivity of SIMMs to lipid extraction. Analyzing a representative number of samples before and after extraction allows researchers to evaluate when to use lipid extraction methods or mathematical models to avoid biased results (Tarroux *et al.,* 2010). If there is substantial variation among the concentration of elements and the food sources connected with the isotopic values used (e.g., C for $\delta^{13}C$), then a concentration-dependent SIMM should be considered (Phillips & Koch, 2002; Philips *et al.,* 2014).

### 2.3.3 Compound-Specific Isotope Analysis

Compound-specific stable isotope analysis (CSIA) enables researchers to exploit the molecular specificity and isotopic signatures of different compounds simultaneously, providing a tool for tracking the origin and eventual fate of matter in ecosystems (Evershed *et al.,* 2007), as

well as many other applications (Schmidt *et al.,* 2004; Bradley *et al.,* 2015). Biochemical components of organic materials that are similar structurally can develop from multiple sources and can potentially reveal differences in isotopic signatures (e.g., palmitic acid found in some soils can originate from microbes, plants, or invertebrates; O'Brien *et al.,* 2002; Jim *et al.,* 2003; Corr *et al.,* 2005). The preferential breakdown of components that are isotopically heavy would result in a lowering of bulk isotopic values (Evershed *et al.,* 2007). Using CSIA paired with structurally diagnostic biomarkers in chemically complex materials can reveal information on biochemical pathways or biological process unobtainable through bulk SIA alone (Lichtfouse, 2000; Schmidt *et al.,* 2004; Bradley *et al.,* 2015). For example, Matos *et al.,* (2018) used CSIA of amino acids to distinguish types of metabolism at different life stages in blowflies (*Calliphora vicina* Robineau-Desvoidy). Essential amino acids did not undergo isotopic fractionation because they were unaffected by blowfly metabolic processes. However, non-essential amino acids were more positive in larvae and pupae but depleted in adults relative to the carrion (Matos *et al.,* 2018). The results suggest that it is possible to exclude carrion as potential larval food sources, and that amino acid-specific CSIA could improve the accuracy of post-mortem interval determinations based on blowfly development.

## 2.4 Stable Isotope Applications in Entomological Research

### 2.4.1 Carbon and Nitrogen Stable Isotopes in Entomology

Entomologists have used carbon and nitrogen isotope signatures to study food preferences (Petelle *et al.,* 1979; Akamatsu *et al.,* 2004; Adams *et al.,* 2016), feeding strategies (Paetzold *et al.,* 2005a; Chari *et al.,* 2018), sperm transfer (Helinski *et al.,* 2007; Helinski *et al.,* 2008), natal origins (Hobson *et al.,* 2012), dispersal patterns (Medeiros *et al.,* 2017; Madeira *et al.,* 2019), disease transmission (Kaufman *et al.,* 2010; Hamer *et al.,* 2014), trophic position (Tillberg *et al.,* 2006; Hyodo, 2015), predator-prey relationships (Paetzold *et al.,* 2005b; Wise *et al.,* 2006), and nitrogen transfer (Täyasu *et al.,* 1994; Nardi *et al.,* 2002; summarized in Table 1). Researchers use naturally occurring differences in isotopes to follow flows and processes. In contrast, enrichment studies (using commercially available isotope-enriched compounds in feeding regimes) provide an opportunity for enriched isotopes to be incorporated in capture-recapture (Opiyo *et al.,* 2016;

McDermott *et al.,* 2019), feeding preference (Spence & Rosenheim, 2005), and resource allocation studies (Oelbermann & Scheu, 2002).

### 2.4.2 Hydrogen and Oxygen Stable Isotopes in Entomology

Hydrogen and oxygen isotopes are less commonly used in entomological research. However, hydrogen and oxygen isotopes exhibit predictable patterns over the earth's surface waters (Bowen *et al.,* 2005; West *et al.,* 2006; West *et al.,* 2009). In North America, $\delta^2H$ and $\delta^{18}O$ tend to become progressively depleted on a southeast to northwest gradient (West *et al.,* 2006; West *et al.,* 2009). Studies determined $\delta^2H$ signatures of keratin in wings of monarch butterflies (*Danaus plexippus* Linnaeus) indicated natal origins when combined with keratin $\delta^{13}C$ signatures (which shows a general pattern of enrichment along a southeast to northeast gradient) in their wings (Wassenaar & Hobson, 1998; Hobson *et al.,* 1999; Flockhart *et al.,* 2013; 2017). Doucett *et al.,* (2007) estimated energy flow partitioning in aquatic ecosystems by evaluating contributions of aquatic and terrestrial sources of $\delta^2H$ to aquatic insects and fish using SIMMs. Stable isotopes of oxygen and carbon were used to identify spruce budworm (*Choristoneura fumiferana* Clem.) outbreaks in a boreal forest of northeastern North America (Simard *et al.,* 2008). Wang *et al.,* (2009) used oxygen and hydrogen isotope analyses of chironomid larvae (Chironomidae: Diptera) to identify the degree to which water and diet influence the $\delta^{18}O$ and $\delta^2H$ signatures of these organisms. The isotope composition of chironomid subfossils explained changes in the oxygen and hydrogen isotope values of paleoenvironments when paired with other geological evidence (Wang *et al.,* 2009).

### 2.4.3 Diet Assimilation and Turnover

Evaluating how different environmental, physiological, biochemical, and behavioural factors influence the rates of assimilation and turnover into specific compounds such as carbohydrates, amino acids, and lipids leads to an improved understanding of an organism's biochemistry (Webb *et al.,* 1998; Gratton & Forbes, 2006). Chamberlain *et al.,* (2004) evaluated the relationship between lipid content and $\delta^{13}C$ values of Collembola and their diet. They found that fatty acid $\delta^{13}C$ values did not reflect those of bulk dietary fatty acids alone. Instead, $\delta^{13}C$

compositions routed into fatty acid biosynthesis, suggesting that fatty acid composition of Collembola is a combination of both diet and biosynthesized sources.

### 2.4.4 Feeding Behaiour

Entomologists have used SIA to evaluate different aspects of feeding behaviour. Researchers used differences in $\delta^{13}C$ values to study food preference in crop pests (Petelle *et al.*, 1979; Prasifka & Heinz, 2004). Trimble and Sagers, (2004) discovered differences in feeding strategies among ants, which were opportunistic foragers at lower elevations and specialized foragers at higher elevations. Another study determined that riparian spiders that forage between aquatic and terrestrial environments obtain greater than 50% of their diet from aquatic insects (Akamatsu *et al*., 2004). In multitrophic systems, entomologists use enriched isotopes to study food-web complexities. Fischer *et al.*, (2003) evaluated symbiotic relationships of ants (*Pheidole bicornis* Forel) and their host-plant. Researchers provided ants with a food source enriched in $^{15}N$ glycine and followed the fate of the nitrogen excreted by the ants, which eventually transferred to the host-plant (Fischer *et al.*, 2003).

### 2.4.5 Dispersal

Knowledge of dispersal distances, locations, and timing is central to our understanding of insect ecology and behaviour and is necessary for effective pest control or conservation efforts. It is possible to use the natural variation of isotopes, or enriched isotopes, to determine the range, migration patterns, or drift of insects. Naturally occurring isotope markers do not require pre-marking individuals because they vary over geographical area and often have distinctive profiles based on local biogeochemical processes (Hood-Nowotny & Knols, 2007). For example, geographic variation in oxygen and hydrogen isotope signatures of insects will reflect the signature of their water source and geographic variation in carbon and nitrogen signatures will reflect their diet (Gratton & Forbes, 2006; Hamer *et al.*, 2014).

Using SIA of insects provides increasingly useful tools in wildlife forensics (Bowen *et al.*, 2005). In the case of pest management, studies investigated the movement patterns of natural enemies and parasitoids of pests (Prasifka & Heinz, 2004). Using an ecophysiological perspective to study insect dispersal, researchers employed enriched isotopes (Macneale *et al.*, 2004; 2005).

In a study attempting to identify the region of origin in an invasive pest (*Helicoverpa armigera*), researchers determined the region of origin for 73.3% of individual moths using a multivariate combination of $\delta^2$ H, $^{87}Sr/^{86}Sr$, $^{208}Pb/^{206}Pb$, and $^{208}Pb/^{207}Pb$ (Holder *et al.,* 2014).

Enrichment methods allow for marking insects with isotopes as an effective method for labelling individuals within populations and determining habitat use when paired with mark-release-recapture techniques (MRR; Hagler & Jackson, 2001; McDermott *et al.,* 2019). Insect MRR protocols should incorporate techniques that do not affect the insect's fecundity or behaviour, are durable, non-toxic, easy to apply, clearly identifiable, cost effective, and retained by the individual (Hagler & Jackson, 2001). Stable isotopes meet these criteria, thus providing opportunities for incorporation into ecological studies as natural tracers (Macneal *et al.,* 2005; Hood-Nowotny *et al.,* 2006). Incorporating enriched isotopes is a minimally invasive method to label a distinct proportion of an ecosystem to determine dispersal patterns (Macneal *et al.,* 2005). One limitation of this technique is that analysis methods for insects are often destructive; therefore, methods that require the researcher to capture marked individuals multiple times are not always possible (Hood-Nowothny & Knols, 2007). Enrichment studies with wild populations are sometimes difficult because they rely on the ability to recapture previously labeled individuals.
In a study evaluating the role of adult mosquitos (*Culex pipiens* Linnaeus) in West Nile Virus transmission, Hamer *et al.,* (2014) implemented enriched isotopes in an MRR study to observe mosquito dispersal patterns. They determined that 90% of female *Culex* mosquitoes remained within 3 km of their larval habitat, corresponding with the distance-limited genetic variation of West Nile Virus in their study region (Hamer *et al.,* 2014). Furthermore, SIA differentiated between flies (*Musca domestica* Linnaeus) that developed on a range of substrates and determined the likely source of nuisance insects, which is useful in pest management practices (Heinrich *et al.,* 2012).

### 2.4.6 Mating and Sperm Competition

Similar to studies of feeding behaviour, the study of insect mating traditionally relied on direct observation or the use of chemical/radioactive tracers (Dame & Schmidt, 1964; Sivinski & Smittle, 1987; Hood-Nowotny & Knols, 2007). Researchers used differences in spermatophores' isotopic signatures in European corn borers (*Ostrinia nubilalis* Hübner) reared on $C_3$ and $C_4$ plants to evaluate assortative mating strategies (Posnard *et al.,* 2004; Malausa *et al.,* 2005). Scientists

examined sperm transfer and multiple mating by tracing the fate of labelled sperm into female spermathecae from male mosquitoes labelled with $^{13}C$ (Helinski *et al.*, 2007; Helinski *et al.*, 2008). Although entomologists use SIA less frequently in mating and sperm competition studies, it provides useful information on host-plant and insect interactions, oviposition preferences, and assortative mating strategies (Ponsard *et al.*, 2004) and offers an area for more research.

## 2.5 Mistakes, Considerations, and Future Directions

### 2.5.1 Use Previous Knowledge and Caution

It is important that entomologists use informed knowledge when considering SIA (Post, 2002; Dalerum & Angerbjörn, 2005; Ben-David & Flaherty, 2012; Phillips *et al.*, 2014). For example, SIA is an attractive tool to determine diet. However, initially, some researchers did not consider that the sample collected was influenced by spatial and temporal scales based on habitat (Flaherty & Ben-David, 2010; Cummings *et al.*, 2012), or assimilation and turnover rates (Kelly, 2000; Robbins *et al.*, 2010; Ben-David *et al.*, 2012), and additional uncertainties (Phillips *et al.*, 2014). For example, in a homogeneous system (e.g. $C_4$ dominated agricultural fields) there is less isotopic variation among food sources. If the composition of specific diet items varies across spatial scales and a species' range, then conclusions using site specific isotopes might be misinformed (Kelly, 2000; Phillips *et al.*, 2014). Additionally, in longitudinal studies it is necessary to account for anthropogenic inputs of carbon and nitrogen that have resulted in atmospheric depletion in $\delta\,^{13}C$ and $\delta\,^{15}N$ through time because this affects isotope signatures in both consumers and their diet (Schell, 2001; Long *et al.*, 2005; Hobson *et al.*, 2010; Ben-David & Flaherty, 2012).

A well-founded understanding of the study system including, but not limited to, its environmental conditions, the composition and availability of primary producers, feeding behaviours, nutrient quality, life stages, and biochemical processes result in a more informative stable isotope study (Fig. 3). To alleviate the possibility of uninformative or erroneous results, researchers should identify potential dietary food items of the study organism prior to sample collection and determine the temporal and spatial variation in sources from the literature or collect samples to quantify the variation at the study site (Ben-David & Flaherty, 2012; Phillips *et al.*, 2014). Most importantly, it is beneficial to plot the data before running a SIMM. If values for the

consumer fall outside of the possible range of potential diet sources, an important source is missing or discrimination factors are incorrect. This highlights the importance of knowing dietary habits of study species before attempting SIA. Developing an understanding of stable isotopes and their behaviour (especially in the system under study) and the available tools, techniques, and outputs as discussed above should help researchers avoid these limitations. Like any experiment, developing a sound hypothesis with testable predictions and a robust design will help ensure that the methodology is appropriate for the questions asked. SIA and SIMMS are not a cure-all and the ability to interpret results is dependent on the questions asked and the experimental design (Phillips *et al.,* 2014).

### 2.5.2 Quality Control

An increasing interest of SIA as a research tool for ecological studies paired with the simplicity of automated analysis has led to a gap in knowledge between ecologists and technicians of isotope ratio mass spectrometry (IRMS) equipment. Additionally, ease of sample preparation and analysis has resulted in a deterioration of the understanding of methodological procedures and the proper dissemination of findings in the ecological literature (Jardine & Cunjak, 2005). When submitting samples for SIA, researchers should review laboratory standards whether from the International Atomic Energy Agency or internal laboratory standards used to calibrate data. In addition, researchers should review the number of sample replicates analyzed within and across runs to ensure data quality and consistency (Jardine & Cunjack, 2005). Samples should be run in duplicate when possible, and results should only be accepted if the variance between duplicate samples does not exceed 0.15‰, and machine linearity does not deviate from 0.99 (Ben-David *et al.,* 1997a; 1997b; Flaherty & Ben-David, 2010). Researchers should provide measures of both precision and accuracy in publications (Stephenson & Lyon, 1982; Schelske & Hodell, 1995; Doucett *et al.,* 1999) to ensure that reviewers can judge the reliability of data. Jardine and Cunjak 2005, recommend 1) reporting accuracy by comparing measured values (mean ± 1 standard deviation; SD) for the calibrated commercially available standards alongside the samples, and 2) reporting precision (mean ± 1SD) by including values for measured standards (across run precision during sample analysis), repeated samples (within run precision) and for a single sample analyzed every time samples are run (across runs precision).

### 2.5.3 Insect Specific Considerations

A number of insect-specific factors can influence SIA. For example, in terrestrial ecosystems, insect carbon and nitrogen isotopic signatures can reflect both trophic interactions and soil microbial processes, such as humidification and fungal development (Hyodo, 2015). In addition, the ectothermic physiology and size of insects need consideration when using SIA because metabolic rate and body size affect how organisms assimilate isotopes into their tissue (Tieszen *et al.*, 1983; Martínez del Rio & Carleton, 2012). Metabolic activity differences influence the rate of isotopic discrimination differently across taxa and affect SIMMs (Post, 2002; Caut *et al.*, 2009; Phillips *et al.*, 2014). Because of the reasons mentioned above, more research using SIA in insect systems is needed.

### 2.5.4 Future Directions for Entomological Studies

Insect model systems provide unique opportunities for entomologists to incorporate SIA in their research to advance our knowledge of insect biology and the stable isotope ecology of insects. Entomologists can employ model systems to evaluate factors that influence isotopic discrimination, isotopic routing, and assimilation rates, which are lacking in insect ecology and are difficult to address experimentally in other taxa (Wolf *et al.*, 2009). Furthermore, entomologists can evaluate how specific compounds incorporate into tissues, gaining a better understanding of properties of insect biochemistry.

The estimation of discrimination factors using feeding trials is an important study area to inform SIA in insects. Many studies document considerable variation in discrimination factors (Hobson *et al.*, 1993; Hobson *et al.*, 1996; Hobson & Cherel, 2006; Caut *et al.*, 2009; Franssen *et al.*, 2017) and SIMMs are sensitive to differences in discrimination factors (Post, 2002; Phillips *et al.*, 2014). Unlike other taxa, insects are amenable to experimental manipulation, have short lifespans, and can easily be reared in laboratory settings. As a result, insects make excellent systems to evaluate the effects of biochemical, physiological, and behavioural factors on incorporation, routing, assimilation and turnover.

Entomologists can explore feeding relationships in systems where direct observation or traditional methods are not feasible (Hood-Nowotny & Knols, 2007; Hyodo, 2015; Quinby *et al.*, 2020), and explore ecological interactions that are complex and cover multiple levels of

organization (Ben-David & Flaherty, 2012). Stable isotopes allow researchers to evaluate the response of individuals to environmental conditions (Norris *et al.*, 2007), explore how individual responses influence fitness (Sorensen *et al.*, 2009), population dynamics (Rubenstein *et al.*, 2002), and community and ecosystem processes (Kennedy *et al.*, 2018; Rosumek *et al.*, 2018). Furthermore, SIA is the only way for paleoentomologists to evaluate foraging ecology and paleoenvironments of extinct insects (Koch, 2007; Wang *et al.*, 2009).

## 2.6 Conclusions

Field studies that use SIA to evaluate ecological problems outnumber experimental studies clarifying mechanisms that describe ecological patterns (Gannes *et al.*, 1997; Wolf *et al.*, 2009). Although SIA provides opportunities for addressing a variety of unknowns in entomological research that use an ecosystem approach, the technique is underutilized. For instance, SIA is used in conservation efforts in other taxa (Hilderbrand *et al.*, 1999; Pain *et al.*, 2004) but is nearly absent in the insect literature, potentially because analysis methods in insects are usually destructive (Hood-Nowothny & Knols, 2007). However, in larger bodied insects, small clippings of tissue (i.e. elytra notches; Fig. 2) are less invasive (Gratton & Forbes, 2006; Quinby *et al.*, 2020). Further studies evaluating routing, assimilation, species-specific discrimination factors, and CSIA provide opportunities for entomologists to add to the field of stable isotope ecology while at the same time gain insight to previously unanswered questions. Incorporating insect model systems into stable isotope ecology will advance the stable isotope ecology of insects and our knowledge of insect biology.

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## 2.8 References


Adams, M.O., Seifert, C.L., Lehner, L., Truxa, C., Wanek, W. & Fiedler, K. (2016) Stable isotope signatures reflect dietary diversity in European forest moths. *Frontiers in Zoology*, **13**, 37.

Akamatsu, F., Hideshige, T.O.D.A. & Okino, T. (2004) Food source of riparian spiders analyzed by using stable isotope ratios. *Ecological Research*, **19**, 655–662.

Bearhop, S., Waldron, S., Votier, S.C., & Furness, R.W. (2002) Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiological and Biochemical Zoology*, **75**, 451–458.

Ben-David, M., Flynn, R.W., & Schell, D.M. (1997a) Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. *Oecologia*, **111**, 280–291.

Ben-David, M., Hanley, T.A., Klein, D.R., & Schell, D.M. (1997b) Seasonal changes in diets of coastal and riverine mink: the role of spawning Pacific salmon. *Canadian Journal of Zoology*, **75**, 803–811.

Ben-David, M. & Flaherty, E.A. (2012). Stable isotopes in mammalian research: a beginner's guide. *Journal of mammalogy*, **93,** 312–328.

Ben-David, M., Newsome, S.D., & Whiteman, J.P. (2012) Lipid and amino acid composition influence incorporation and discrimination of $^{13}C$ and $^{15}N$ in mink. *Journal of Mammalogy*, **93**, 399–412.

Bodin, N., Le Loc'h, F. & Hily, C. (2007) Effect of lipid removal on carbon and nitrogen stable isotope ratios in crustacean tissues. *Journal of Experimental Marine Biology and Ecology*, **341**, 168–175.

Bowen, G.J., Wassenaar, L.I., & Hobson, K.A. (2005) Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia*, **143**, 337–348.

Bradley, C.J., Wallsgrove, N.J., Choy, C.A., Drazen, J.C., Hetherington, E.D., Hoen, D.K., & Popp, B.N. (2015) Trophic position estimates of marine teleosts using amino acid compound specific isotopic analysis. *Limnology and Oceanography: Methods*, **13**, 476–493.

Brauns, M., Boëchat, I.G., de Carvalho, A.P.C., Graeber, D., Gücker, B., Mehner, T. & von Schiller, D. (2018) Consumer-resource stoichiometry as a predictor of trophic discrimination ($\Delta^{13}C$, $\Delta^{15}N$) in aquatic invertebrates. *Freshwater Biology*, **63**, 1240-1249.

Blüthgen, N., Gebauer, G., & Fiedler, K. (2003) Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. *Oecologia*, **137**, 426–435.

Carleton, S.A. & Martíez del Rio, C. (2005) The effect of cold-induced increased metabolic rate on the rate of $^{13}C$ and $^{15}N$ incorporation in house sparrows (*Passer domesticus*). *Oecologia*, **144**, 226–232.

Caut, S., Roemer, G.W., Donlan, C.J., & Courchamp, F. (2006) Coupling stable isotopes with bioenergetics to estimate interspecific interactions. *Ecological Applications*, ***16***, 1893–1900.

Caut, S., Angulo, E., & Courchamp, F. (2009) Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, **46**, 443–453.

Caut, S., Angulo, E., Courchamp, F., & Figuerola, J. (2010) Trophic experiments to estimate sotope discrimination factors. *Journal of Applied Ecology*, **47**, 948–954.

Chamberlain, P.M., Bull, I.D., Black, H.I., Ineson, P., & Evershed, R.P. (2004) Lipid content and carbon assimilation in Collembola: implications for the use of compound-specific carbon isotope analysis in animal dietary studies. *Oecologia*, **139,** 325–335.

Chari, L.D., Moyo, S., & Richoux, N.B. (2018) Trophic ecology of adult male Odonata. I. Dietary niche metrics by foraging guild, species, body size, and location. *Ecological Entomology*, **43**, 1–14.

Collier, K.J., Bury, S. & Gibbs, M. (2002) A stable isotope study of linkages between stream and terrestrial food webs through spider predation. *Freshwater biology*, **47**, 1651–1659.

Corr, L.T., Sealy, J.C., Horton, M.C., & Evershed, R.P. (2005) A novel marine dietary indicator utilising compound-specific bone collagen amino acid $\delta^{13}C$ values of ancient humans. *Journal of Archaeological Science*, **32**, 321–330.

Cummings, D.O., Buhl, J., Lee, R.W., Simpson, S.J., & Holmes, S.P. (2012). Estimating niche width using stable isotopes in the face of habitat variability: a modelling case study in the marine environment. *PloS one*, **7**, e40539.

Dalerum, F. & Angerbjörn, A. (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia*, **144**, 647–658.

Dame, D.A., & Schmidt, C.H. (1964) $P^{32}$-labeled semen for mosquito mating studies. *Journal of Economic Entomology*, **57**, 669–672.

DeNiro, M.J. & Epstein, S. (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science*, **197**, 261–263.

DeNiro, M.J., & Epstein, S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et cosmochimica acta*, **42**, 495–506.

DeNiro, M.J., & Epstein, S. (1981) Isotopic composition of cellulose from aquatic organisms. *Geochimica et Cosmochimica Acta*, **45**, 1885–1894.

Derbridge, J.J., Merkle, J.A., Bucci, M.E., Callahan, P., Koprowski, J.L., Polfus, J.L., & Krausman, P.R. (2015). Experimentally derived $\delta^{13}C$ and $\delta^{15}N$ discrimination factors for gray wolves and the impact of prior information in Bayesian mixing models. *PloS one*, **10,** e0119940.

deVries, M.S., Martínez del Rio, C., Tunstall, T.S., & Dawson, T.E. (2015) Isotopic incorporation rates and discrimination factors in mantis shrimp crustaceans. *PLoS One*, **10**, e0122334.

Divine, L.M., Bluhm, B.A., Mueter, F.J., & Iken, K. (2017) Diet analysis of Alaska Arctic snow crabs (Chionoecetes opilio) using stomach contents and $\delta^{13}C$ and $\delta^{15}N$ stable isotopes. *Deep Sea Research Part II: Topical Studies in Oceanography*, **135**, 124–136.

Doucett, R.R., Hooper, W., & Power, G. (1999) Identification of anadromous and nonanadromous adult brook trout and their progeny in the Tabusintac River, New Brunswick, by means of multiple-stable-isotope analysis. *Transactions of the American Fisheries Society*, **128**, 278–288.

Doucett, R.R., Marks, J.C., Blinn, D.W., Caron, M., & Hungate, B.A. (2007) Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. *Ecology*, **88**, 1587–1592.

Erhardt, E.B., Wolf, B.O., Ben-David, M., & Bedrick, E.J. (2014) Stable isotope sourcing using sampling. *Open Journal of Ecology*, **4**, 289–298.

Evershed, R.P., Bull, I.D., Corr, L.T., Crossman, Z.M., van Dongen, B.E., Evans, C.J., Jim, S., Mottram, H.R., Mukherjee, A.J., & Pancost, R.D. (2007) Compound-specific stable isotope analysis in ecology and paleoecology. *Stable isotopes in ecology and environmental science*, **2,** 480–541.

Feldhaar, H., Gebauer, G. & Blüthgen, N. (2010) Stable isotopes: past and future in exposing secrets of ant nutrition (Hymenoptera: Formicidae). *Myrmecological News*, **13**, 3–13.

Fernandes, R., Millard, A.R., Brabec, M., Nadeau, M.J., & Grootes, P. (2014) Food reconstruction using isotopic transferred signals (FRUITS): a Bayesian model for diet reconstruction. *PloS one*, **9**, e87436.

Fischer, R. C., Wanek, W., Richter, A., & Mayer, V. (2003). Do ants feed plants? A $^{15}N$ labelling study of nitrogen fluxes from ants to plants in the mutualism of *Pheidole* and *Piper*. *Journal of Ecology*, **91**, 126–134.

Flaherty, E.A. & Ben-David, M. (2010). Overlap and partitioning of the ecological and isotopic niches. *Oikos*, **119**, 1409–1416.

Flockhart, D.T., Wassenaar, L.I., Martin, T.G., Hobson, K.A., Wunder, M.B. & Norris, D.R. (2013) Tracking multi-generational colonization of the breeding grounds by monarch butterflies in eastern North America. *Proceedings of the Royal Society B: Biological Sciences*, **280**, p.20131087.

Flockhart, D.T., Brower, L.P., Ramirez, M.I., Hobson, K.A., Wassenaar, L.I., Altizer, S. & Norris, D.R. (2017) Regional climate on the breeding grounds predicts variation in the natal origin of monarch butterflies overwintering in Mexico over 38 years. *Global change biology*, **23**, 2565–2576.

Franssen, N.R., Gilbert, E.I., James, A.P. & Davis, J.E. (2017). Isotopic tissue turnover and discrimination factors following a laboratory diet switch in Colorado pikeminnow (*Ptychocheilus lucius*). *Canadian Journal of Fisheries and Aquatic Sciences*, **74**, 265-272.

Freude, C. & Blaser, M. (2016). Carbon isotope fractionation during catabolism and anabolism in acetogenic bacteria growing on different substrates. *Applied and. Environmental Microbiology*, **82**, 2728-2737.

Fry, B. & Arnold, C. (1982) Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). *Oecologia*, **54**, 200–204.

Fry, B. (2006) *Stable isotope ecology Vol. 521*. Springer, New York, USA.

Gannes, L.Z., O'Brien, D.M., & Del Rio, C.M. (1997). Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology*, **78**, 1271–1276.

Gannes, L.Z., Del Rio, C.M., & Koch, P. (1998). Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative biochemistry and physiology Part A: Molecular & integrative physiology*, **119**, 725–737.

Gómez, C., Larsen, T., Popp, B., Hobson, K.A., & Cadena, C.D. (2018) Assessing seasonal changes in animal diets with stable-isotope analysis of amino acids: a migratory boreal songbird switches diet over its annual cycle. *Oecologia*, **187**, 1–13.

Gratton, C., & Forbes, A.E. (2006) Changes in $\delta^{13}C$ stable isotopes in multiple tissues of insect predators fed isotopically distinct prey. *Oecologia*, **147**, 615–624. doi:10.1007/s00442-005-0322-y.

Griffiths, H. (1991) Applications of stable isotope technology in physiological ecology. *Functional Ecology*, **5**, 254–269.

Hagler, J.R., & Jackson, C.G. (2001) Methods for marking insects: current techniques and future prospects. *Annual Review of Entomology*, **46**, 511–543.

Hamer, G.L., Anderson, T.K., Donovan, D.J., Brawn, J.D., Krebs, B.L., Gardner, A.M., Ruiz, M.O., Brown, W.M., Kitron, U.D., Newman, C.M. & Goldberg, T.L. (2014). Dispersal of adult Culex mosquitoes in an urban West Nile virus hotspot: a mark-capture study incorporating stable isotope enrichment of natural larval habitats. *PLoS Neglected Tropical Diseases*, **8**, e2768.

Haubert, D., Langel, R., Scheu, S., & Ruess, L. (2005) Effects of food quality, starvation and life stage on stable isotope fractionation in Collembola. *Pedobiologia*, **49**, 229–237.

Heinrich, K., Weaver, R.J., & Bell, H.A. (2012) Determining the source of house flies (*Musca domestica*) using stable isotope analysis. *Pest Management Science*, **68**, 31–37.

Helinski, M.E., Hood-Nowotny, R., Mayr, L. & Knols, B.G. (2007). Stable isotope-mass spectrometric determination of semen transfer in malaria mosquitoes. *Journal of Experimental Biology*, **210**, 1266–1274.

Helinski, M.E., Hood, R.C. & Knols, B.G. (2008). A stable isotope dual-labelling approach to detect multiple insemination in un-irradiated and irradiated *Anopheles arabiensis* mosquitoes. *Parasites & Vectors*, **1**, 9.

Hilderbrand, G.V., Schwartz, C.C., Robbins, C.T., Jacoby, M.E., Hanley, T.A., Arthur, S.M., & Servheen, C. (1999) The importance of meat, particularly salmon, to body size, population productivity, and conservation of North American brown bears. *Canadian Journal of Zoology*, **77**, 132–138.

Hobson, K.A., Alisauskas, R.T. & Clark, R.G. (1993) Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. *The Condor*, **95**, 388–394.

Hobson, K.A., Piatt, J.F. & Pitocchelli, J. (1994) Using stable isotopes to determine seabird trophic relationships. *Journal of Animal Ecology*, 63, 786–798.

Hobson, K.A., Schell, D.M., Renouf, D., & Noseworthy, E. (1996) Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 528–533.

Hobson, K.A., Wassenaar, L.I. & Taylor, O.R. (1999) Stable isotopes (δD and δ 13 C) are geographic indicators of natal origins of monarch butterflies in eastern North America. *Oecologia*, **120**, 397–404.

Hobson, K.A., & Cherel, Y. (2006) Isotopic reconstruction of marine food webs using cephalopod beaks: new insight from captively raised *Sepia officinalis*. *Canadian Journal of Zoology*, **84**, 766–770.

Hobson, K.A., Barnett-Johnson, R., & Cerling, T. (2010) Using isoscapes to track animal migration. *Isoscapes*. Springer, Dordrecht, Netherlands. 273–298.

Hobson, K.A., Soto, D.X., Paulson, D.R., Wassenaar, L.I. & Matthews, J.H. (2012) A dragonfly ($\delta^2$H) isoscape for North America: a new tool for determining natal origins of migratory aquatic emergent insects. *Methods in Ecology and Evolution*, **3**, 766–772.

Hood-Nowotny, R., Mayr, L., & Knols, B.G. (2006) Use of carbon-13 as a population marker for *Anopheles arabiensis* in a sterile insect technique (SIT) context. *Malaria Journal*, **5**, 6.

Hood-Nowotny, R. & Knols, B.G. (2007) Stable isotope methods in biological and ecological studies of arthropods. *Entomologia Experimentalis et Applicata*, **124**, 3–16.

Holder, P.W., Armstrong, K., Van Hale, R., Millet, M.A., Frew, R., Clough, T.J., & Baker, J.A. (2014) Isotopes and trace elements as natal origin markers of *Helicoverpa armigera*–an experimental model for biosecurity pests. *PloS one*, **9**, p.e92384.

Hopkins III, J.B., & Ferguson, J.M. (2012) Estimating the diets of animals using stable isotopes and a comprehensive Bayesian mixing model. *PloS one*, **7**, e28478.

Hopkins III, J.B., Ferguson, J.M., Tyers, D.B. & Kurle, C.M. (2017). Selecting the best stable isotope mixing model to estimate grizzly bear diets in the Greater Yellowstone Ecosystem. *PloS one*, **12,** e0174903.

Hyodo, F. (2015) Use of stable carbon and nitrogen isotopes in insect trophic ecology. *Entomological Science*, **18**, 295–312.

Jardine, T.D. & Cunjak, R.A. (2005) Analytical error in stable isotope ecology. *Oecologia*, **144**, 528–533.

Jim, S., Jones, V., Copley, M.S., Ambrose, S.H., & Evershed, R.P. (2003) Effects of hydrolysis on the $\delta^{13}C$ values of individual amino acids derived from polypeptides and proteins. *Rapid Communications in Mass Spectrometry*, **17,** 2283–2289.

Kaufman, M.G., Pelz-Stelinski, K.S., Yee, D.A., Juliano, S.A., Ostrom, P.H. & Walker, E.D. (2010) Stable isotope analysis reveals detrital resource base sources of the tree hole mosquito, *Aedes triseriatus*. *Ecological Entomology*, **35**, 586–593.

Kelly, J.F. (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology*, **78**, 1–27.

Kennedy, S.R., Dawson, T.E. & Gillespie, R.G. (2018) Stable isotopes of Hawaiian spiders reflect substrate properties along a chronosequence. *PeerJ*, **6**, p.e4527.

Koch, P.L., Zachos, J.C., & Dettman, D.L. (1995) Stable isotope stratigraphy and paleoclimatology of the Paleogene Bighorn Basin (Wyoming, USA). *Palaeogeography, Palaeoclimatology, Palaeoecology*, **115**, 61–89.

Koch, P. L. (2007) Isotopic study of the biology of modern and fossil vertebrates. *Stable isotopes in ecology and environmental science*, **2**, 99–154.

Karasov, W.H. & Martínez del Rio, C. (2007) Nitrogen Isotopes. *Physiological ecology: how animals process energy, nutrients, and toxins*. Princeton University Press, New Jersey, USA. 466-472.

Kurle, C.M., Koch, P.L., Tershy, B.R. & Croll, D.A. (2014) The effects of sex, tissue type, and dietary components on stable isotope discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$) in mammalian omnivores. *Isotopes in Environmental and Health Studies*, **50**, 307–321.

Lee, S.H., Schell, D.M., McDonald, T.L. & Richardson, W.J. (2005) Regional and seasonal feeding by bowhead whales *Balaena mysticetus* as indicated by stable isotope ratios. *Marine Ecology Progress Series*, **285**, 271–287.

Lichtfouse, E. (2000) Compound-specific isotope analysis. Application to archaelogy, biomedical sciences, biosynthesis, environment, extraterrestrial chemistry, food science, forensic science, humic substances, microbiology, organic geochemistry, soil science and sport. *Rapid Communications in Mass Spectrometry*, **14**, 1337–1344.

Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A., & Lutcavage, M.E. (2008) Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *Journal of Animal Ecology*, **77**, 838–846.

Long, E.S., Sweitzer, R.A., Diefenbach, D.R., & Ben-David, M. (2005) Controlling for anthropogenically induced atmospheric variation in stable carbon isotope studies. *Oecologia*, **146**, 148-156.

Lorrain, A., Paulet, Y.M., Chauvaud, L., Dunbar, R., Mucciarone, D. & Fontugne, M. (2004) $\delta^{13}C$ variation in scallop shells: increasing metabolic carbon contribution with body size?. *Geochimica et Cosmochimica Acta*, **68**, 3509–3519.

Macneale, K.H., Peckarsky, B.L., & Likens, G.E. (2004) Contradictory results from different methods for measuring direction of insect flight. *Freshwater Biology*, **49**, 1260–1268.

Macneale, K.H., Peckarsky, B.L. & Likens, G.E. (2005) Stable isotopes identify dispersal patterns of stonefly populations living along stream corridors. *Freshwater Biology*, **50**, 1117–1130.

Madeira, F., di Lascio, A., Costantini, M.L., Rossi, L., Rösch, V. & Pons, X. (2019) Intercrop movement of heteropteran predators between alfalfa and maize examined by stable isotope analysis. *Journal of Pest Science*, **92**, 757–767.

Malausa, T., Bethenod, M.T., Bontemps, A., Bourguet, D., Cornuet, J.M., & Ponsard, S. (2005) Assortative mating in sympatric host races of the European corn borer. *Science*, **308**, 258–260.

Martínez del Rio, C., & Wolf, B.O. (2005) Mass-balance models for animal isotopic ecology. *Physiological and ecological adaptations to feeding in vertebrates*, 141–174.

Martínez del Rio, C., Wolf, N., Carleton, S.A. & Gannes, L.Z. (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews*, **84**, 91–111.

Martínez del Rio, C., & Carleton, S.A. (2012). How fast and how faithful: the dynamics of isotopic incorporation into animal tissues. *Journal of Mammalogy*, **93**, 353–359.

Matos, M.P., Konstantynova, K.I., Mohr, R.M., & Jackson, G.P. (2018) Analysis of the $^{13}C$ isotope ratios of amino acids in the larvae, pupae and adult stages of *Calliphora vicina* blow flies and their carrion food sources. *Analytical and bioanalytical chemistry*, **410**, 7943–7954.

McCutchan Jr, J.H., Lewis Jr, W.M., Kendall, C., & McGrath, C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102,** 378–90.

McDermott, E.G., Mullens, B.A., Mayo, C.E., Roark, E.B., Maupin, C.R., Gerry, A.C., *et al.*, (2019) Laboratory evaluation of stable isotope labeling of *Culicoides* (Diptera: Ceratopogonidae) for adult dispersal studies. *Parasites & vectors*, **12**, 411.

Medeiros, M.C., Boothe, E.C., Roark, E.B. & Hamer, G.L. (2017) Dispersal of male and female *Culex quinquefasciatus* and *Aedes albopictus* mosquitoes using stable isotope enrichment. *PLoS Neglected Tropical Diseases*, **11**, p.e0005347.

Moore, J.W. & Semmens, B.X. (2008). Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology letters*, **11**, 470-480.

Nardi, J.B., Mackie, R.I., & Dawson, J.O. (2002) Could microbial symbionts of arthropod guts contribute significantly to nitrogen fixation in terrestrial ecosystems?. *Journal of Insect Physiology*, **48**, 751–763.

Newsome, S.D., Martínez del Rio, C., Bearhop, S., & Phillips, D.L. (2007) A niche for isotopic ecology. *Frontiers in Ecology and the Environment*, 5, 429–436.

Newsome, S.D., Yeakel, J.D., Wheatley, P.V. & Tinker, M.T. (2012) Tools for quantifying isotopic niche space and dietary variation at the individual and population level. *Journal of Mammalogy*, **93**, 329-341.

Nienstedt, K.M., & Poehling, H.M. (2004) Prey to predator transfer of enriched $^{15}$N-contents: basic laboratory data for predation studies using $^{15}$N as marker. *Entomologia experimentalis et applicata*, **112**, 183–190.

Norris, D.R., Arcese, P., Preikshot, D., Bertram, D.F., & Kyser, T.K. (2007) Diet reconstruction and historic population dynamics in a threatened seabird. *Journal of Applied Ecology*, **44**, 875–884.

O'Brien, D.M., Fogel, M.L., & Boggs, C.L. (2002) Renewable and nonrenewable resources: amino acid turnover and allocation to reproduction in Lepidoptera. *Proceedings of the National Academy of Sciences*, **99**, 4413–4418.

Oelbermann, K. & Scheu, S. (2002). Stable isotope enrichment ($\delta^{15}$N and $\delta^{13}$C) in a generalist predator (*Pardosa lugubris*, Araneae: Lycosidae): effects of prey quality. *Oecologia*, **130**, 337-344.

Opiyo, M.A., Hamer, G.L., Lwetoijera, D.W., Auckland, L.D., Majambere, S. & Okumu, F.O. (2016). Using stable isotopes of carbon and nitrogen to mark wild populations of *Anopheles* and *Aedes* mosquitoes in South-Eastern Tanzania. *PloS one*, **11**, p.e0159067.

Ostrom, P.H., Colunga-Garcia, M., & Gage, S.H. (1996) Establishing pathways of energy flow for insect predators using stable isotope ratios: field and laboratory evidence. *Oecologia*, **109**, 108–113.

Paetzold, A., Schubert, C. J., & Tockner, K. (2005a) Aquatic terrestrial linkages along a braided-river: riparian arthropods feeding on aquatic insects. *Ecosystems*, **8**, 748–759.

Paetzold, A., & Tockner, K. (2005b) Effects of riparian arthropod predation on the biomass and abundance of aquatic insect emergence. *Journal of the North American Benthological Society*, **24**, 395–402.

Pain, D.J., Green, R.E., Gieβing, B., Kozulin, A., Poluda, A., Ottosson, U., Flade, M. & Hilton, G.M. (2004) Using stable isotopes to investigate migratory connectivity of the globally threatened aquatic warbler *Acrocephalus paludicola*, *Oecologia*, **138**, 168–174.

Parnell, A., & Jackson, A. (2008) The SIAR Package: stable isotope analysis in R.

Parnell, A.C., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., Jackson, A.L., Grey, J., Kelly, D.J. & Inger, R. (2013). Bayesian stable isotope mixing models. *Environmetrics*, **24**, 387–399.

Pearson, S.F., Levey, D.J., Greenberg, C.H., & Martínez del Rio, C. (2003). Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia*, **135**, 516–523.

Pethybridge, H., Choy, C.A., Logan, J.M., Allain, V., Lorrain, A., Bodin, N. *et al.*, (2018) A global meta-analysis of marine predator nitrogen stable isotopes: Relationships between trophic structure and environmental conditions. *Global Ecology and Biogeography*, **27**, 1043–1055.

Petelle, M., Haines, B., & Haines, E. (1979) Insect food preferences analysed using $^{13}C/^{12}C$ ratios. *Oecologia*, **38**, 159–166.

Peterson, B.J. & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*. **18**, 293–320.

Phillips, D.L. & Koch, P.L. (2002) Incorporating concentration dependence in stable isotope mixing models. *Oecologia*, **130**, 114–125.

Phillips, D.L., Inger, R., Bearhop, S., Jackson, A.L., Moore, J.W., Parnell, A.C., Semmens, B.X., & Ward, E.J. (2014) Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology*, **92**, 823–835.

Podlesak, D.W. & McWilliams, S.R. (2006) Metabolic routing of dietary nutrients in birds: effects of diet quality and macronutrient composition revealed using stable isotopes. *Physiological and Biochemical Zoology*, **79**, 534–549.

Polischuk, S.C., Hobson, K.A. & Ramsay, M.A. (2001) Use of stable-carbon and-nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. *Canadian Journal of Zoology*, **79**, 499–511.

Ponsard, S., Bethenod, M.T., Bontemps, A., Pélozuelo, L., Souqual, M.C., & Bourguet, D. (2004) Carbon stable isotopes: a tool for studying the mating, oviposition, and spatial distribution of races of European corn borer, *Ostrinia nubilalis*, among host plants in the field. *Canadian Journal of Zoology*, **82**, 1177–1185.

Post, D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, **83**, 703–718.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., & Montana, C.G. (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, **152**, 179–189.

Prasifka, J., & Heinz, K. (2004) The use of C3 and C4 plants to study natural enemy movement and ecology, and its application to pest management. *International Journal of Pest Management*, **50**, 177–181.

Quinby, B.M., Feldman, N.S., Flaherty, E.A., Belk, M.C., Smith, A.D.F., & Creighton, J.C. (2020) Isotopic discrimination between carrion and elytra clippings of lab-reared American burying beetles (*Nicrophorus americanus*): Implications for conservation and evaluating feeding relationships in the wild. *Rapid Communications in Mass Spectrometry*, (*in press*).

Reich, K.J., Bjorndal, K.A. & Martínez del Rio, C. (2008) Effects of growth and tissue type on the kinetics of $^{13}C$ and $^{15}N$ incorporation in a rapidly growing ectotherm. *Oecologia*, **155**, 651–663.

Robbins, C.T., Felicetti, L.A., & Florin, S.T. (2010) The impact of protein quality on stable nitrogen isotope ratio discrimination and assimilated diet estimation. *Oecologia*, **162**, 571–579.

Rosing, M.N., Ben-David, M., & Barry, R.P. (1998) Analysis of stable isotope data: AK nearest-neighbors randomization test. *The Journal of Wildlife Management*, **62**, 380–388.

Rosumek, F.B., Blüthgen, N., Brückner, A., Menzel, F., Gebauer, G., & Heethoff, M. (2018) Unveiling community patterns and trophic niches of tropical and temperate ants using an integrative framework of field data, stable isotopes and fatty acids. *PeerJ*, **6**, p.e5467.

Rubenstein, D.R., Chamberlain, C.P., Holmes, R.T., Ayres, M.P., Waldbauer, J.R., Graves, G.R., & Tuross, N.C. (2002) Linking breeding and wintering ranges of a migratory songbird using stable isotopes. *Science*, **295**, 1062–1065.

Sagouis, A., Cucherousset, J., Villéger, S., Santoul, F., & Boulêtreau, S. (2015) Non-native species modify the isotopic structure of freshwater fish communities across the globe. *Ecography*, **38**, 979–985.

Santi-Júnior, A.D., Botteon, V.W., Mastrangelo, T., & Moreira, M.Z. (2018) Trophic ecology of citrus pests based on stable isotope analysis. *Scientia Agricola*, **75**, 504–508.

Sharp, Z. (2007) Nitrogen. *Principles of stable isotope geochemistry 2nd ed*. Pearson education, London, UK. 242.

Schell, D. M., S. M. Saupe, & Haubenstock, N. (1989) Natural isotope abundances in bowhead whale (*Balaena mysticetus*) baleen: markers of aging and habitat usage. *Stable isotopes in ecological research*, Springer, New York, USA. 260–269.

Schell, D.M. (2001) Carbon isotope ratio variations in Bering Sea biota: the role of anthropogenic carbon dioxide. *Limnology and Oceanography*, **46**, 999-1000.

Schelske, C.L. & Hodell, D.A. (1995) Using carbon isotopes of bulk sedimentary organic matter to reconstruct the history of nutrient loading and eutrophication in Lake Erie. *Limnology and Oceanography*, **40**, 918–929.

Schimmelman A. (2011) Carbon, nitrogen and oxygen stable isotope ratios in chitin. *Chitin Formation and Diagenesis*. Springer, Dordrecht. Neaherlands. 81–103.

Schmidt, T.C., Zwank, L., Elsner, M., Berg, M., Meckenstock, R.U., & Haderlein, S.B. (2004) Compound-specific stable isotope analysis of organic contaminants in natural environments: a critical review of the state of the art, prospects, and future challenges. *Analytical and Bioanalytical Chemistry*, **378**, 283–300.

Schwarcz, H.P. (1991) Some theoretical aspects of isotope paleodiet studies. *Journal of Archaeological Science*, **18**, 261–275.

Scrimgeour, C.M., Gordon, S.C., Handley, L.L., & Woodford, J.A.T. (1995) Trophic levels and anomalous $\delta^{15}N$ of insects on raspberry (*Rubus idaeus L.*). *Isotopes in Environmental and Health Studies*, **31**, 107–115.

Semenina, E.E. & Tiunov, A.V. (2011). Trophic fractionation ($\Delta^{15}N$) in Collembola depends on nutritional status: A laboratory experiment and mini-review. *Pedobiologia*, **54**, 101–109.

Semmens, B.X., & Moore, J.W. (2008) MixSIR: a Bayesian stable isotope mixing model.

Simard, S., Elhani, S., Morin, H., Krause, C., & Cherubini, P. (2008) Carbon and oxygen stable isotopes from tree-rings to identify spruce budworm outbreaks in the boreal forest of Québec. *Chemical Geology*, **252**, 80–87.

Sivinski, J., & Smittle, B. (1987) Male transfer of materials to mates in the Caribbean fruit fly, *Anastrepha suspensa* (Diptera: Tephritidae). *Florida Entomologist*, 233-238.

Sorensen, M.C., Hipfner, J.M., Kyser, T.K., & Norris, D.R. (2009) Carry-over effects in a Pacific seabird: stable isotope evidence that pre-breeding diet quality influences reproductive success. *Journal of Animal Ecology*, **78**, 460–467.

Sotiropoulos, M.A., Tonn, W.M., & Wassenaar, L.I. (2004) Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: potential consequences for food web studies. *Ecology of Freshwater Fish*, **13**, 155–160.

Spence, K.O. & Rosenheim, J.A. (2005) Isotopic enrichment in herbivorous insects: a comparative field-based study of variation. *Oecologia*, **146**, 89–97.

Stephenson, R.L. & Lyon, G.L. (1982) Carbon-13 depletion in an estuarine bivalve: detection of marine and terrestrial food sources. *Oecologia*, **55**, 110–113.

Stewart, K.M., Bowyer, R.T., Kie, J., Dick, B.L. & Ben-David, M. (2003) Niche partitioning among mule deer, elk, and cattle: do stable isotopes reflect dietary niche? *Ecoscience*, **10**, 297–302.

Stock, B.C., & Semmens, B.X. (2013) MixSIAR, version 1.0.

Sulzman, E.W. (2007). Stable isotope chemistry and measurement: a primer. *Stable isotopes in ecology and environmental science 2nd Edition*, Blackwell Publishing, Malden, MA, USA.

Sweeting, C.J., Polunin, N.V.C., & Jennings, S. (2006) Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Communications in Mass Spectrometry*, **20**, 595–601.

Tarroux, A., Ehrich, D., Lecomte, N., Jardine, T.D., Bêty, J., & Berteaux, D. (2010) Sensitivity of stable isotope mixing models to variation in isotopic ratios: evaluating consequences of lipid extraction. *Methods in Ecology and Evolution*, **1**, 231–241.

Tayasu, I. (1998) Use of carbon and nitrogen isotope ratios in termite research. *Ecological Research*, **13**, 377–387.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G. & Slade, N.A. (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. *Oecologia*, **57**, 32–37.

Tieszen, L.L., & Fagre, T. (1993). Effect of diet quality and composition on the isotopic composition of respiratory $CO_2$, bone collagen, bioapatite, and soft tissues. In *Prehistoric human bone*, Springer, Berlin, Heidelberg. 121–155.

Tillberg, C.V., McCarthy, D.P., Dolezal, A.G. & Suarez, A.V. (2006) Measuring the trophic ecology of ants using stable isotopes. *Insectes sociaux*, **53**, 65–69.

Trapp, S.E., Smith, W.P., & Flaherty, E.A. (2017) Diet and food availability of the Virginia northern flying squirrel (Glaucomys sabrinus fuscus): implications for dispersal in a fragmented forest. *Journal of Mammalogy*, **98**, 1688–1696.

Trimble, S.T., & Sagers, C.L. (2004) Differential host use in two highly specialized ant-plant associations: evidence from stable isotopes. *Oecologia*, **138**, 74–82.

Vander Zanden, M.J., Casselman, J.M., & Rasmussen, J.B. (1999) Stable isotope evidence for the food web consequences of species invasions in lakes. *Nature*, **401**, 464.

Vander Zanden, M.J., Clayton, M.K., Moody, E.K., Solomon, C.T. & Weidel, B.C. (2015) Stable isotope turnover and half-life in animal tissues: a literature synthesis. *PloS one*, **10**, p.e0116182.

Voigt, C.C., Rex, K., Michener, R.H., & Speakman, J.R. (2008) Nutrient routing in omnivorous animals tracked by stable carbon isotopes in tissue and exhaled breath. *Oecologia*, **157**, 31–40.

Wang, Y.V., O'brien, D.M., Jenson, J., Francis, D., & Wooller, M.J. (2009) The influence of diet and water on the stable oxygen and hydrogen isotope composition of Chironomidae (Diptera) with paleoecological implications. *Oecologia*, **160**, 225–233.

Ward, E.J., Semmens, B.X., & Schindler, D.E. (2010). Including source uncertainty and prior information in the analysis of stable isotope mixing models. *Environmental Science & Technology*, **44**, 4645-4650.

Wassenaar, L.I. & Hobson, K.A. (1998) Natal origins of migratory monarch butterflies at wintering colonies in Mexico: new isotopic evidence. *Proceedings of the National Academy of Sciences*, **95**, 15436–15439.

Webb, S.C., Hedges, R.E., & Simpson, S.J. (1998) Diet quality influences the $\delta^{13}C$ and $\delta^{15}N$ of locusts and their biochemical components. *Journal of Experimental Biology*, **201**, 2903–2911.

West, J.B., Bowen, G.J., Cerling, T.E., & Ehleringer, J.R. (2006) Stable isotopes as one of nature's ecological recorders. *Trends in Ecology & Evolution*, **21**, 408–414.

West, J.B., Bowen, G.J., Dawson, T.E. & Tu, K.P. eds., (2009) *Isoscapes: understanding movement, pattern, and process on Earth through isotope mapping*. Springer Science and Business Media. Berlin, Germany.

Wolf, N., Carleton, S.A., & Martínez del Rio, C. (2009) Ten years of experimental animal isotopic ecology. *Functional Ecology*, **23**, 17–26.

Wise, D.H., Moldenhauer, D.M., & Halaj, J. (2006) Using stable isotopes to reveal shifts in prey consumption by generalist predators. *Ecological Applications*, **16**, 865–876.

Yurkowski, D.J., Hussey, N.E., Semeniuk, C., Ferguson, S.H., & Fisk, A.T. (2015) Effects of lipid extraction and the utility of lipid normalization models on $\delta^{13}C$ and $\delta^{15}N$ values in Arctic marine mammal tissues. *Polar Biology,* **38**, 131–143.

**Table 2.1.** Entomological Research using Stable Isotopes

Examples of the application of stable isotopes in entomology.

| Study Topics | Invertebrate Study System | Isotopes Used | References |
|---|---|---|---|
| **Diet** | | | |
| Food preference | Coleoptera, Lepidoptera, & Hymenoptera; Mosquitoes; Ants: Moths | $^{13}C$; $^{13}C$ & $^{15}N$; $^{13}C$ & $^{15}N$; $^{13}C$ & $^{15}N$ | Petelle *et al.,* 1979; Akamatsu *et al.,* 2004; Feldhaar *et al.,* 2010 Adams *et al.,* 2016 |
| Food web structure | Ants; Ants; Aquatic insects & Spiders; Benthic invertebrates | $^{13}C$ & $^{15}N$; $^{13}C$ & $^{15}N$; $^{13}C$ & $^{15}N$; $^{2}H$ | Blüthgen *et al.,* 2003; Feldhaar *et al.,* 2010; Paetzold *et al.,* 2005; Doucett *et al.,* 2007 |
| Dietary niche | Ants; Odonata; Ants; Coleoptera | $^{13}C$ & $^{15}N$; $^{13}C$ & $^{15}N$; $^{13}C$ & $^{15}N$; $^{13}C$ & $^{15}N$ | Feldhaar *et al.,* 2010; Chari *et al.,* 2018; Rosumek *et al.,* 2018; Santi-Júnior *et al.,* 2018 |
| Resource contribution to biomass | Spiders; Mosquitoes | $^{13}C$ & $^{15}N$; $^{13}C$ & $^{15}N$ | Collier *et al.,* 2002, Oelbermann & Scheu, 2004, Akamatsu *et al.,* 2004; Kaufman *et al.,* 2010 |

Table 2.1 continued

| | | | |
|---|---|---|---|
| Trophic enrichment, discrimination, and fractionation | Beetles; | $^{13}C$ & $^{15}N$; | Scrimgeour *et al.,* 1995; |
| | Beetles; | $^{13}C$ & $^{15}N$; | Ostrom *et al.,* 1996; |
| | Termites; | $^{13}C$ & $^{15}N$; | Tayasu, 1998; |
| | General insects; | $^{13}C$ & $^{15}N$; | McCutchan *et al.,* 2003; |
| | Herbivorous insects; | $^{13}C$ & $^{15}N$; | Spence & Rosenheim, 2005; |
| | Collembola; | $^{15}N$; | Haubert *et al.,* 2005 |
| | Invertebrates; | $^{13}C$ & $^{15}N$; | Caut *et al.,* 2009; |
| | Ants | $^{13}C$; | Feldhaar *et al.,* 2010 |
| | Collembola; Ephemeroptera; Blowflies; Beetles | $^{13}C$ & $^{15}N$; | Semenina & Tiunov, 2011; Brauns *et al.,* 2018; Matos *et al.,* 2018; Quinby *et al.,* 2020 |
| Trophic relationships | Coleoptera & | $^{15}N$; | Scrimgeour *et al.,* 1995; |
| | Aphids; | $^{13}C$ & $^{15}N$; | Tillberg *et al.,* 2006; |
| | Ants; | $^{13}C$ & $^{15}N$; | Hyodo, 2015 |
| | Moths | $^{13}C$ & $^{15}N$ | Adams *et al.,* 2016 |
| Feeding Strategies | Ants; | $^{13}C$ & $^{15}N$; | Trimble & Sagers, 2004; |
| | Spiders; | $^{13}C$ & $^{15}N$; | Paetzold *et al.,* 2005; |
| | Odonata; | $^{13}C$ & $^{15}N$; | Chari *et al.,* 2018; |
| | Coleoptera | $^{13}C$ & $^{15}N$ | Santi-Júnior *et al.,* 2018 |
| **Movement** | | | |
| Population marker | Mosquitoes; | $^{13}C$; | Hood-Nowotny *et al.,* 2006; |
| | Mosquitoes; | $^{13}C$ & $^{15}N$ | Opiyo *et al.,* 2016; |
| | Biting midges | $^{13}C$ & $^{15}N$ | McDermott *et al.,* 2019 |

Table 2.1 continued

| | | | |
|---|---|---|---|
| Dispersal | Coleoptera; | $^{13}C$; | Ponsard *et al.*, 2004; |
| | European corn | $^{13}C$; | Prasifka & Heinz, 2004; |
| | borer | $^{15}N$; | Macneale *et al.*, 2004 & |
| | Stoneflies; | $^{15}N$; | Macneale *et al.*, 2005; |
| | Mosquitoes; | $^{13}C$ & $^{15}N$; | Hamer *et al.*, 2014; |
| | Mosquitoes; | $^{13}C$ & $^{15}N$ | Mederios *et al.*, 2017; |
| | Heteroptera | | Madeira *et al.*, 2019 |
| Multi-generational colonization of breeding grounds | Butterflies | $^{2}H$ & $^{13}C$ | Flockhart *et al.*, 2013 |
| Natal origin | Butterflies; | $^{2}H$ & $^{13}C$; | Wassenaar & Hobson, |
| | Butterflies; | $^{2}H$ & $^{13}C$ | 1998; |
| | Houseflies; | $^{13}C$ & $^{15}N$; | Hobson *et al.*, 1999; |
| | Dragonflies; | $^{2}H$; | Heinrich *et al.*, 2012; |
| | Cotton | $^{2}H$, $^{87}Sr$, $^{207}Pb$, & | Hobson *et al.*, 2012; |
| | bollworm; | $^{208}Pb$; | Holder *et al.*, 2014 |
| | Butterflies | $^{2}H$ & $^{13}C$ | Flockhart *et al.*, 2017 |
| **Predator-Prey Relationships** | | | |
| Nitrogen transfer from prey to predator | Aphids, flies, beetles, & Spiders | $^{15}N$ | Nienstedt & Poehling, 2004 |
| Host specificity | Ants | $^{13}C$ & $^{15}N$ | Trimble & Sagers, 2004 |
| Effects of predation on abundance and biomass of aquatic insect emergence | Aquatic insects & Spiders | $^{13}C$ & $^{15}N$ | Paetzold & Tockner, 2005 |

Table 2.1 continued

| | | | |
|---|---|---|---|
| Shifts in prey consumption | Spiders | $^{13}C$ & $^{15}N$ | Wise *et al.*, 2006 |
| **Disease Transmission** | | | |
| Tick-borne diseases | Ticks | $^{13}C$ & $^{15}N$ | Schmidt *et al.*, 2010 |
| Feeding Habits | Mosquitoes | $^{13}C$, $^{15}N$, & $^{34}S$ | Njabo *et al.*, 2013 |
| West Nile Virus | Mosquitoes | $^{13}C$ & $^{15}N$ | Hamer *et al.*, 2014 |
| **Mating** | | | |
| Spermatophore as host plant indicator & ovipositioning preference | European corn borer | $^{13}C$ | Ponsard *et al.*, 2004 |
| Assortative mating in sympatric host | European corn borer | $^{13}C$ | Malausa *et al.*, 2005 |
| Sperm transfer | Mosquitoes | $^{13}C$; $^{13}C$ & $^{15}N$ | Helinski *et al.*, 2007; Helinski *et al.*, 2008 |
| **Energy Flow** | | | |
| Nitrogen transfer | Termites; Termites; Ants | $^{13}C$ & $^{15}N$; $^{13}C$ & $^{15}N$; $^{15}N$ | Tayasu, 1998; Nardi *et al.*, 2002; Fischer *et al.*, 2003 |
| Quantifying energy flows in agroecosystems | Beetles | $^{13}C$ & $^{15}N$ | Ostrom *et al.*, 1996 |
| Nitrogen fluxes to plants | Ants | $^{15}N$ | Fischer *et al.*, 2003 |
| Aquatic subsidies to terrestrial food webs | Aquatic insects & Spiders | $^{13}C$ & $^{15}N$ | Paetzold *et al.*, 2005a |

Table 2.1 continued

| | | | |
|---|---|---|---|
| Terrestrial subsidies to aquatic food webs | Benthic invertebrates | $^{2}H$ | Doucett *et al.,* 2007 |
| Terrestrial subsidies through trophic positions | Spiders | $^{13}C$ & $^{15}N$ | Kennedy *et al.,* 2018 |
| **Effects on isotope signatures** | | | |
| Diet quality influences isotope signature and biochemical components | Locust | $^{13}C$ & $^{15}N$ | Webb *et al.,* 1998 |
| Diet and water | Chironomidae | $^{2}H$ & $^{18}O$ | Wang *et al.,* 2009 |
| Nutritional status | Collembola | $^{15}N$ | Semenina & Tiunov, 2011 |
| Lipid extraction | Aquatic invertebrates | $^{13}C$ & $^{15}N$ | Logan *et al.,* 2008 |
| Lipid content and carbon assimilation | Collembola | $^{13}C$ | Chamberlain *et al.,* 2004 |
| Isotope incorporation rate | Beetles; General Insects | $^{13}C$; $^{13}C$, $^{15}N$, & $^{18}O$ | Gratton & Forbes, 2006; Schimmelman, 2011 |
| Tissue type | Beetles; General Insects | $^{13}C$; $^{13}C$, $^{15}N$, & $^{18}O$ | Gratton & Forbes, 2006; Schimmelman, 2011 |
| Food quality, starvation and life stage impacts on isotope fractionation | Collembola | $^{13}C$ & $^{15}N$ | Haubert *et al.,* 2005 |

Table 2.1 continued

| | | | |
|---|---|---|---|
| Amino acid nitrogen recycling during fasting | Coleoptera | $^{15}N$ | Scrimgeour *et al.*, 1995 |
| Dietary sources contribution to amino acids in eggs | Lepidoptera | $^{13}C$ | O'Brien *et al.*, 2002 |
| Amino acids at different life stages | Blowflies | $^{13}C$ | Matos *et al.*, 2018 |
| Turnover and half-life in tissues | Invertebrates | $^{13}C$, $^{15}N$, & $^{34}S$ | Vander Zanden *et al.*, 2015 |
| **Insect Pest** | | | |
| Crop pest | Herbivorous and Parasitic Insects | $^{13}C$ | Petelle *et al.*, 1979 |
| Pest management | Coleoptera; Flies | $^{13}C$; $^{13}C$ & $^{15}N$ | Prasifka & Heinz, 2004; Heinrich *et al.*, 2012 |
| Insect pest outbreaks | Spruce budworm | $^{13}C$ & $^{18}O$ | Simard *et al.*, 2008 |
| **Additional Reviews** | | | |
| Stable isotopes in termite research | Termites | $^{13}C$ & $^{15}N$ | Tayasu, 1998 |
| Stable isotope methods in biology and ecology | General Arthropods | $^{2}H$, $^{13}C$, $^{15}N$, $^{18}O$, $^{34}S$, & $^{84}Sr$ | Hood-Nowotny & Knols, 2007 |
| Ant nutrition | Ants | $^{13}C$ & $^{15}N$ | Feldhaar *et al.*, 2010 |

Table 2.1 continued

| | | | |
|---|---|---|---|
| Stable isotopes evaluating Collembola | Collembola | $^{15}N$ | Semenina & Tiunov, 2011 |
| Stable isotopes in trophic ecology | General Insects | $^{13}C$ & $^{15}N$ | Hyodo, 2015 |

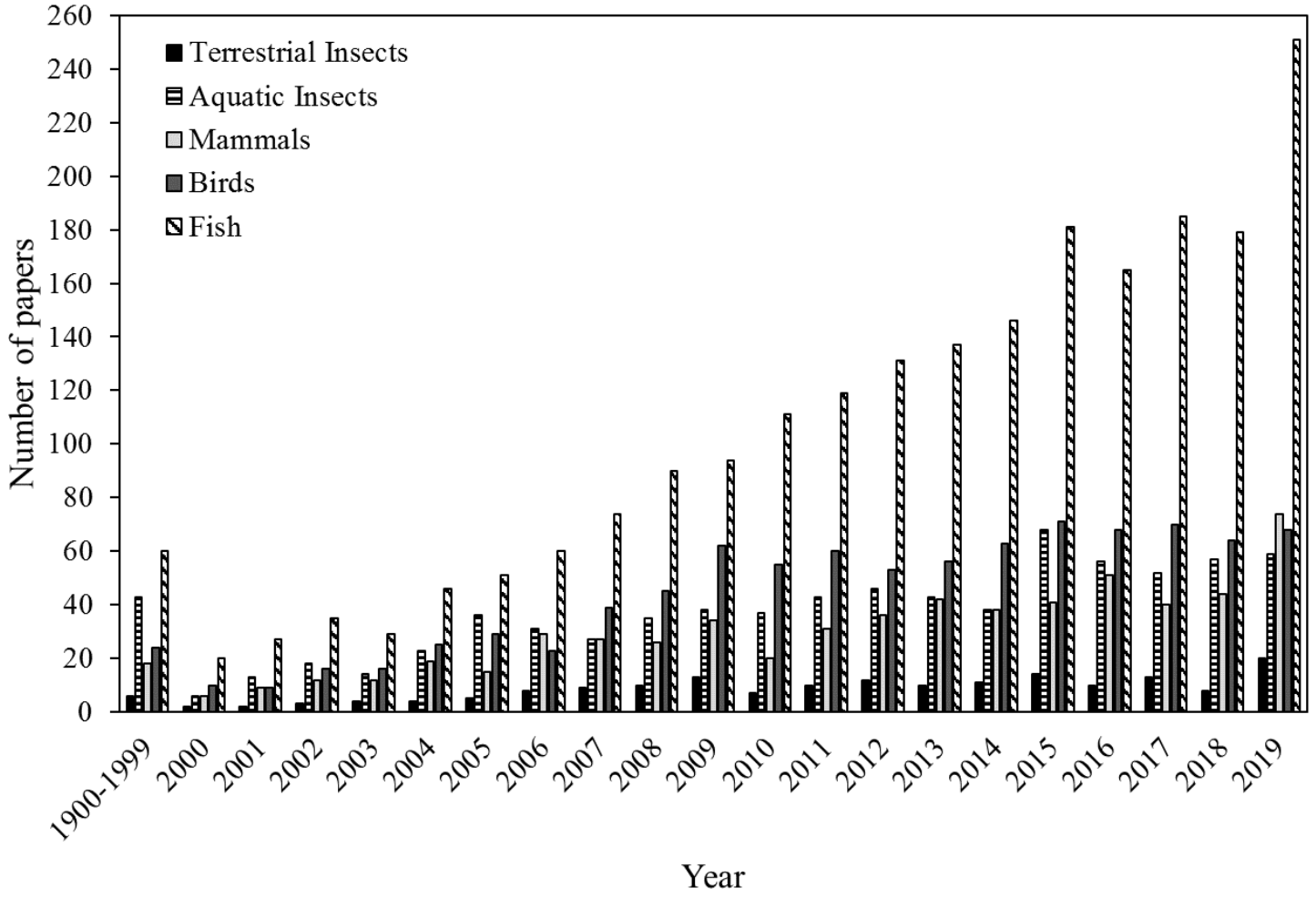


**Figure 2.1.** Publications Using Stable Isotopes in Wildlife Research

Total number of published papers in which stable isotope analysis was used to determine migration, diet, niche, parasite-host interactions or condition of terrestrial insects, aquatic insects, mammals, birds, and fish. We conducted our literature search using Web of Science and the terms “stable isotopes” and “insects/mammals/birds/fish”.

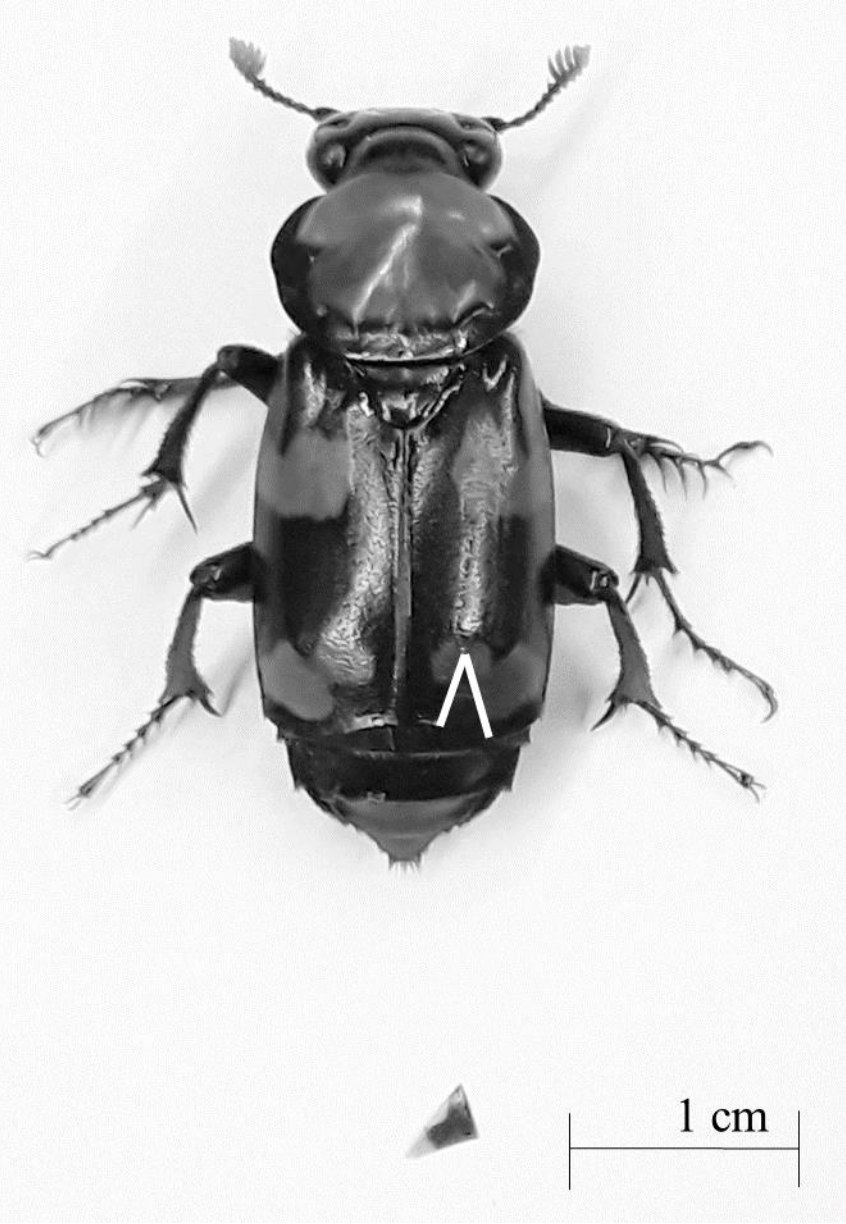


**Figure 2.2.** Burying Beetle Elytral Notch

Example of a minimally invasive elytral notch used to determine stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) in the Federally Endangered American burying beetle (*Nicrophorus americanus*; Quinby *et al.*, 2020).

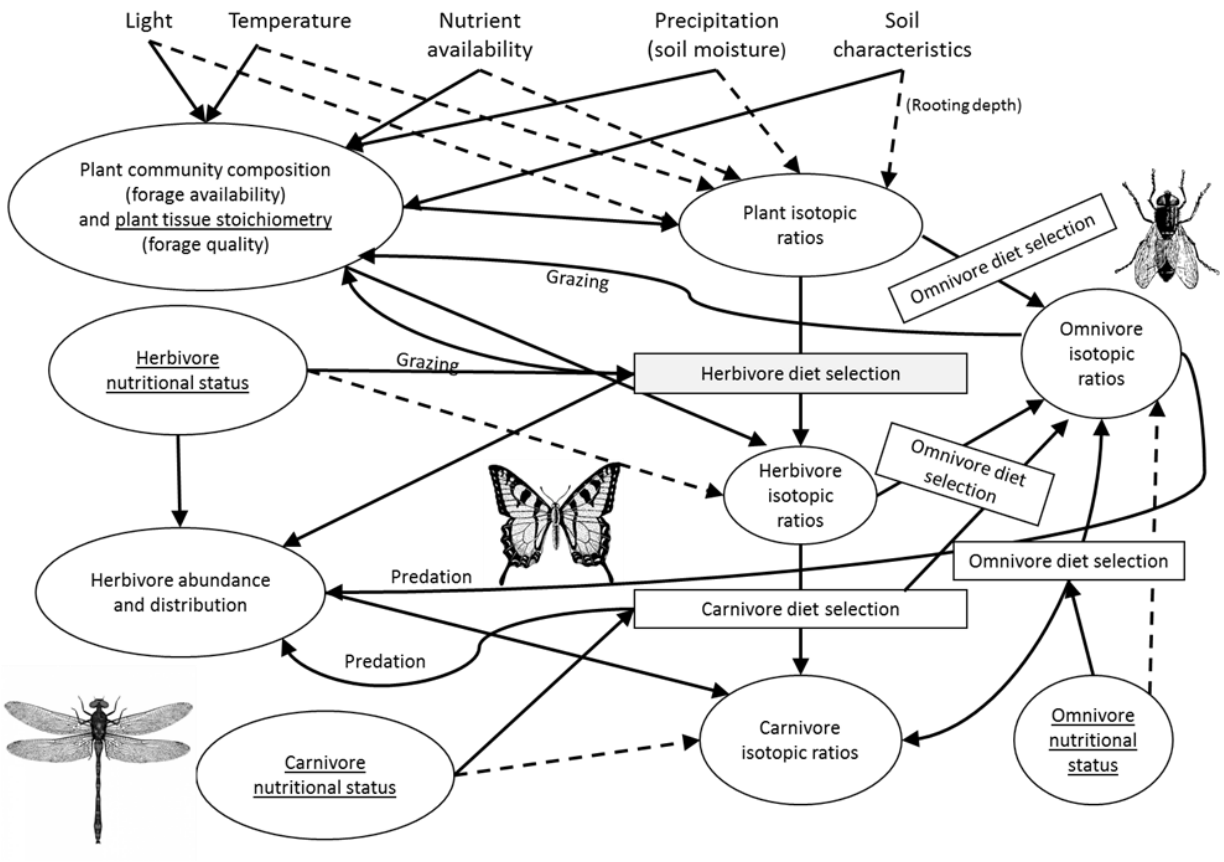


**Figure 2.3.** Factors that Influence Stable Isotope Ratios

Chemical reactions and biological events that effect stable isotope signatures of adult herbivorous, carnivorous, and omnivorous insects, including biochemical processes, underlined physical processes, and rectangles surround the behavioural processes. Ecological interactions are denoted by solid lines, whereas aspects influencing enzymatic reactions and diffusion rates (i.e., nutrient cycling, nutrient routing, and photosynthesis) are denoted with dotted lines. Isotopic values from tissues of a consumer that is omnivorous is influenced by multiple ecological properties including physiological processes, behavioural processes, and various effects from the ecosystem. Additional considerations for insects as interactions between processes such trophic enrichment during metamorphosis; soil microbial processes; decomposition; and methane-derived C for insects that have aquatic stages will also influence stable isotope ratios (Hyodo, 2015). Modified from Ben-David *et al.*, (2001) and Ben-David & Flaherty, (2012).

# CHAPTER 3. EVALUATION OF THE VERTEBRATE CARRION RESOURCES USED BY THE AMERICAN BURYING BEETLE (*NICROPHORUS AMERICANUS*)

Brandon M. Quinby, J. Curtis Creighton, and Elizabeth A. Flaherty

## 3.1 Highlights

- American burying beetles use natural carrion for reproduction.
- Nantucket Island American burying beetles rely on provisioned carrion for reproduction.
- Co-occurring burying beetles exhibit large niche overlap.
- Ring-necked pheasant is an important resource on Block Island but not Nantucket.
- Long-term provisioning of quail may be necessary for successful recruitment.

## 3.2 Abstract

The last naturally occurring American burying beetle, *Nicrophorus americanus* (ABB) on Nantucket Island, Massachusetts was recorded in 1926. Beginning in 1993, laboratory-reared offspring of wild-caught individuals from Block Island, Rhode Island were reintroduced onto Nantucket. After an initially successful reintroduction, the population shows little evidence of recruitment and likely requires provisioning of quail carcasses for long-term success. A key requirement of the ABB's life cycle is the availability of small vertebrate carcasses used for breeding. Despite over 30 years of research, we know little about the preferred carrion base necessary to support a healthy ABB population. We investigated carrion use and feeding relationships of local burying beetles within an extant and reintroduced population using stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) conducted on elytral samples collected from live-captured specimens. Our results suggested that ABBs are not specializing on avian or mammalian carrion and are using both natural and provisioned carrion for reproduction. On Block Island, estimates for the highest proportional dietary resource for ABBs was ring-necked pheasant (*Phasianus colchicus*) at 28% for 2017 and 2018. On Nantucket Island, where pheasant are less abundant, estimates for the highest proportional dietary resource for ABBs in 2017 was small mammals at 50%, and granivorous birds at 65% in 2018. Additionally, we observed large niche overlap in both populations. For successful management and reintroduction, conservation managers must consider

the availability of natural carrion resources. Lastly, we recommend long-term provisioning of quail at reintroduction sites for successful recruitment.

## 3.3 Introduction

Anthropogenic habitat alteration can lead to a change in ecological niche structure that reduces essential resources for many species (Severns and Warren, 2008; Preston et al., 2012). Changes in resource availability has a negative impact on species that specialize on these depleted resources, which can change community composition and species interactions (Jonsson et al., 2015; Seidl et al., 2017). For example, Berg and Ellers (2010) determined that species exhibiting plasticity in resource requirements as a result of changes in resource availability can result in an enlarged fundamental niche for that species and causes a reduction of vacant niches for others. Furthermore, plasticity in the proportional resource uptake for a species results in the expansion of its realized niche, causing a reduction in the possibility for coexistence with other species (Berg and Ellers, 2010).

In most terrestrial ecosystems competition between invertebrate decomposers and vertebrate scavengers direct the role of carrion energy transfer in food webs (DeVault et al., 2011). In carrion feeding guilds competition for resources can be intense, and insects and microbes have evolved efficient ways of monopolizing carrion (Janzen, 1977; Burkepile et al., 2006). However, anthropogenic habitat alteration has a significant impact of the available carrion base by changing community structure (Gibbs and Stanton, 2001; Wilson and Wolkovich, 2011). For example, habitat alteration changes the relative abundance of different size classes of small mammals: larger species tend to disappear and smaller species increase in population size (Nupp and Swihart, 2000). Habitat alteration also can lead to an increase in vertebrate scavengers that in turn changes competitive interactions among the vertebrate and invertebrate carrion feeding guilds (DeVault et al., 2004).

Burying beetles are an essential component in carrion food webs of many terrestrial ecosystems (Parmenter and MacMahon, 2009; Barry et al., 2019). These beetles use carrion for both nonreproductive feeding and for reproduction as food for their offspring (Scott, 1998). For reproduction burying beetles specialize on small vertebrate carcasses, which developing young feed on after the carcass is preserved and buried by their parents. Because carrion is highly sought after and unpredictable in space and time (Hanksi and Cambefort 1991; Darimont et al., 2008),

available carcasses for reproduction is hypothesized to be the limiting resource for burying beetle species (Lomolino and Creighton, 1996).

Among burying beetle species, carrion size used for reproduction is highly correlated with body size (Scott, 1998). For example, the federally endangered *Nicrophorus americanus* (ABB) is the largest member of the North American burying beetle guild and generally uses larger carcasses for reproduction (range between 80-200 g; Kozol et al., 1988). This makes larger burying beetle species such as the ABB disproportionately impacted by habitat fragmentation because of a loss of optimal-sized carcasses for reproduction (Nupp and Swihart, 2000). The concurrent increase in smaller mammal species that can be used only by smaller burying beetle species and an increase in vertebrate scavengers associated with habitat fragmentation potentially changes the dynamics of interspecific competition for carrion resources (DeVault et al., 2011; Hopwood et al., 2016a). Thus, from a conservation perspective, effective management of the ABB requires both an understanding of its critical habitat and limiting resources and an understanding of how potential anthropogenic changes in the habitat impacts community structure and competitive interactions among species (Poole et al., 2014).

Burying beetles partition resources depending on breeding season, daily activity patterns, habitat use, and carcass size used for reproduction (Trumbo, 1990; Lomolino et al., 1995; Creighton, 2005; Cook et al., 2019). For breeding, it is hypothesized that burying beetles have broad preferences for a variety of small vertebrate carrion types but a strong preference for carrion size (Lomolino and Creighton, 1996; Scott, 1998; Hocking et al., 2007; Quinby et al., 2020). Furthermore, it is highly likely that burying beetles exhibit a nested hierarchy in ecological niches that includes specialist species with a narrow niche breadth, generalist species with a wider niche breadth, or a combination of both (Araújo et al., 2010; Robertson et al., 2015; Quinby et al., 2020).

Although burying beetle ecology and behavior has been extensively studied over the last 30 years (Kozol et al., 1988; Eggert et al., 1998; Creighton, 2005; Cook et al., 2019), we know little about what burying beetles actually use for breeding under natural conditions. Using stable isotopes, researchers recently evaluated feeding relationships of co-occurring, wild-caught burying beetles (Quinby et al., 2020). They determined that co-occurring burying beetles from their study area were all using a similar carrion base of mammalian carrion for reproduction (Quinby et al., 2020). However, due to small sample sizes of potential reproductive carrion, their study was unable to distinguish among small vertebrate species used by burying beetles for reproduction in the wild

(Quinby et al., 2020). Additionally researchers used stable isotopes to characterize within- and among-population variation in dietary niche in populations and communities of burying beetles by evaluating niche partitioning among species focused on differentiation in carcass use in relation to body size (Ikeda et al., 2006) and niche variation associated with terrestrial and marine carrion (Hocking et al., 2007). However, there are still many unknowns in regards to burying beetle resource use under natural conditions especially in areas of conservation concern.

The ABB was listed as a federally endangered species in 1989 (Federal Register 1989). Historically, ABBs were distributed across 35 states three Canadian provinces in eastern and central temperate forested areas of North America but has recently undergone a dramatic decline in its abundance and geographic range (Anderson, 1982; Lomolino et al., 1995; Backlund et al., 2008; Mckenna-Foster et al., 2016). Today, extant populations are constrained to the western and eastern limits of its historic range including, Arkansas, Kansas, Nebraska, Oklahoma, Rhode Island, South Dakota, and Texas (Anderson, 1982; U.S. Fish and Wildlife Service, 1991; Leasure and Hoback, 2017). Sikes and Raithel (2002) proposed several hypotheses to explain the species decline, but it is likely that the primary influences leading to its endangered status are its specialized breeding behavior and large body size. Reintroduction efforts are underway or have been attempted in Massachusetts (on Nantucket Island), Missouri, and Ohio that exhibit varying degrees of success (Barnhart and Brown, 2002; Selbo, 2009; Mckenna-Foster et al., 2016; Perrotti and Mckenna-Foster, 2019).

Efforts to reintroduce ABBs to Nantucket Island began in 1993 with initial success (Mckenna-Foster et al., 2016). However, the 2016 surveys on Nantucket resulted in the lowest number of captures in the 23 years of organized surveys (L. Perrotti pers. comm.) suggesting that the population is not self-sustaining and may require human assistance for long-term maintenance (Mckenna-Foster et al., 2016). Although the ABB has been studied extensively relative to habitat use (Creighton et al., 1993; Lomolino et al., 1995; Bedick et al., 1999), its feeding relationships and availability of preferred food sources under natural conditions has received relatively little attention (Quinby et al., 2020), and knowledge of what species and sizes of small vertebrates ABBs are using for reproduction is lacking. Furthermore, it is unclear how the reintroduction of one species of burying beetles following local extirpation affects resource partitioning among the extant species. This challenges appropriate management of habitat for extant and reintroduced populations. Therefore, characterizing suitable habitat and managing existing and reintroduced

ABB populations depend on knowing the distribution and availability of all reproductive carrion sources. Furthermore, interspecific interactions among burying beetles can influence reproduction and establishment of reintroduced ABBs. It is therefore important to understand interspecific interactions including potential competition among members of the burying beetle community, especially in respect to carrion used for reproduction, to better evaluate factors affecting population dynamics.

Stable isotope analysis provides indirect techniques to evaluate the community ecology and resource use of species, and can offer insights into the feeding ecology and trophic relationships of organisms (Fleming et al., 1993; Voigt et al., 2008; Dammhahn et al., 2015). Furthermore, stable isotope analysis is beneficial because it can reveal differences in the overall composition of diet (Gratton and Forbes, 2006; Flaherty et al., 2010; Chikaraishi et al., 2011), niche partitioning (Wolf et al., 2002; Blüthgen et al., 2003; Barnum et al., 2013), and trophic position (Tooker and Hanks, 2004; Tillberg et al., 2006; Lorrain et al., 2009) among species that are problematic or impossible to identify using traditional methods (i.e., direct observation, gut content analysis, and pigment tracing; Pearson et al., 2003; Hood-Nowotny and Knols, 2007; Flaherty et al., 2010; Ruhl et al., 2020). As organisms consume prey items the isotopic values of assimilated prey are incorporate into the consumer's tissues to varying degrees (DeNiro and Epstein, 1978; Ruhl et al., 2020). Using the isotopic composition of diet items and tissue samples from consumers and including diet-tissue discrimination (Hobson and Clark, 1992; Dalerum and Angerbjörn, 2005), researchers can incorporate mathematical mixing models into their research to approximate the relative contributions of dietary items to a consumer's diet (Parnell et al., 2013; Phillips et al., 2014).

The goals of our current study were to provide conservation managers with information on carrion resources ABBs use *in situ* for reproduction by evaluating the isotopic signatures of co-occurring burying beetles, locally available carrion and provisioned quail carrion. Burying beetle larval diet is restricted to the single small vertebrate carcass that their parents bury and preserve using oral and anal secretions (Scott, 1998). As result, the body tissues of the adult beetles, including the elytra, reflect the isotopic signature of their larval food source (Gratton and Forbes, 2006; Schimmelman, 2011; Quinby et al., 2020). Using elytron clippings, we evaluated the isotopic niche of co-occurring burying beetles between an extant population on Block Island, Rhode Island and a reintroduced population on Nantucket Island, Massachusetts to provide insight

into intra-population factors that influence resource use and feeding relationships between populations. An understanding of feeding relationships will provide critical information necessary to manage existing ABB populations and to inform ongoing reintroduction efforts and habitat management. We hypothesized that ABBs within the extant Block Island population are using locally available ring-necked pheasant (*Phasianus colchicus*) as a reproductive resource (Sikes and Raithel, 2002). Within this population, we hypothesized that the availability of a large reliable carrion source (ring-necked pheasant) allows for niche separation between ABBs and other local burying beetle species on Block Island because smaller local burying beetle species are less capable of using pheasants for reproduction (Creighton et al., 2009; Hopwood et al., 2016b). However, on Nantucket Island, the ring-necked pheasant is extremely rare (Mckenna-Foster et al., 2016), and we hypothesized that ABBs rely on smaller carrion, which in turn increases niche overlap and competition among burying beetles in that population. Additionally, we predicted that the reintroduced ABB population relies more heavily on provisioned farm-raised quail for reproduction when compared to the extant population.

## 3.4 Materials and Methods

### 3.4.1 Study Area

We conducted our study on Block Island, Rhode Island (Lat. 41.1617°N, Long. 71.5843°W; Fig. 1A), and Nantucket Island, Massachusetts (Lat. 41.2835°N, Long. 70.0995°W; Fig. 1B). Study sites largely were composed of maritime shrub thickets, costal moraine grassland, and agricultural pastures on Block Island (Kozol et al., 1988; Amaral et al., 1997), and sandplain grassland, costal heathland, and mixed forest on Nantucket Island (Mckenna-Foster et al., 2019, 2016). Vegetation communities on the islands largely consist of scrub oak (*Quercus ilicifolia*) and pitch pine (*Pinus rigida*) among a mosaic of low grasses and shrubs (Kozol et al., 1988; Amaral et al., 1997; Mckenna-Foster et al., 2019, 2016).

### 3.4.2 Sample Collection

#### *Burying Beetle Sampling*

In the summers of 2017 and 2018 (12 June – 30 June), we used pitfall traps to collect burying beetles. We followed the ongoing trapping protocol originally described by Kozol (1991).

Pitfall traps contained a 946 ml mason jar buried flush to the top level of the soil. We placed a screw-on mesh lid on the top of a small plastic container full of aged chicken inside of each pitfall trap. Before baiting traps, we aged it for 7-8 days in a plastic container maintained at room temperature. We also placed a moist sponge in each trap to help prevent beetle desiccation. We covered each jar with a square piece of hardware cloth with a 3 x 3 cm hole in the center that allowed beetles access to the trap and helped prevent disturbance from other wildlife. We placed a disposable aluminum pan lid over the pitfall trap and staked it down with ground staples to exclude rain and provide shade. We checked traps every morning between 0600 and 1000 hr EST thus ensuring we removed all burying beetles before environmental temperatures became lethally warm for beetles. We collected all ABBs captured in single occupancy containers to await provisioning, and a subset of the other burying beetle species captured were frozen until they were processed for analysis (Tables 1, 2).

On Nantucket Island, we collaborated with the United States Fish and Wildlife Service (USFWS), Roger Williams Park Zoo (RWPZ), Maria Mitchell Association of Nantucket (MMA), and Nantucket Conservation (NC) on pitfall trapping efforts that began in 1993 (Perrotti and Mckenna-Foster, 2019). During the summer of 2017, we set traps on the night of 12 June and trapped continuously until 28 June. For the summer of 2018, we set traps on the night of 13 June and trapped continuously until 25 June. We placed 60 total pitfall traps spaced 20 m apart at 12 sites arranged in individual linear transects in eastern Nantucket Island for a total sampling effort of 720 trap-nights/yr.

On Block Island, we collaborated with the Nature Conservancy (TNC), Rhode Island Department of Environmental Management (RDEM), USFW, and RWPZ during their annual monitoring of the ABB population, which began in 1991 (Raithel et al., 2006). The annual Block Island survey involves three consecutive nights of pitfall trapping during the last week of June. During the summers of 2017 and 2018, we set traps on the night of 28 June and trapped continuously until 30 June. We placed 50 total pitfall traps, 20 m apart from one another, at three sites arranged in individual linear transects in southwest Block Island, for a total sampling effort of 150 trap-nights per year.

We collected tissue for stable isotope analysis from four species of burying beetles (ABB, *Nicrophorus orbicollis*, *Nicrophorus tomentosus*, and *Nicrophorus marginatus*; Table A1). To determine feeding relationships of ABBs, we analyzed stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) using a

small clip from the elytra of live-captured specimens (Fig. 2); we used the whole elytra for all other burying beetle species. We collected all samples for stable isotope analysis during the peak reproductive season in mid-summer.

### *Small Mammal and Bird Sampling*

To compare to the signatures of the burying beetle species and determine carrion food sources, we collected whole blood, muscle tissue, and feather samples from locally available small mammals and birds for 2017 and 2018 on both Block Island and Nantucket Island (Tables 1, 2). We collected samples from all potential small mammal species on both islands and based the avian species sampled on bird surveys previously conducted by RDEM and Massachusetts Division of Fisheries and Wildlife. Body mass of most sampled species ranged between 100-300 g (Schwartz and Schwartz, 2001) and could provide a suitable carrion base for ABBs. The goal in our sampling was to represent variation in size and functional groups (e.g., herbivores versus insectivores) of the small vertebrate fauna. We followed recommendations by the Ornithological Council (Fair et al., 2010) and the American Society of Mammologists (Sikes et al., 2016) for all small mammal and bird surveys. None of the potential carrion species collected were of conservation concern on Block Island or Nantucket Island, and Purdue University's Institutional Animal Care and Use Committee (PACUC) approved all methods involving live vertebrates (PACUC protocol No. 1705001577). We handled all live mammals in accordance with State Permits (180.17SCM and 062.18SCM for Nantucket Island; 2017-32-W and 2018-14-W for Block Island). We handled all live invertebrates in accordance with State Permits (182.17SCI and 064.18SCI for Nantucket Island; 2017-32-W and 2018-14-W for Block Island) and Federal Permits (TE-41559C-1). We handled all live birds in accordance with State Permits (181.17SCB and 063.18SCB for Nantucket Island; 2017-32-W and 2018-14-W for Block Island) under Federal Banding Permit Numbers (22795 Nantucket Island; and 09636 Block Island).

We live trapped locally available small mammals in the summers of 2017 and 2018 (28 June – 30 September; Table A1). We collected ≤100 μL of whole blood samples from small mammals using submandibular venipuncture (Berl et al., 2017). The basic sampling scheme at each small mammal trapping site consisted of a 1 × 5 trapping line transect of Sherman live-traps (LAFAHD Folding Live Capture, H. B. Sherman, Tallahassee, FL, USA; 7.62 × 8.89 × 22.86 cm) each separated by 20 m. Additionally, we mist-netted during the summers of 2017 and 2018 in

collaboration with ongoing bird monitoring surveys conducted by MMA and TNC. We opportunistically removed molt feathers from birds and stored the samples at –18° C in a disposable plastic bag (Podlesak et al., 2005; Ruhl et al., 2020; Vitz and Rodewald, 2012; Table A1, A2). We collected ≤100 μL of whole blood samples from birds using brachial venipuncture (Pearson et al., 2003; Podlesak et al., 2005; Ruhl et al., 2020; Table 1, 2). To capture birds we used a basic sampling scheme at each mist-netting site consisting of 3, 12 m long, 30 mm mesh, 2 tier, black, tethered, nylon mist nets. During the summers of 2017 and 2018, MMA and TNC provided additional feather and muscle tissue (1-3 g) samples from mammals and birds previously donated and frozen (Tables A1, A2). We stored all samples of vertebrate muscle and blood tissue in microcentrifuge tubes and feather samples in plastic bags in a freezer at –18 ℃ until processing.

### 3.4.3 Stable Isotope Sample Analysis

We prepared all samples for isotope analysis in the Wildlife Physiology Laboratory at Purdue University. We prepared feathers by washing them with a 2:1 mixture of chloroform and methanol (Hobson and Bairlein, 2003; Ruhl et al., 2020) and dried consumer (burying beetle) tissues and prey items in an oven for 48 hr at 60°C. We cut feathers into small pieces using scissors then used a mixer mill (Retsch MM 200, Glen Mills Inc., Clinton, NJ), to grind and homogenize invertebrate and muscle tissue samples. To maximize sample yield for ABB elytral clips or whole blood samples from mammals and birds, we used a mortar and pestle. Finally, we weighed all samples in miniature tin weigh boats (3 mm × 5mm; Costech Analytical Tech Inc., Balencia, California, USA) using a Sartorius microbalance (model CPA2P; Sartorius, Arvada, Colorado, USA).

If sample quantity allowed, we submitted samples in duplicate for analysis to the University of Wyoming Stable Isotope Facility (UWSIF; University of Wyoming, Laramie, WY). We analyzed all samples for $\delta^{13}C$ and $\delta^{15}N$ using an elemental analyzer and isotope ratio mass spectrometer (Thermo Finnigan Delta Plus XP, Costeck 4010 and Carlo Erba 1110 Elemental Analyzer, Costec Zero Blank Autosampler, Finnigan Conflo III Interface). During analysis, UWSIF used the standards PeeDee Belemnite (for $\delta^{13}C$) and atmospheric air (for $\delta^{15}N$). Additionally, they used alfalfa, chitin, glutamic 1, glutamic 2, keratin, liver, and whole blood as reference materials for quality control. Mean standard uncertainty was 0.1 for $\delta^{13}C$ and 0.09 for $\delta^{15}N$. To determine if lipid correction using mathematical calculations was needed, we calculated

the carbon-to-nitrogen (C:N) mass ratio of each sample ( Post, 2002; Cherel et al., 2005). We employed mathematical corrections to account for effects of lipids on $\delta^{13}C$ values for beetle species and carrion categories with C/N ratios > 4 (Post, 2002; Post et al., 2007). We used sample results for analysis only if the two subsamples' variance did not exceed the standards' variance, which ranged between 0.1–0.3‰ based on individual runs (Ben-David and Flaherty, 2012). To complete all analyses for each sample, we used the mean values of the two subsamples for $\delta^{13}C$ and $\delta^{15}N$.

### 3.4.4 Statistical Analysis

We used multivariate analysis of variance (MANOVA) with post hoc Tukey's HSD (Zar, 2014) and a *K* nearest-neighbor randomization test (Rosing et al., 1998), before incorporation into stable isotope mixing models in program R (v. 3.6.1, (R Core Team 2019) to evaluate isotopic signatures among vertebrate dietary items to ensure that all of the potential vertebrate prey were significantly different in bivariate mixing space. We combined data for diet items that did not differ significantly ($P>0.05$) in $\delta^{13}C$ and $\delta^{15}N$. In addition, we used a MANOVA in program R to assess differences in isotopic signature among seasons and localities for species of burying beetles and potential prey (Table 1, 2, A2). We determined different dietary source profiles among burying beetle species based on ongoing ABB management plans (Mckenna-Foster et al., 2016; Perrotti and Mckenna-Foster, 2019). We did not include farm-raised quail in the source profiles of *N. orbicollis, N. marginatus,* or *N. tomentosus* because we did not detect any evidence of carcass takeover on farm-raised quail from these species after provisioning (L. Perrotti personal communication).

We used the dual-isotope linear mixing model package SISUS (Stable Isotope Sourcing Using Sampling; Erhardt et al., 2014) to determine the relative contribution of potential food items to the diet of burying beetles in a Bayesian framework. To avoid issues with model convergence (Moore and Semmens, 2008), we used SISUS because it allows users to specify the number of probabilistic exact solutions derived from the mixing model and it outperforms deterministic methods (Erhardt et al., 2014). We adjusted source estimates for isotopic signatures using diet-consumer discrimination factors published in the literature for ABBs ($\Delta_{birds}\ ^{13}C = –1.3‰$ and $\Delta_{birds}\ ^{15}N = 2.4$ ‰; $\Delta_{mammals}\ ^{13}C = 0.6$ ‰ and $\Delta_{mammals}\ ^{15}N = 2.9$ ‰; Quinby et al. 2020). There are known changes in discrimination associated with light availability and decomposition (Gebauer and Schulze, 1991; West et al., 2006). Because *N. marginatus* is diurnal (Bedick et al., 2006) we

adjusted isotopic source estimates using discrimination factors reported in the literature ($\Delta_{birds}\ ^{13}C = -2.5‰$ and $\Delta_{birds}\ ^{15}N = 2‰$; $\Delta_{mammals}\ ^{13}C = -1.4‰$ and $\Delta_{mammals}\ ^{15}N = 2‰$; (Hyodo, 2015; Matos et al., 2018). If we had determined *a priori* to test for the contribution of a source of interest (e.g., proportional contribution of farm-raised quail for ABBs) we included them in mixing models. We also used kernel utilization density (KUD) methods with the rKIN package in program R to estimate isotopic niche space and percent overlap for the 50%, 75%, and 95% contours (Eckrich et al., 2020). Due to constraints in sample size, we combined Nantucket Island ABBs from 2017 and 2018 for KUD methods, and we did not include *N. tomentosus* in KUD calculations on Nantucket Island.

## 3.5 Results

### 3.5.1 Reproductive Carrion

The final assemblage of potential reproductive carrion resources used in mixing models contained insectivorous birds, pheasant, farm raised quail, Norway rats, and native small mammals on Block Island (Table A2.) and generalist birds, insectivorous birds, granivorous birds, farm raised quail, Norway rats, and small mammals on Nantucket Island (Table A2). We combined reproductive food resources into 5 and 7 distinct groups based on results of the K nearest-neighbor test ($P < 0.01$) for Block Island and Nantucket Island, respectively.

### 3.5.2 Stable Isotope Analysis

***Block Island***

On Block Island, we collected tissue samples from 69 ABBs, 29 *N. orbicollis*, 23 *N. tomentosus*, and 52 *N. marginatus*. We found a significant difference in both $\delta^{13}C$ values ($F_{(4,130)} = 19.56$; $p<0.01$; partial $\eta^2 = 0.376$) and $\delta^{15}N$ values ($F_{(4,130)} = 9.59$; $p<0.01$; partial $\eta^2 = 0.228$) among different species of wild-caught burying beetles (Table 1; Fig. 3A). Mean isotopic signatures for $\delta^{13}C$ and $\delta^{15}N$ for all Block Island burying beetles did not differ between years (Table 1). Block Island ABBs had significantly different $\delta^{13}C$ values than *N. orbicollis, N. marginatus,* and *N. tomentosus* (Table 1). The $\delta^{15}N$ values for Block Island ABBs were significantly different from *N. marginatus,* but not *N. orbicollis* and *N. tomentosus* (Table 1).

Additionally, $\delta^{13}C$ and $\delta^{15}N$ values differed significantly among potential reproductive carrion ($F_{(8,128)} = 84.23$; $p<0.01$; Wilk's $\Lambda = 0.025$; partial $\eta^2 = 0.84$; Table 1; Fig. 3A). The $\delta^{13}C$ values were significantly different between farm-raised quail and all other potential reproductive carrion (Table 1). Values for $\delta^{13}C$ for Norway rats did not differ significantly from insectivorous birds, pheasant, or small mammals (Table 1). Values for $\delta^{13}C$ between small mammals and insectivorous birds or pheasant did not differ significantly (Table 1). Additionally, values for $\delta^{13}C$ in insectivorous birds and pheasant were not significantly different (Table 1).

The $\delta^{15}N$ values were significantly different between quail and all other potential reproductive carrion (Table 1). Values for $\delta^{15}N$ in small mammals were significantly different from Norway rats, insectivorous birds, and pheasant (Table 1). The $\delta^{15}N$ values in Norway rats differed from $\delta^{15}N$ values pheasant but not insectivorous birds (Table 1). Additionally, $\delta^{15}N$ values in insectivorous birds and pheasant were significantly different between groups (Table 1).

***Nantucket Island***

We collected tissue samples from 30 ABBs, 65 *N. orbicollis,* 3 *N. tomentosus,* and 36 *N. marginatus* on Nantucket Island. We found a significant difference in $\delta^{13}C$ ($F_{(3,75)} = 14.42$; $p<0.01$; partial $\eta^2 = 0.37$) and $\delta^{15}N$ values ($F_{(3,75)} = 19.47$; $p<0.01$; partial $\eta^2 = 0.44$) among different species of wild-caught burying beetles (Table 2; Fig. 3B). The mean isotopic signatures for Nantucket Island burying beetles are summarized in Table 2. The mean isotopic signatures for Nantucket Island burying beetles did not differ significantly between years except for ABBs (Table 2).

Additionally, $\delta^{13}C$ and $\delta^{15}N$ values differed significantly among potential reproductive carrion ($F_{(8,210)} = 52.56$; $p<0.01$; Wilk's $\Lambda = 0.111$; partial $\eta^2 = 0.67$; Table 2; Fig. 3B). The $\delta^{13}C$ values were significantly different between sea birds and all other potential reproductive carrion except farm raised quail (Table 2). Values for $\delta^{13}C$ between native small mammals and Norway rats did not differ significantly (Table 2). Additionally, values for $\delta^{13}C$ between small mammals and insectivorous, granivorous, and generalist birds did not differ significantly, but they significantly differed between small mammals and farm-raised quail (Table 2). Values for $\delta^{13}C$ in Norway rats and insectivorous, granivorous, or generalist birds were not significantly different (Table 2). However, there was a significant difference between $\delta^{13}C$ values between quail and

insectivorous, granivorous, and generalist birds, as well as between quail and Norway rats (Table 2).

The $\delta^{15}N$ values were significantly different between seabirds and all other potential reproductive carrion (Table 2). Values for $\delta^{15}N$ in small mammals and quail did not differ between one another, however, $\delta^{15}N$ values in small mammals differed from $\delta^{15}N$ values in insectivorous, granivorous, generalist birds, and Norway rats (Table 2). Additionally, $\delta^{15}N$ values in insectivorous, granivorous, and generalist birds and Norway rats were not significantly different between groups (Table 2).

### 3.5.3 Mixing Model Analysis

Based on the results of the stable isotope mixing model, Block Island burying beetles used all available carrion categories for reproduction. The largest estimated proportional reproductive carrion resource for all Block Island burying beetle species except *N. marginatus* was pheasant (Table 3). Estimates for *N. marginatus* suggested a larger proportion of sampled individuals relied on insectivorous birds for reproductive carrion (Table 3).

Similar to Block Island populations, Nantucket Island burying beetles used carrion from all available carrion categories for reproduction. For ABBs sampled in 2017, the largest estimated proportional reproductive carrion resource was small mammals; however, for ABBs sampled in 2018 it was granivorous birds (Table 3). For all other Nantucket Island burying beetles, the largest estimated proportional reproductive carrion resource was small mammals (Table 3).

### 3.5.4 KUD Analysis

We identified a significant amount of niche overlap among all burying beetle species on both islands using the KUD analysis (Table 6; Fig. 4). On Block Island, we observed $\geq$50% overlap of core-niche-space estimates among ABBs, *N. orbicollis*, and *N. tomentosus* but not for *N. marginatus* (Table 6). Isotopic niche-space estimates on Block Island were largest for ABBs (Table 6). Among ABBs, *N. orbicollis*, and *N. marginatus* on Nantucket Island, we observed $<$50% overlap of core-niche-space estimates (Table 6). Similar to Block Island beetles, ABBs on Nantucket Island had the largest isotopic niche-space estimates (Table 6).

## 3.6 Discussion

American burying beetles from Block Island used a broad range of carrion including both naturally available carrion and provisioned quail for reproduction (Table 3). Our results did not identify a significant reproductive carrion preference for Block Island ABBs and the largest estimated proportional source contribution for any reproductive carrion source was ring-necked pheasant (28%; Table 3). Similarly, non-endangered burying beetle species on Block Island used a variety of naturally available carrion for reproduction, however they did not use provisioned quail (Table 3). Avian reproductive carrion resources were important for all Block Island burying beetle species, comprising 61-97% of the estimated proportional source contributions (Table 3).

All four co-occurring species on Block Island used ring-necked pheasant for reproductive carrion (Table 3). Furthermore, ring-necked pheasant is estimated to be the largest proportional source contribution for reproductive carrion among all Block Island burying beetles except for *N. marginatus* (Table 4). We observed evidence of large niche overlap among all Block Island burying beetles (Table 4; Fig. 4A) suggesting that they were competing for similar reproductive carrion resources to raise their young. This was contrary to our hypothesis that the large size and availability of ring-necked pheasant would allow for niche separation among ABBs and co-occurring burying beetle species on Block Island. The larger niche-space estimates for ABBs are likely a result of provisioned farm-raised quail (Table 4).

Similar to Block Island, Nantucket Island ABBs are using a broad range of both naturally available carrion and provisioned quail for reproduction (Table 4). Our results did not identify a significant reproductive carrion preference for ABBs. In 2017, the largest estimated proportional source contribution for any reproductive carrion source was for small mammals at 50%, however, in 2018 it was for granivorous birds at 65% (Table 4). Non-endangered burying beetles on Nantucket Island were using a variety of naturally available carrion but they are not using provisioned quail for reproduction (Table 4). The largest estimated proportional reproductive carrion source contribution for non-endangered co-occurring burying beetles was native small mammals for *N. marginatus* on Nantucket Island at 86%; however, all other proportional reproductive carrion source estimates were < 70% among non-endangered burying beetle species on Nantucket Island (Table 4). Mammalian reproductive carrion resources were important for most Nantucket Island burying beetle species, comprising 55-86% of estimated proportional source contributions (Table 4). Conversely, for 2018 ABBs our results estimated a heavy reliance on avian

reproductive carrion resources that comprised 90% of estimated proportional source contributions (Table 4). Unlike burying beetles from Block Island, Nantucket Island beetles do not appear to be using ring-necked pheasant as a reproductive resource (Table 4). This is most likely because ring-necked pheasants are in low abundance and are unreliable as a carrion resource (Mckenna-Foster et al., 2016).

Classical theories on niche variation (Gause, 1934; Van Valen, 1965) and competitive exclusion (MacArthur, 1958; Sugihara, 1980) are often centered around specific species and focus on their interactions while ignoring intraspecific variability in trophic position (Zalewski et al., 2014). However, research on character displacement (Kirschel et al., 2019) and competitive release (Segre et al., 2016) as well as food web dynamics, all focus on the importance of intraspecific variability over temporal and spatial scales (Bolnick et al., 2011; Zalewski et al., 2014) in organizing trophic relationships (Nakazawa et al., 2010), competitive interactions and species associations (Lichstein et al., 2007; Bolnick et al., 2011).

In the present study, our ability to sample all co-occurring burying beetle species allowed us to assess variability in the isotopic niche space among and within species simultaneously. On both Block Island and Nantucket Island, we observed evidence of large niche overlap between all burying beetle species (Fig. 4B), suggesting that they are competing for similar reproductive carrion resources to raise their young. Additionally, the largest species niche-space estimate on Block Island and Nantucket Island were for ABBs, which is likely a result of provisioned farm-raised quail (Table 6). Our results are similar to studies reporting high niche overlap between species of similar functional diversity (Semenyuk and Tiunov, 2011; Zalewski et al., 2014).

Quinby et al. (2020) did not compare niche overlap between co-occurring species, however, they determined that co-occurring burying beetles in Oklahoma used mammalian carrion and not avian carrion for reproduction. Similarly, Holloway and Schnell (1997) identified significant correlations of habitat use of ABBs with the number of individual mammals; the biomass of mammals, biomass of mammals plus birds; and the numbers of species of mammals in western Arkansas. In our study, we identified large overlap in carrion resources used for reproduction that included both avian and mammalian carrion as main components of ABBs reproductive carrion using isotopic analysis of elytral clippings. These findings differ from western populations; however, the observed large niche overlap may also be influenced by a lack of functionally diverse potential reproductive carrion and its availability at our study sites (Semenyuk and Tiunov, 2011).

Because of differences in the carrion base used across the extant range of ABBs, our study suggest that a range-wide management prescription may not be suitable for conservation of ABBs. However, future studies determining the abundance of potential reproductive carrion as well as resource selectivity, in the context of isotopic diet analysis, is needed to provide clarity for resource use between populations and over time to compare all ABB populations more thoroughly.

Temporal variability in the isotopic signatures of potential reproductive carrion items may have contributed to the variation in carrion use detected by our stable isotope analysis (Goetz et al., 2017; Ruhl et al., 2020). We detected moderate variation within isotopic levels of prey tissues (Appendix Table A1); however, to account for this variability in our isotope analyses we included standard deviations for each reproductive carrion resource contribution in SISUS mixing models. Additionally, we only included potential reproductive carrion items in final analyses if they were isotopically distinct as determined by preliminary MANOVA and K-nearest neighbor tests (Rosing et al., 1998; Phillips et al., 2005, 2014). Lastly, a two-way ANOVA confirmed that variation among-groups explained much of the variation in total isotopic signatures.

## 3.7 Conclusions

Studies evaluating within and among population variation in dietary niche and resource use has significant usefulness for the field of conservation and management, especially with *Nicrophorus* beetles. With recent habitat alteration and the extirpation of ABBs from more than 90% of its historic range, our understanding of the extent to which reintroduction affects feeding relationships and resource use within reintroduced populations is important for conservation efforts. We used stable isotope analysis to evaluate the diet of larval ABBs within an extant and reintroduced population using elytral clippings. Our results provide information to the intricate trophic associations within these systems.

Based on our results, management of ABBs should consider long term provisioning of farm-raised quail that may supplement a potential lack of naturally occurring reproductive carrion resources at reintroduction sites. We suggest evaluating small mammal and bird abundances at reintroduction sites to determine the size and availability of potential reproductive carrion. Specifically, managers should consider the habitat matrix at reintroduction sites and select sites that promote larger bodied small vertebrates that ABBs prefer. Furthermore, studies evaluating

inter- and intraspecific competition for carrion resources will provide managers with vital information needed to conserve endangered populations.

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## 3.9 References

Amaral, M., Kozol, A. & French, T. (1997) Conservation status and reintroduction of the endangered American burying beetle. *Northeastern Naturalist*, **4**, 121.

Anderson, R.S. (1982) On the decreasing abundance of *Nicrophorus americanus* Olivier (Coleoptera: Silphidae) in eastern North America. *The Coleopterist Bulletin*, **36**, 362–365.

Araújo, M.S., Bolnick, D.I. & Layman, C.A. (2011) The ecological causes of individual specialisation. *Ecology Letters*, **14**, 948–958.

Araújo, M.S., Martins, E.G., Cruz, L.D., Fernandes, F.R., Linhares, A.X., Reis, S.F.D., *et al.* (2010) Nested diets: a novel pattern of individual-level resource use. *Oikos*, **119**, 81–88.

Backlund, D.C., Marrone, G.M., Williams, C.K. & Tilmon, K. (2008) Population estimate of the endangered American burying beetle, *Nicrophorus americanus* Olivier (Coleoptera: Silphidae) in South Dakota. *The Coleopterists Bulletin*, **62**, 9–15.

Barnhart, M.C. & Brown, R. (2002) *A survey for American burying beetles in southwest Missouri*. Missouri State University, Springfield.

Barnum, T.R., Verburg, P., Kilham, S.S., Whiles, M.R., Lips, K.R., Colón-Gaud, C., *et al.* (2013) Use of stable isotope ratios to characterize potential shifts in the isotopic niches of grazing insects following an amphibian decline in a neotropical stream. *Journal of Tropical Ecology*, **29**, 291–299.

Barry, J.M., Elbroch, L.M., Aiello-Lammens, M.E., Sarno, R.J., Seelye, L., Kusler, A., *et al.* (2019) Pumas as ecosystem engineers: ungulate carcasses support beetle assemblages in the Greater Yellowstone Ecosystem. *Oecologia*, **189**, 577–586.

Bedick, J.C., Hoback, W.W. & Albrecht, M.C. (2006) High water-loss rates and rapid dehydration in the burying beetle, *Nicrophorus marginatus*. *Physiological Entomology*, **31**, 23–29.

Bedick, J.C., Ratcliffe, B.C., Hoback, W.W. & Higley, L.G. (1999) Distribution, ecology, and population dynamics of the American burying beetle [*Nicrophorus americanus* Olivier (Coleoptera, Silphidae)] in south-central Nebraska, USA. *Journal of Insect Conservation*, **3**, 171–181.

Ben-David, M. & Flaherty, E.A. (2012) Stable isotopes in mammalian research: a beginner's guide. *Journal of Mammalogy*, **93**, 312–328.

Berl, J.L., Voorhees, M.W., Wu, J.W., Flaherty, E.A. & Swihart, R.K. (2017) Collection of blood from wild-caught mice (*Peromyscus*) via submandibular venipuncture. *Wildlife Society Bulletin*, **41**, 816–820.

Blüthgen, N., Gebauer, G. & Fiedler, K. (2003) Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. *Oecologia*, **137**, 426–435.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., *et al.* (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution*, **26**, 183–192.

Bolnick, D.I., Svanback, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D., *et al.* (2003) The ecology ofiIndividuals: Incidence and implications of individual specialization. *The American Naturalist*, **161**, 28.

Burkepile, D.E., Parker, J.D., Woodson, C.B., Mills, H.J., Kubanek, J., Sobecky, P.A., *et al.* (2006) Chemically Mediated Competition Between Microbes and Animals: Microbes as Consumers in Food Webs. *Ecology*, **87**, 2821–2831.

Capitán, J.A., Cuenda, S. & Alonso, D. (2017) Stochastic competitive exclusion leads to a cascade of species extinctions. *Journal of Theoretical Biology*, **419**, 137–151.

Cherel, Y., Hobson, K.A. & Hassani, S. (2005) Isotopic discrimination between food and blood and feathers of captive penguins: Implications for dietary studies in the wild. *Physiological and Biochemical Zoology*, **78**, 106–115.

Chikaraishi, Y., Ogawa, N.O., Doi, H. & Ohkouchi, N. (2011) $^{15}N/^{14}N$ ratios of amino acids as a tool for studying terrestrial food webs: a case study of terrestrial insects (bees, wasps, and hornets). *Ecological Research*, **26**, 835–844.

Creighton, J.C. (2005) Population density, body size, and phenotypic plasticity of brood size in a burying beetle. *Behavioral Ecology*, **16**, 1031–1036.

Creighton, J.C., Heflin, N.D. & Belk, M.C. (2009) Cost of reproduction, resource quality, and terminal investment in a burying beetle. *The American Naturalist*, **174**, 673–684.

Creighton, J.C., Vaughn, C.C. & Chapman, B.R. (1993) Habitat preference of the endangered American burying beetle (*Nicrophorus americanus*) in Oklahoma. *The Southwestern Naturalist*, **38**, 275–277.

Dalerum, F. & Angerbjörn, A. (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia*, **144**, 647–658.

Dammhahn, M., Rakotondramanana, C.F. & Goodman, S.M. (2015) Coexistence of morphologically similar bats (*Vespertilionidae*) on Madagascar: stable isotopes reveal fine-grained niche differentiation among cryptic species. *Journal of Tropical Ecology*, **31**, 153–164.

Darimont, C.T., Paquet, P.C. & Reimchen, T.E. (2008) Spawning salmon disrupt trophic coupling between wolves and ungulate prey in coastal British Columbia. *BMC Ecology*, **8**, 14.

DeNiro, M.J. & Epstein, S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, **42**, 495–506.

DeVault, T.L., Brisbin, Jr., I.L. & Rhodes, Jr., O.E. (2004) Factors influencing the acquisition of rodent carrion by vertebrate scavengers and decomposers. *Canadian Journal of Zoology*, **82**, 502–509.

DeVault, T.L., Jr, O.E.R. & Shivik, J.A. (2003) Scavenging by vertebrates: behavioral, ecological, and evolutionary perspectives on an important energy transfer pathway in terrestrial ecosystems. *Oikos*, **102**, 225–234.

DeVault, T.L., Olson, Z.H., Beasley, J.C. & Rhodes, O.E. (2011) Mesopredators dominate competition for carrion in an agricultural landscape. *Basic and Applied Ecology*, **12**, 268–274.

Eckrich, C.A., Albeke, S.E., Flaherty, E.A., Bowyer, R.T. & Ben-David, M. (2020) rKIN: Kernel-based method for estimating isotopic niche size and overlap. *Journal of Animal Ecology*, 1365-2656.13159.

Eckrich, C.A., Flaherty, E.A. & Ben-David, M. (2018) Functional and numerical responses of shrews to competition vary with mouse density. *PLOS ONE*, **13**, e0189471.

Erhardt, E.B., Wolf, B.O., Ben-David, M. & Bedrick, E.J. (2014) Stable isotope sourcing using sampling. *Open Journal of Ecology*, **04**, 289–298.

Fair, H., Paul, E. & Jones, J. (2010) Eds. Guidelines to the use of wild birds in research. Washington, D.C.: Ornithological Council.

Flaherty, E.A., Ben-David, M. & Smith, W.P. (2010) Diet and food availability: implications for foraging and dispersal of Prince of Wales northern flying squirrels across managed landscapes. *Journal of Mammalogy*, **91**, 79–91.

Fleming, T.H., Nuñez, R.A. & Sternberg, L. da S.L. (1993) Seasonal changes in the diets of migrant and non-migrant nectarivorous bats as revealed by carbon stable isotope analysis. *Oecologia*, **94**, 72–75.

Gause, G.F. (1934) *The Struggle for Existence*. Courier Dover Publications, Zoological Institute, Moscow.

Gebauer, G. & Schulze, E.-D. (1991) Carbon and nitrogen isotope ratios in different compartments of a healthy and a declining *Picea abies* forest in the Fichtelgebirge, NE Bavaria. *Oecologia*, **87**, 198–207.

Gibbs, J.P. & Stanton, E.J. (2001) Habitat fragmentation and arthropod community change: carrion beetles, phoretic mites, and flies. *Ecological Applications*, **11**, 79–85.

Goetz, K.T., Burns, J.M., Hückstädt, L.A., Shero, M.R. & Costa, D.P. (2017) Temporal variation in isotopic composition and diet of Weddell seals in the western Ross Sea. *Deep Sea Research Part II: Topical Studies in Oceanography*, **140**, 36–44.

Gratton, C. & Forbes, A.E. (2006) Changes in $\delta^{13}C$ stable isotopes in multiple tissues of insect predators fed isotopically distinct prey. *Oecologia*, **147**, 615–624.

Hobson, K.A. & Bairlein, F. (2003) Isotopic fractionation and turnover in captive Garden Warblers (*Sylvia borin*): implications for delineating dietary and migratory associations in wild passerines. *Canadian Journal of Zoology*, **81**, 1630–1635.

Hobson, K.A. & Clark, R.G. (1992) Assessing Avian Diets Using Stable Isotopes I: Turnover of $^{13}C$ in Tissues. *The Condor*, **94**, 181–188.

Hocking, M.D., Darimont, C.T., Christie, K.S. & Reimchen, T.E. (2007) Niche variation in burying beetles ( *Nicrophorus* spp.) associated with marine and terrestrial carrion. *Canadian Journal of Zoology*, **85**, 437–442.

Holloway, A.K. & Schnell, G.D. (1997) Relationship between numbers of the endangered American burying beetle *Nicrophorus americanus* Olivier (Coleoptera: Silphidae) and available food resources. *Biological Conservation*, **81**, 145–152.

Hood-Nowotny, R. & Knols, B.G.J. (2007) Stable isotope methods in biological and ecological studies of arthropods. *Entomologia Experimentalis et Applicata*, **124**, 3–16.

Hopwood, P.E., Moore, A.J., Tregenza, T. & Royle, N.J. (2016) Niche variation and the maintenance of variation in body size in a burying beetle. *Ecological Entomology*, **41**, 96–104.

Hyodo, F. (2015) Use of stable carbon and nitrogen isotopes in insect trophic ecology. *Entomological Science*, **18**, 295–312.

Ikeda, H., Kubota, K., Kagaya, T. & Abe, T. (2006) Niche differentiation of burying beetles (Coleoptera: Silphidae: Nicrophorinae) in carcass use in relation to body size: Estimation from stable isotope analysis. *Applied Entomology and Zoology*, **41**, 561–564.

Janzen, D.H. (1977) Why Fruits Rot, Seeds Mold, and Meat Spoils. *The American Naturalist*, **111**, 691–713.

Kirschel, A.N.G., Seddon, N. & Tobias, J.A. (2019) Range-wide spatial mapping reveals convergent character displacement of bird song. *Proceedings of the Royal Society B*, **286**, 20190443.

Kozol, A.J. (1991) Appendix 2. Survey protocol for *Nicrophorus americanus*, the American burying beetle [pp. 1–6]. US Fish and Wildlife Service, American burying beetle (*Nicrophorus americanus*) Recovery Plan. Newton Corner, MA.

Kozol, A.J., Scott, M.P. & Traniello, J.F.A. (1988) The American burying beetle, *Nicrophorus americanus*: studies on the natural history of a declining species. *Psyche: A Journal of Entomology*, **95**, 167–176.

L'Hérault, V., Franke, A., Lecomte, N., Alogut, A. & Bêty, J. (2013) Landscape heterogeneity drives intra-population niche variation and reproduction in an arctic top predator. *Ecology and Evolution*, **3**, 2867–2879.

Lichstein, J.W., Dushoff, J., Levin, S.A. & Pacala, S.W. (2007) Intraspecific Variation and Species Coexistence. *The American Naturalist*, **170**, 807–818.

Lomolino, M.V. & Creighton, J.C. (1996) Habitat selection, breeding success and conservation of the endangered American burying beetle *Nicrophorus americanus*. *Biological Conservation*, **77**, 235–241.

Lomolino, M.V., Creighton, J.C., Schnell, G.D. & Certain, D.L. (1995) Ecology and conservation of the endangered American burying beetle (*Nicrophorus americanus*). *Conservation Biology*, **9**, 605–614.

Lorrain, A., Graham, B., Ménard, F., Popp, B., Bouillon, S., Breugel, P. van, *et al.* (2009) Nitrogen and carbon isotope values of individual amino acids: a tool to study foraging ecology of penguins in the Southern Ocean. *Marine Ecology Progress Series*, **391**, 293–306.

MacArthur, R.H. (1958) Population ecology of some warblers of northeastern coniferous forests. *Ecology*, **39**, 599–619.

Maldonado, K., Bozinovic, F., Newsome, S.D. & Sabat, P. (2017) Testing the niche variation hypothesis in a community of passerine birds. *Ecology*, **98**, 903–908.

Matos, M.P.V., Konstantynova, K.I., Mohr, R.M. & Jackson, G.P. (2018) Analysis of the $^{13}C$ isotope ratios of amino acids in the larvae, pupae and adult stages of *Calliphora vicina* blow flies and their carrion food sources. *Analytical and Bioanalytical Chemistry*, **410**, 7943–7954.

Mckenna-Foster, A., O'Dell, D., Sorrows, E., Laurenzano, J. & Perrotti, L. (2019) Vertebrate scavenger species composition on Nantucket Island, Massachusetts and implications for *Nicrophorus americanus* Olivier (American burying beetle) reintroduction. *Northeastern Naturalist*, **26**, 52.

Mckenna-Foster, A., Perrotti, L., Blyth, J., LoPresti, E. & Kennedy, R.S. (2016) Measuring success of a reintroduced population of the American burying beetle (*Nicrophorus americanus* Olivier) to Nantucket Island, MA. *Journal of Insect Conservation*, **20**, 895–904.

Moore, J.W. & Semmens, B.X. (2008) Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology Letters*, **11**, 470–480.

Nakazawa, T., Sakai, Y., Hsieh, C., Koitabashi, T., Tayasu, I., Yamamura, N., *et al.* (2010) Is the Relationship between Body Size and Trophic Niche Position Time-Invariant in a Predatory Fish? First Stable Isotope Evidence. *PLoS ONE*, **5**.

Nupp, T.E. & Swihart, R.K. (2000) Landscape-level correlates of small-mammal assemblages in forest fragments of farmland. *Journal of Mammalogy*, **81**, 15.

Parmenter, R.R. & MacMahon, J.A. (2009) Carrion decomposition and nutrient cycling in a semiarid shrub–steppe ecosystem. *Ecological Monographs*, **79**, 637–661.

Parnell, A.C., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., *et al.* (2013) Bayesian stable isotope mixing models: Baysian stable isotope mixing models. *Environmetrics*, n/a-n/a.

Pearson, S.F., Levey, D.J., Greenberg, C.H. & Martínez del Rio, C. (2003) Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia*, **135**, 516–523.

Penteriani, V., Gallardo, M. & Roche, P. (2002) Landscape structure and food supply affect eagle owl (*Bubo bubo*) density and breeding performance: a case of intra-population heterogeneity. *Journal of Zoology*, **257**, 365–372.

Perrotti, L. & Mckenna-Foster, A. (2019) A report on the 25 year effort to establish the American burying beetle (*Nicrophours americanus* Olivier) to Nantucket Island, Massachusetts. Presented at the Entomology, St. Louis, MO.

Phillips, D.L., Inger, R., Bearhop, S., Jackson, A.L., Moore, J.W., Parnell, A.C., *et al.* (2014) Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology*, **92**, 823–835.

Phillips, D.L., Newsome, S.D. & Gregg, J.W. (2005) Combining sources in stable isotope mixing models: alternative methods. *Oecologia*, **144**, 520–527.

Podlesak, D.W., McWilliams, S.R. & Hatch, K.A. (2005) Stable isotopes in breath, blood, feces and feathers can indicate intra-individual changes in the diet of migratory songbirds. *Oecologia*, **142**, 501–510.

Post, D.M. (2002) Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, **83**, 703–718.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J. & Montaña, C.G. (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, **152**, 179–189.

Quinby, B.M., Feldman, N.S., Flaherty, E.A., Belk, M.C., Smith, A.D.F. & Creighton, J.C. (2020) Isotopic discrimination between carrion and elytra clippings of lab-reared American burying beetles (*Nicrophorus americanus*): Implications for conservation and evaluation of feeding relationships in the wild. *Rapid Communications in Mass Spectrometry*, **n/a**.

Raithel, C.J., Ginsberg, H.S. & Prospero, M.L. (2006) Population trends and flight behavior of the American burying beetle, *Nicrophours americanus* (Coleoptera: Silphidae), on Block Island, RI. *Journal of Insect Conservation*, **10**, 317–322.

Robertson, A., McDonald, R.A., Delahay, R.J., Kelly, S.D. & Bearhop, S. (2015) Resource availability affects individual niche variation and its consequences in group-living European badgers *Meles meles*. *Oecologia*, **178**, 31–43.

Rosing, M.N., Ben-David, M. & Barry, R.P. (1998) Analysis of stable isotope data: A K nearest-neighbors randomization test. *The Journal of Wildlife Management*, **62**, 380–388.

Ruhl, P.J., Flaherty, E.A. & Dunning, J.B. (2020) Using stable isotopes of plasma, red blood cells, feces, and feathers to assess mature-forest bird diet during the post-fledging period. *Canadian Journal of Zoology*, **98**, 39–46.

Schimmelman, A. (2011) Carbon, nitrogen and oxygen stable isotope ratios in chitin. In *Chitin Formation and Diagenesis. Edited by Gupta N.S.* Springer, Dordrecht, pp. 81–103.

Schwartz, C.W. & Schwartz, E.R. (2001) *The wild mammals of Missouri*. University of Missouri Press.

Scott, M.P. (1998) The ecology and behavior of burying beetles. *Annual Review of Entomology*, **43**, 595–618.

Segre, H., DeMalach, N., Henkin, Z. & Kadmon, R. (2016) Quantifying Competitive Exclusion and Competitive Release in Ecological Communities: A Conceptual Framework and a Case Study. *PLOS ONE*, **11**, e0160798.

Selbo, S.M. (2009) Reintroducing rare beetles to Ohio. *Endangered Species Update*, **26**, S17.

Semenyuk, I.I. & Tiunov, A.V. (2011) Isotopic signature ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) confirms similarity of trophic niches of millipedes (Myriapoda, diplopoda) in a temperate deciduous forest. *Biology Bulletin*, **38**, 283–291.

Sikes, D.S. & Raithel, C.J. (2002) A review of hypotheses of decline of the endangered American burying beetle (Silphidae: *Nicrophorus americanus* Olivier). *Journal of Insect Conservation*, **6**, 103–113.

Sikes, R.S. & The Animal Care and Use Committee of the American Society of Mammologists. (2016) Guidelines of the American Society of Mammologists for the use of wild mammals in research and education: *Journal of Mammalogy*, **97**, 663–688.

Sugihara, G. (1980) Minimal Community Structure: An Explanation of Species Abundance Patterns. *The American Naturalist*, **116**, 770–787.

Tillberg, C.V., McCarthy, D.P., Dolezal, A.G. & Suarez, A.V. (2006) Measuring the trophic ecology of ants using stable isotopes. *Insectes Sociaux*, **53**, 65–69.

Tooker, J.F. & Hanks, L.M. (2004) Trophic Position of the Endophytic Beetle, *Mordellistena aethiops* Smith (Coleoptera: Mordellidae). *Environmental Entomology*, **33**, 291–296.

Van Valen, L. (1965) Morphological variation and width of ecological niche. *The American Naturalist*, **99**, 377–390.

Vitz, A.C. & Rodewald, A.D. (2012) Using stable isotopes to investigate the dietary trophic level of fledgling songbirds. *Journal of Field Ornithology*, **83**, 73–84.

Voigt, C.C., Grasse, P., Rex, K., Hetz, S.K. & Speakman, J.R. (2008) Bat breath reveals metabolic substrate use in free-ranging vampires. *Journal of Comparative Physiology B*, **178**, 9–16.

West, J.B., Bowen, G.J., Cerling, T.E. & Ehleringer, J.R. (2006) Stable isotopes as one of nature's ecological recorders. *TRENDS in Ecology and Evolution*, **21**, 408–414.

Wolf, B.O., Martínez del Rio, C. & Babson, J. (2002) Stable isotopes reveal that saguaro fruit provides different resources to two desert dove species. *Ecology*, **83**, 1286–1293.

Zalewski, M., Dudek, D., Tiunov, A.V., Godeau, J.-F., Okuzaki, Y., Ikeda, H., *et al.* (2014) High Niche Overlap in the Stable Isotope Space Of Ground Beetles. *Annales Zoologici Fennici*, **51**, 301–312.

Zar, J.H. (2014) *Biostatistical analysis*. 5th edn. Pearson Education Limited, London, U.K.

**Table 3.1.** Stable Isotope Results in Beetles and Carrion

Sample size (*n*) and mean isotopic signature (± *SD*) for $\delta^{13}C$ and $\delta^{15}N$ for burying beetles [*Nicrophorus americanus* (Na); *N. orbicollis* (No), *N. marginatus* (Nm), and *N. tomentosus* (Nt)] and each collected diet item [Insectivorous birds (IB); Pheasant (Ph); Farm-raised quail (FRQ); Norway rats (NR); Small mammals (SM); Generalist birds (Gen B); Granivorous birds (Gra B); Insectivorous birds (IB); Sea birds (SB)] for from Block Island (BI) and Nantucket Island (NI). Difference in $\delta^{13}C$ and $\delta^{15}N$ values calculated from a multivariate analysis of variance (MANOVA) with a post hoc Tukey's multiple comparison test (Zar 2014) and a k-nearest neighbor analysis (Rosing et al. 1998).

| Block Island | | | Tukey's HSD | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Burying beetles | $\delta^{13}C$ | *n* | 2017 ABB | 2018 ABB | 2017 No | 2018 No | 2017 Nm | 2018 Nm | 2018 Nt |
| 2017 ABB | –24.73 ± 1.63 | 39 | – | > 0.05 | **< 0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| 2018 ABB | –24.25 ± 1.54 | 26 | > 0.05 | – | **< 0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| 2017 No | –26.04 ± 0.54 | 4 | **< 0.01** | **<0.01** | – | > 0.05 | **<0.01** | **<0.01** | **<0.01** |
| 2018 No | –25.68 ± 0.98 | 17 | **< 0.01** | **<0.01** | > 0.05 | – | **<0.01** | **<0.01** | **<0.01** |
| 2017 Nm | –26.83 ± 0.88 | 15 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | – | > 0.05 | **<0.01** |
| 2018 Nm | –27.13 ± 0.71 | 15 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | > 0.05 | – | **<0.01** |
| 2018 Nt | –25.82 ± 1.18 | 19 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** | – |
| | $\delta^{15}N$ | *n* | 2017 ABB | 2018 ABB | 2017 No | 2018 No | 2017 Nm | 2018 Nm | 2018 Nt |
| 2017 ABB | 7.24 ± 1.29 | 39 | – | > 0.05 | 0.565 | 0.565 | **<0.01** | **<0.01** | 0.409 |
| 2018 ABB | 8.40 ± 1.70 | 26 | > 0.05 | – | 0.565 | 0.565 | **<0.01** | **<0.01** | 0.409 |
| 2017 No | 6.57 ± 1.01 | 4 | 0.565 | 0.565 | – | > 0.05 | **<0.01** | **<0.01** | 1.000 |
| 2018 No | 7.94 ± 2.64 | 17 | 0.565 | 0.565 | > 0.05 | – | **<0.01** | **<0.01** | 1.000 |
| 2017 Nm | 5.62 ± 1.69 | 15 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | – | > 0.05 | **<0.01** |
| 2018 Nm | 5.99 ± 0.88 | 15 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | > 0.05 | – | **<0.01** |
| 2018 Nt | 7.54 ± 1.48 | 19 | 0.409 | 0.409 | 1.000 | 1.000 | **<0.01** | **<0.01** | – |
| Diet Items | $\delta^{13}C$ | *n* | IB | Ph | FRQ | NR | SM | | |
| IB | -24.79 ± 0.69 | 26 | – | 0.461 | **<0.01** | 0.748 | 0.891 | | |
| Ph | -24.79 ± 0.59 | 12 | 0.461 | – | **<0.01** | 0.253 | 0.061 | | |
| FRQ | -20.22 ± 0.30 | 18 | **<0.01** | **<0.01** | – | **<0.01** | **<0.01** | | |
| NR | -25.31 ± 1.92 | 2 | 0.748 | 0.253 | **<0.01** | – | 0.923 | | |
| SM | -24.97 ± 0.51 | 13 | 0.891 | 0.061 | **<0.01** | 0.923 | – | | |

Table 3.1 continued

| | $\delta^{15}N$ | *n* | IB | Ph | FRQ | NR | SM |
|---|---|---|---|---|---|---|---|
| IB | 6.59 ± 0.49 | 26 | – | **<0.01** | **<0.01** | 1.000 | **<0.01** |
| Ph) | 4.42 ± 0.92 | 12 | **<0.01** | – | **<0.01** | **<0.01** | **0.02** |
| FRQ | 3.61 ± 0.47 | 18 | **<0.01** | **<0.01** | – | **<0.01** | **<0.01** |
| NR | 6.57 ± 1.00 | 2 | 1.000 | **<0.01** | **<0.01** | – | **<0.01** |
| SM | 5.06 ± 0.46 | 13 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | – |

**Table 3.2.** Stable Isotope Results in Beetles and Carrion

Sample size (*n*) and mean isotopic signature (± *SD*) for $\delta^{13}C$ and $\delta^{15}N$ for burying beetles [*Nicrophorus americanus* (Na); *N. orbicollis* (No), *N. marginatus* (Nm), and *N. tomentosus* (Nt)] and each collected diet item [Insectivorous birds (IB); Pheasant (Ph); Farm-raised quail (FRQ); Norway rats (NR); Small mammals (SM); Generalist birds (Gen B); Granivorous birds (Gra B); Insectivorous birds (IB); Sea birds (SB)] for from Block Island (BI) and Nantucket Island (NI). Difference in $\delta^{13}C$ and $\delta^{15}N$ values calculated from a multivariate analysis of variance (MANOVA) with a post hoc Tukey's multiple comparison test (Zar 2014) and a k-nearest neighbor analysis (Rosing et al. 1998).

| Nantucket Island | | | Tukey's HSD | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Burying beetles | $\delta^{13}C$ | *n* | 2017 ABB | 2018 ABB | 2017 No | 2018 No | 2017 Nm | 2018 Nm | 2018 Nt |
| 2017 ABB | –23.50 ± 2.15 | 22 | – | 1.000 | **<0.01** | **<0.01** | **<0.01** | 0.914 | 0.914 |
| 2018 ABB | –23.38 ± 1.02 | 8 | 1.000 | – | 0.078 | **<0.01** | **<0.01** | 0.942 | 0.942 |
| 2017 No | –25.43 ± 1.18 | 32 | **<0.01** | 0.078 | 0.634 | 0.427 | 0.427 | 1.000 | 1.000 |
| 2018 No | –25.87 ± 1.47 | 33 | **<0.01** | 0.078 | – | 0.427 | 0.427 | 1.000 | 1.000 |
| 2017 Nm | –26.45 ± 1.10 | 34 | **<0.01** | **<0.01** | 0.427 | – | >0.05 | 1.000 | 1.000 |
| 2018 Nm | –26.76 ± 1.02 | 32 | **<0.01** | **<0.01** | 0.427 | >0.05 | – | 1.000 | 1.000 |
| 2018 Nt | –25.41 ± 2.44 | 3 | 0.914 | 0.942 | 1.000 | 1.000 | 1.000 | – | – |
| | $\delta^{15}N$ | *n* | 2017 ABB | 2018 ABB | 2017 No | 2018 No | 2017 Nm | 2018 Nm | 2018 Nt |
| 2017 ABB | 6.29 ± 0.96 | 22 | – | **0.046** | **<0.01** | **<0.01** | **<0.01** | **<0.01** | 0.974 |
| 2018 ABB | 8.93 ± 1.40 | 8 | **0.046** | – | **<0.01** | **<0.01** | **<0.01** | **<0.01** | 0.904 |
| 2017 No | 5.88 ± 1.03 | 32 | **<0.01** | **<0.01** | – | >0.05 | 0.770 | 0.770 | 1.000 |
| 2018 No | 5.60 ± 1.23 | 33 | 0.983 | **<0.01** | 0.341 | – | 0.770 | 0.770 | 1.000 |
| 2017 Nm | 5.27 ± 1.00 | 34 | 0.282 | **<0.01** | 0.770 | 0.770 | – | >0.05 | 0.974 |
| 2018 Nm | 5.32 ± 1.00 | 32 | 0.283 | **<0.01** | 0.770 | 0.770 | >0.05 | – | 0.974 |
| 2018 Nt | 6.73 ± 1.11 | 3 | 1.000 | 0.904 | 1.000 | 1.000 | 0.974 | 0.974 | – |

Table 3.2 continued

| Diet Items | $\delta^{13}C$ | *n* | IB | FRQ | NR | SM | Gen B | Gra B | SB |
|---|---|---|---|---|---|---|---|---|---|
| FRQ | -20.22 ± 0.30 | 18 | **<0.01** | – | **<0.01** | **<0.01** | **<0.01** | **<0.01** | 0.071 |
| Gen B | -24.72 ± 1.21 | 31 | **<0.01** | **<0.01** | 1.000 | 1.000 | – | **<0.01** | **<0.01** |
| Gran B | -22.85 ± 2.01 | 13 | **<0.01** | **<0.01** | 1.000 | 1.000 | **<0.01** | – | **<0.01** |
| IB | -25.48 ± 0.89 | 4 | – | **<0.01** | 1.000 | 1.000 | **<0.01** | **<0.01** | **<0.01** |
| SB | -19.65 ± 4.26 | 6 | **<0.01** | 0.071 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | – |
| NR | -24.78 ± 1.30 | 7 | 1.000 | **<0.01** | – | 1.000 | 1.000 | 1.000 | **<0.01** |
| SM | -24.80 ± 1.33 | 90 | 1.000 | **<0.01** | 1.000 | – | 1.000 | 1.000 | **<0.01** |
| | $\delta^{15}N$ | *n* | IB | FRQ | NR | SM | Gen B | Gra B | SB |
| FRQ (NI/BI) | 3.61 ± 0.47 | 18 | 0.100 | – | **<0.01** | 0.321 | **<0.01** | **<0.01** | **<0.01** |
| Gen B (NI) | 6.63 ± 1.77 | 31 | 1.000 | **<0.01** | **<0.01** | **<0.01** | – | 1.000 | **<0.01** |
| Gran B (NI) | 7.49 ± 2.56 | 13 | 1.000 | **<0.01** | 1.000 | **<0.01** | 1.000 | – | **<0.01** |
| IB (NI) | 6.25 ± 1.20 | 4 | 1.000 | 0.100 | 1.000 | **<0.01** | 1.000 | 1.000 | **<0.01** |
| SB (NI) | 12.01 ± 3.06 | 6 | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** | **<0.01** | – |
| NR (NI) | 6.45 ± 1.39 | 7 | 1.000 | **<0.01** | – | **<0.01** | 1.000 | 1.000 | **<0.01** |
| SM (NI) | 2.57 ± 1.77 | 90 | **<0.01** | 0.321 | **<0.01** | – | **<0.01** | **<0.01** | **<0.01** |

**Table 3.3.** Proportional Composition of Reproductive Carrion to Burying Beetles

Relative contribution to the reproductive diet of (*Nicrophorus americanus*; *N. orbicollis*, *N. marginatus*, and *N. tomentosus*) from Block Island (BI) and Nantucket Island (NI). We estimated proportions of reproductive diet items relative to different burying beetle species using dual-isotope mixing model.

| Species | Reproductive Carrion | Relative Contribution | |
|---|---|---|---|
| | | Block Island | Nantucket Island |
| *N. americanus* | Farm-raised quail | 0.16 | — |
| | Pheasant | 0.28 | — |
| | Norway rat | 0.13 | — |
| | Small mammals | 0.26 | — |
| | Insectivorous birds | 0.17 | — |
| 2017 *N. americanus* | Farm-raised quail | — | 0.32 |
| | Norway rat | — | 0.05 |
| | Small mammals | — | 0.50 |
| | Insectivorous birds | — | 0.05 |
| | Granivorous birds | — | 0.04 |
| | Generalist birds | — | 0.04 |
| 2018 *N. americanus* | Farm-raised quail | — | 0.23 |
| | Norway rat | — | 0.09 |
| | Small mammals | — | 0.01 |
| | Insectivorous birds | — | 0.01 |
| | Granivorous birds | — | 0.65 |
| | Generalist birds | — | 0.01 |
| *N. orbicollis* | Pheasant | 0.60 | — |
| | Norway rat | 0.02 | 0.00 |
| | Small mammals | 0.03 | 0.68 |
| | Insectivorous birds | 0.35 | 0.32 |
| | Granivorous birds | — | 0.00 |
| | Generalist birds | — | 0.00 |

Table 3.3 Continued

| | | | |
|---|---|---|---|
| *N. tomentosus* | Pheasant | 0.67 | — |
| | Norway rat | 0.01 | 0.14 |
| | Small mammals | 0.02 | 0.47 |
| | Insectivorous birds | 0.30 | 0.14 |
| | Granivorous birds | — | 0.09 |
| | Generalist birds | — | 0.16 |
| *N. marginatus* | Pheasant | 0.37 | — |
| | Norway rat | 0.04 | 0.01 |
| | Small mammals | 0.05 | 0.86 |
| | Insectivorous birds | 0.54 | 0.10 |
| | Granivorous birds | — | 0.01 |
| | Generalist birds | — | 0.02 |

**Table 3.4.** Niche Overlap Between Burying Beetles on Block Island

Percent overlap estimates of isotopic niche space for Block Island burying beetles (*N. americanus*, *N. orbicollis*, *N. tomentosus*, and *N. marginatus*) generated using kernel utilization density methods for at 50%, 75%, and 95% contour levels.
**Note:** A dash (—) indicates 100% overlap.

| | *N. americanus* | | | *N. orbicollis* | | | *N. tomentosus* | | | *N. marginatus* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50% | 75% | 95% | 50% | 75% | 95% | 50% | 75% | 95% | 50% | 75% | 95% |
| *N. americanus* | | | | | | | | | | | | |
| 50% | — | — | — | 0.37 | 0.56 | 0.87 | 0.67 | 0.88 | 0.98 | 0.01 | 0.16 | 0.41 |
| 75% | 0.47 | — | — | 0.20 | 0.40 | 0.61 | 0.40 | 0.59 | 0.77 | 0.06 | 0.18 | 0.36 |
| 95% | 0.22 | 0.46 | — | 0.09 | 0.19 | 0.37 | 0.21 | 0.39 | 0.56 | 0.08 | 0.15 | 0.29 |
| *N. orbicollis* | | | | | | | | | | | | |
| 50% | 0.86 | — | — | — | — | — | 0.75 | 0.99 | — | 0.02 | 0.40 | 0.87 |
| 75% | 0.59 | 0.83 | 0.95 | 0.45 | — | — | 0.58 | 0.83 | 0.99 | 0.12 | 0.45 | 0.78 |
| 95% | 0.45 | 0.66 | 0.87 | 0.22 | 0.48 | — | 0.43 | 0.67 | 0.93 | 0.13 | 0.33 | 0.60 |
| *N. tomentosus* | | | | | | | | | | | | |
| 50% | 0.70 | 0.89 | — | 0.33 | 0.56 | 0.88 | — | — | — | 0.00 | 0.16 | 0.55 |
| 75% | 0.45 | 0.64 | 0.94 | 0.22 | 0.41 | 0.68 | 0.50 | — | — | 0.11 | 0.26 | 0.54 |
| 95% | 0.27 | 0.45 | 0.73 | 0.12 | 0.26 | 0.50 | 0.27 | 0.54 | — | 0.13 | 0.27 | 0.51 |
| *N. marginatus* | | | | | | | | | | | | |
| 50% | 0.01 | 0.27 | 0.75 | 0.02 | 0.23 | 0.53 | 0.00 | 0.43 | — | — | — | — |
| 75% | 0.16 | 0.38 | 0.72 | 0.17 | 0.43 | 0.65 | 0.15 | 0.50 | 0.99 | 0.49 | — | — |
| 95% | 0.19 | 0.36 | 0.62 | 0.17 | 0.34 | 0.54 | 0.25 | 0.48 | 0.86 | 0.22 | 0.46 | — |

**Table 3.5.** Niche Overlap Between Burying Beetles on Nantucket Island

Percent overlap estimates of isotopic niche space for Nantucket Island burying beetles (*N. americanus*, *N. orbicollis*, *N. tomentosus*, and *N. marginatus*) generated using kernel utilization density methods for at 50%, 75%, and 95% contour levels.
**Note:** A dash (—) indicates 100% overlap.

| | *N. americanus* | | | *N. orbicollis* | | | *N. marginatus* | | |
|---|---|---|---|---|---|---|---|---|---|
| | 50% | 75% | 95% | 50% | 75% | 95% | 50% | 75% | 95% |
| *N. americanus* | | | | | | | | | |
| 50% | — | — | — | 0.18 | 0.32 | 0.51 | 0.02 | 0.14 | 0.30 |
| 75% | — | — | — | 0.17 | 0.34 | 0.50 | 0.06 | 0.16 | 0.35 |
| 95% | 0.57 | 0.46 | — | 0.15 | 0.28 | 0.49 | 0.08 | 0.17 | 0.33 |
| *N. orbicollis* | | | | | | | | | |
| 50% | 0.31 | 0.64 | 0.98 | — | — | — | 0.32 | 0.74 | — |
| 75% | 0.28 | 0.63 | 0.92 | 0.48 | — | — | 0.31 | 0.57 | 0.95 |
| 95% | 0.22 | 0.43 | 0.76 | 0.23 | 0.47 | — | 0.16 | 0.34 | 0.64 |
| *N. marginatus* | | | | | | | | | |
| 50% | 0.06 | 0.31 | 0.77 | 0.46 | 0.93 | — | — | — | — |
| 75% | 0.17 | 0.41 | 0.76 | 0.50 | 0.78 | — | 0.47 | — | — |
| 95% | 0.19 | 0.45 | 0.75 | 0.34 | 0.67 | 0.96 | 0.24 | 0.52 | — |

**Table 3.6.** Isotopic Niche-Space Estimates

Isotopic niche-space estimates generated using kernel utilization density methods for (*Nicrophorus americanus*; *N. orbicollis*, *N. marginatus*, and *N. tomentosus*) from Block Island (BI) and Nantucket Island (NI) for elytra samples at 50%, 75%, and 95% contour levels.

| Species | Contour (%) | Elytra | Population |
|---|---|---|---|
| *N. americanus* | 50 | 9.30 | BI |
| | 75 | 19.79 | BI |
| | 95 | 43.29 | BI |
| *N. orbicollis* | 50 | 3.97 | BI |
| | 75 | 8.75 | BI |
| | 95 | 18.10 | BI |
| *N. marginatus* | 50 | 4.50 | BI |
| | 75 | 9.24 | BI |
| | 95 | 20.04 | BI |
| *N. tomentosus* | 50 | 8.95 | BI |
| | 75 | 18.01 | BI |
| | 95 | 33.51 | BI |
| *N. americanus* | 50 | 12.70 | NI |
| | 75 | 26.84 | NI |
| | 95 | 47.45 | NI |
| *N. orbicollis* | 50 | 7.08 | NI |
| | 75 | 14.64 | NI |
| | 95 | 30.85 | NI |
| *N. marginatus* | 50 | 4.97 | NI |
| | 75 | 10.61 | NI |
| | 95 | 20.62 | NI |

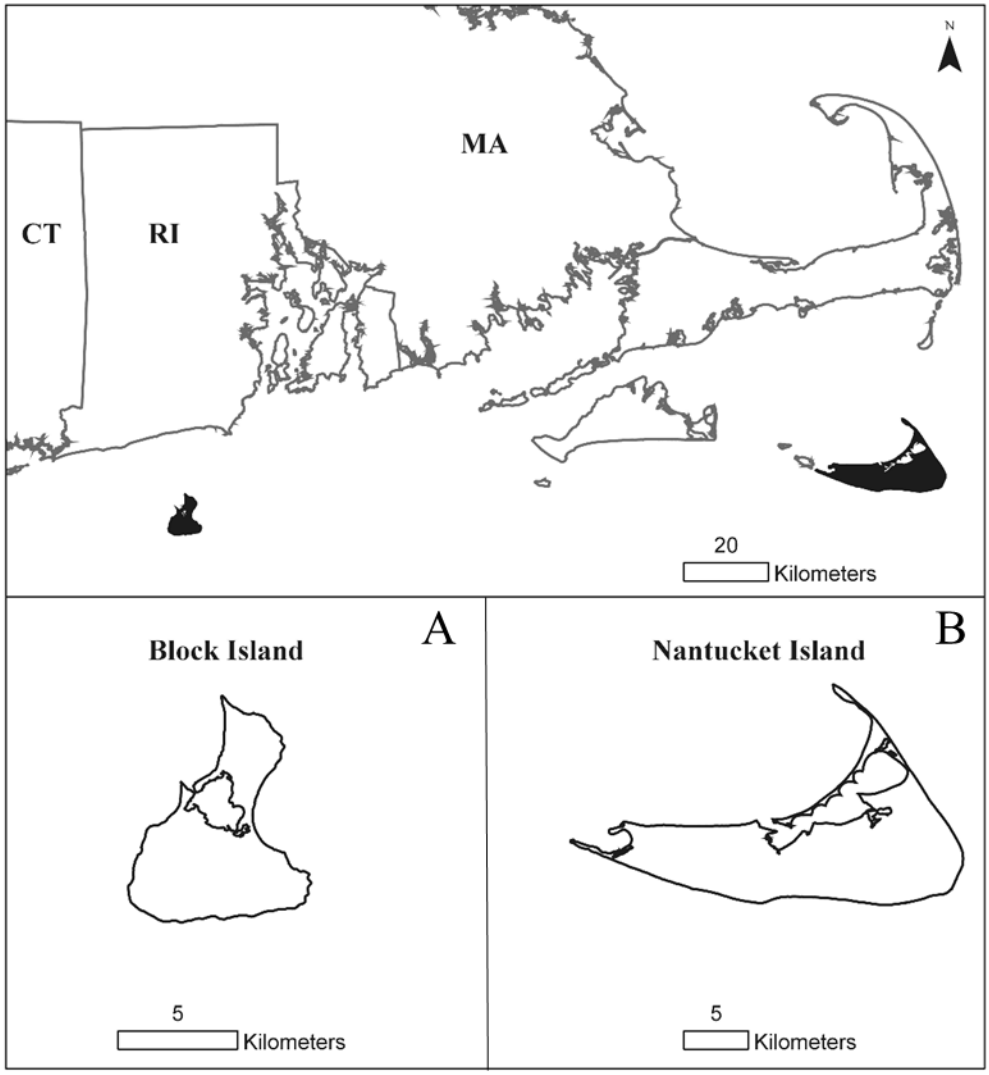


**Figure 3.1.** Study Sites

Study area and location of extant (A) and reintroduced (B) populations of the Federally Endangered American burying beetle (*Nicrophorus americanus*).


**Figure 3.2.** Burying Beetle Elytra Notch

Example of an elytral notch used to determine stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) in the Federally Endangered American burying beetle (*Nicrophorus americanus*; Quinby *et al.* 2020).

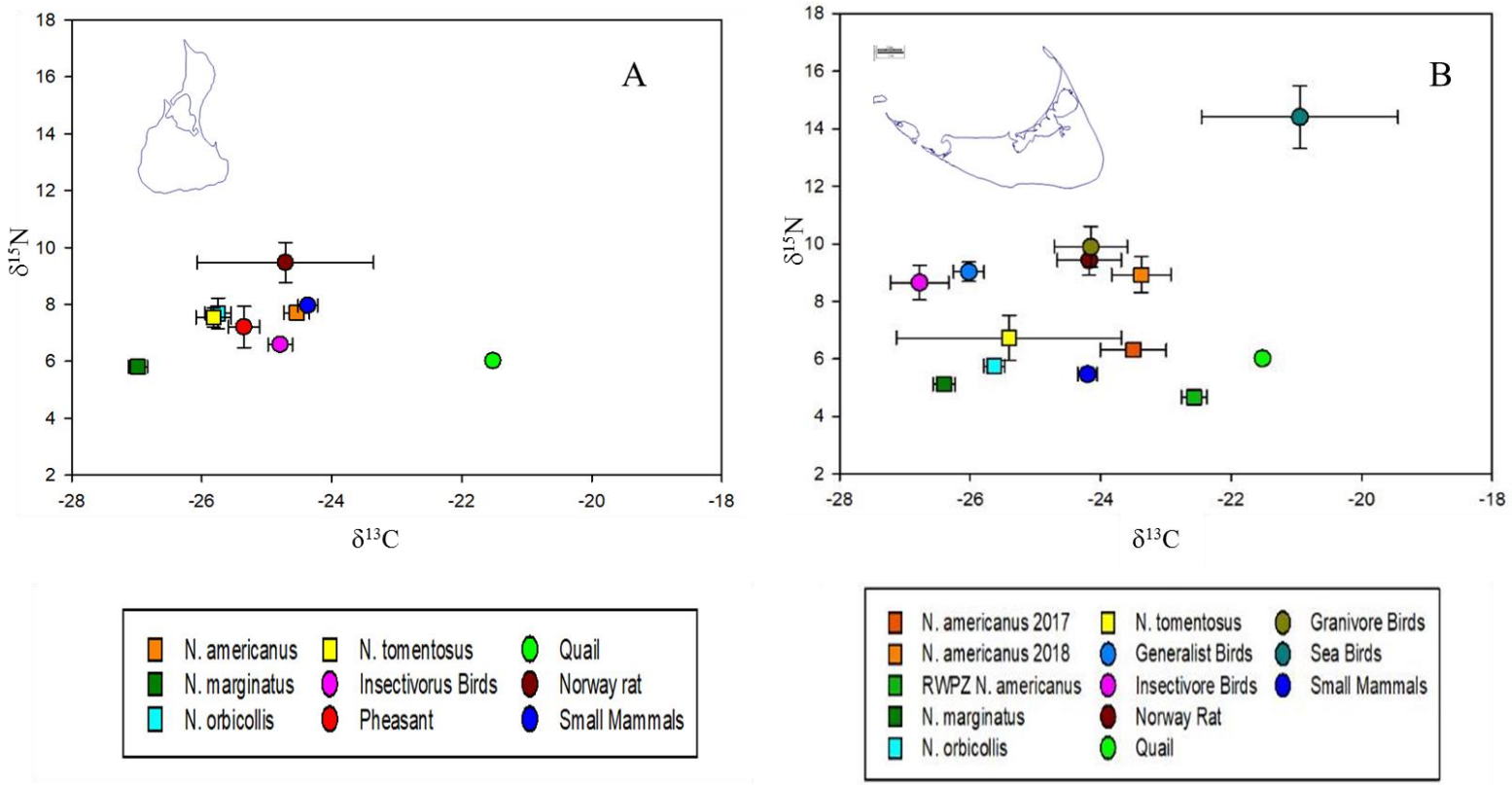


**Figure 3.3.** Stable Isotope Space Between Burying Beetles and Reproductive Carrion

Range of isotopic means (± *SD*) for groups of potential reproductive carrion items (circles) for (*Nicrophorus americanus*; *N. orbicollis*, *N. marginatus*, and *N. tomentosus*; squares) from Block Island (A) and Nantucket Island (B) collected in the summer of 2017 and 2018.

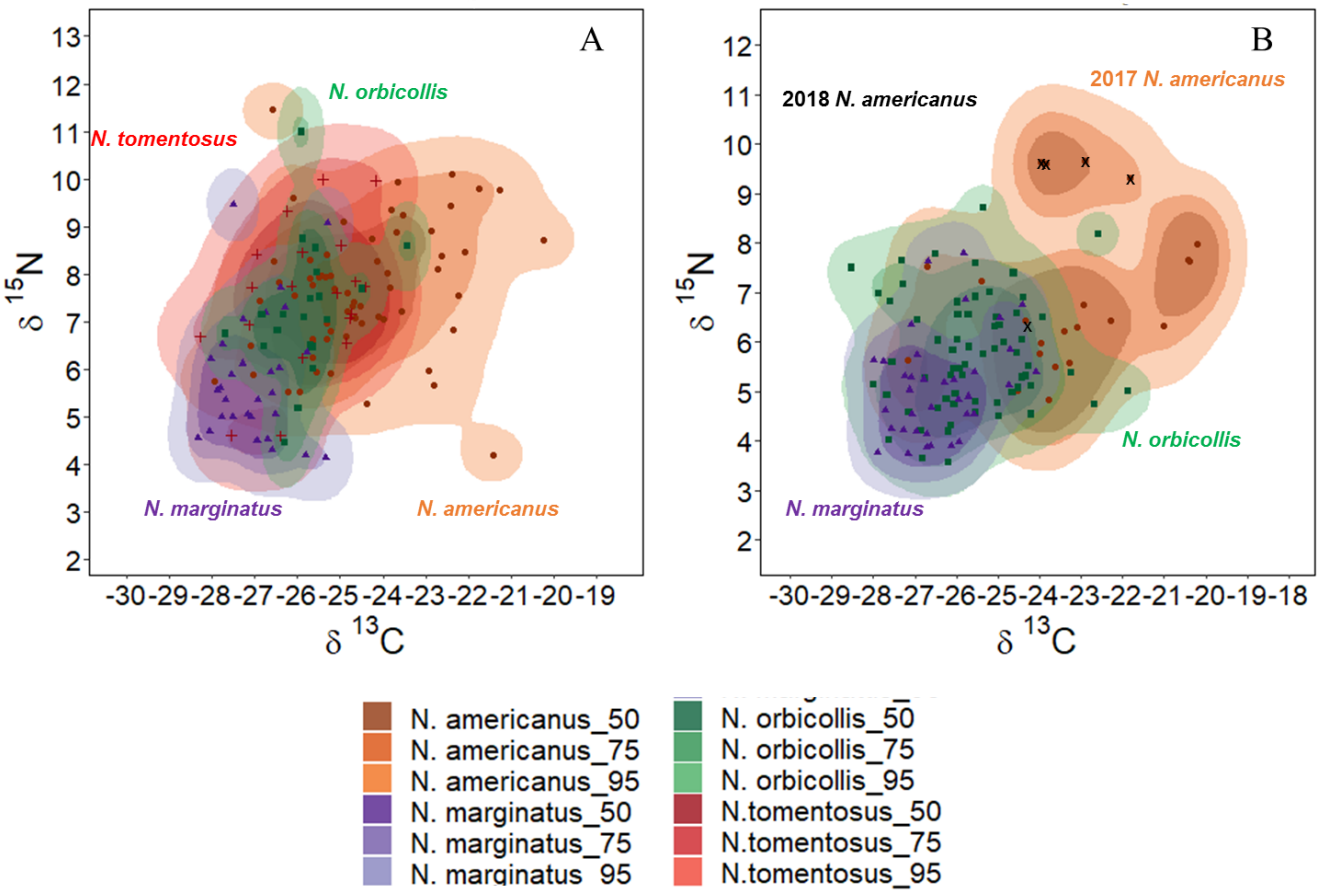


**Figure 3.4.** Isotopic Niche-Space Estimates

Isotopic niche-space estimates generated using kernel utilization density methods for (*Nicrophorus americanus*; *N. orbicollis*, *N. marginatus*, and *N. tomentosus*) from Block Island (A) and Nantucket Island (B) at 50%, 75%, and 95% contour levels collected in the summer of 2017 and 2018. The large amount of overlap in contour levels among co-occurring species on each island indicates that beetles in both populations are using similar carrion for reproduction.

# CHAPTER 4. ENERGETICS OF REPRODUCTION AND PREHATCHING PARENTAL CARE IN BURYING BEETLES

Brandon M. Quinby, J. Curtis Creighton, and Elizabeth A. Flaherty

## 4.1 Summary Statement

Our study indicated that sexual development and prehatching parental care are periods of elevated metabolic activity in *Nicrophorus orbicollis* and provide insight into life-history tradeoffs associated with resource quality.

## 4.2 Abstract

Life-history theory dictates that there are trade-offs between current and future reproductive attempts. These trade-offs are associated with the allocation of assimilated resources to growth, reproduction, and self-maintenance. Additionally, they directly influence life-history characteristics such as breeding behavior, clutch size, lifespan, fecundity, and parental care. Trade-offs associated with the cost of reproduction are studied extensively in respect to longevity of individuals, however, the energetics underlying this relationship are not well understood. We used the burying beetle *Nicrophorus orbicollis* to examine the energetics of individual males and females during sexual maturation, and the energy expenditures of breeding pairs during carcass preparation to quantify the energetic costs associated with reproduction and prehatching parental care. There were no differences in resting metabolic rate (RMR) between male and female *N. orbicollis* during sexual maturity. However, metabolic activity decreased over time following eclosion and stabilized by day 13, indicating a potential reproductive diapause. We evaluated the effects of resource quality (carcass size) on metabolic costs associated with prehatching parental care. Carcass size did not significantly influence the metabolic rate of parents; however, the number of days needed to prepare small carcasses was significantly shorter compared to larger carcasses. Beetle pairs on larger carcasses experienced significantly greater metabolic cost over the course of parental care. These observations indicate that sexual development and prehatching parental care are periods of elevated metabolic activity and provide further insight into life-history

tradeoffs associated with resource quality. Furthermore, these results highlight energy use in association with ideal carcass size.

## 4.3 Introduction

A central concept of life-history theory is the cost of reproduction, which states that there are trade-offs between current and future reproductive events (Williams, 1966). Trade-offs associated with the cost of reproduction influence fundamental life history characteristics such as the timing of breeding (Low et al., 2015), clutch size and inter-clutch intervals (Forsman, 2001), parental care (Gilbert and Manica, 2010), and lifespan and fecundity (Creighton et al., 2009; Trumbo and Rauter, 2014). The relationship between resource availability and energy use, especially during reproduction, provides critical information on trade-offs associated with an organism's life history strategies such as resource allocation to growth, reproduction, and body maintenance (Jervis et al., 2008). Furthermore, variation in resource quality may affect how an organism allocates resources and accumulates reproductive costs over a lifetime (Fox and Mousseau, 1996; Creighton et al., 2009). High quality resources may reduce reproductive costs by allowing for a sufficient allocation of energy to both offspring and individual somatic maintenance (van Noordwijk and de Jong, 1986; Pärt, 2001). Conversely, these high-quality resources may require additional effort to acquire and process, as well as defend, from competitors (Trumbo, 1992). If reproduction outcompetes other organismic processes for limited resources or supplies of energy, then future reproduction and survival are affected (Calow, 1979; Schaub and von Hirschheydt, 2009). As such, optimal resources should lead to the best balance in energetic cost; however, it is unclear how resource variation affects the buildup of added reproductive costs, and how these reproductive costs shape the metabolic physiology of an organism.

Survival trade-offs associated with the cost of reproduction are studied extensively (Magnhagen, 1991; Harshman and Zera, 2007; Creighton et al., 2009; Dale, 2016; Macario et al., 2017); however, the physiological mechanisms underlying this relationship are not well understood (Fedorka et al., 2004; Fowler and Williams, 2017). The field of ecological energetics integrates metabolic processes along with external and internal constraints to the individual organism to track the quantities of nutrients and ingested energy and the subsequent distribution by the organism to fitness-enhancing processes (Tomlinson et al., 2014; Llandres et al., 2015). Ecological energetic models are often used to assess responses to global changes (Llandres et al.,

2015), evaluate the structure and stability of food webs (Getz, 2011; Lercari et al., 2015), characterize ecological niche (Kearney et al., 2010; Friedlaender et al., 2011), and researchers also apply them to conservation physiology and biology (Raubenheimer et al., 2012; Birnie-Gauvin et al., 2017). A central assumption of the metabolic theory of ecology suggests that metabolic rate dictates the pace of biological processes at all levels of organization from the organism to the ecosystem as a whole (Brown et al., 2004; Price et al., 2012; Pontzer et al., 2014; Glazier, 2015). Therefore, studies that integrate behavior, energy metabolism, and life histories are key to addressing conservation questions with broad ecosystem implications (Foster and Vincent, 2004; Biro and Stamps, 2010; Auer et al., 2016).

Burying beetles (Silphidae: *Nicrophorus*) are large-bodied invertebrates found throughout most of North America that may be impacted by varying resource availability, specifically the availability and size of small vertebrate carcasses used for reproduction (Lomolino and Creighton, 1996; Scott, 1998). Burying beetles are ideal model organisms to evaluate the relationship between resource quality and reproductive costs because they use discrete, quantifiable resources for reproduction (Creighton et al., 2009). When a breeding pair secures a carcass, both parents work to preserve the carcass for their young. Parents provide biparental care during the carcass preparation stage, which includes burying the carcass, removing feathers fur or scales, forming the carcass into a sphere, and applying antimicrobial secretions (Scott, 1998; Creighton et al., 2009; Trumbo et al., 2016; Miller et al., 2019). The pair mates repeatedly as they prepare the carcass, and females will begin to oviposit 12–48 h after locating a carcass (Scott and Traniello, 1990; Scott, 1998). After approximately five days, larvae start to arrive on the carcass to feed at an opening prepared by the parents. Parents provide the young with regurgitated tissue from the carcass beginning at their first instar stage. As young develop through the third instar, they begin to rely on self-feeding until they disperse into the surrounding soil where they pupate (Scott, 1998). For large-bodied species (e.g. *N. americanus* and *N. orbicollis*) larvae complete development and disperse to the soil to pupate in approximately six to eight days (Scott and Traniello, 1990; Scott, 1998). Adult beetles emerge from the soil after approximately four to five weeks (length of pupation), and become sexually mature by three weeks after emerging from the soil (Trumbo, 2009).

The preservation of a carcass and reproduction is costly in burying beetles (Creighton et al., 2009). These costs increase with increasing carcass size, and physiological trade-offs

associated with resource use influence a females' fecundity and longevity (Creighton et al., 2009), which can ultimately affect survival and population abundance. In their study, Creighton et al. (2009) determined that females manipulated to overproduce offspring on larger carcasses suffered a reduction in fecundity and lifespan when compared to control females, and females given larger carcasses (30 g) reproduced fewer times and had a shorter lifespan than females given smaller carcasses (20 g). Additionally, females fed low-quality diets produce fewer eggs when given a carcass for reproduction (Trumbo and Robinson, 2004). These data suggest that a resource-allocation trade-off may be one mechanism underlying the cost of reproduction that can result in fewer offspring during a reproductive attempt.

Burying beetles can use a range of carcass sizes from only a few grams to several hundred grams, and brood size and offspring body size increase with carcass size (Scott, 1998); however when females are physiologically unable to produce the number of offspring that is optimal for a particular carcass size, both parents and offspring use the additional resources to grow to a larger size (Trumbo, 1990). Thus, for burying beetles, carcass size represents a quantifiable measurement of resource quality (Trumbo, 1992). While the costs and benefits associated with carcasses of different sizes may vary considerably, they are also a rare and ephemeral reproductive resource that is limiting for burying beetles (Scott, 1998). For the federally endangered American burying beetle (*Nicrophorus americanus*), the availability of appropriately sized carcasses is vital for management and recovery of the species (Mckenna-Foster et al., 2016). However, very little is known about this relationship, and a critical first step in conservation and management of this species is to evaluate how resource quality (i.e. carcass size and age) affects reproductive costs.

Although burying beetles have been studied extensively (Creighton et al., 1993; Schnell et al., 2008; Trumbo, 2017), investigations into the effects on metabolic rate and energetic costs during reproduction and carcass preparation are few (Trumbo and Rauter, 2014). *Nicrophorus orbicollis* is the sister species to the American burying beetle (Sikes and Venables, 2013), and shares a geographic range similar to the historic range of *N. americanus* (Anderson, 1982; Wilson et al., 1984; Scott, 1998). With such an expansive range, *N. orbicollis* populations experience a wide environmental gradient, similar to historic populations of *N. americanus*, and this environmental gradient is expected to influence resource quantity and quality (Ho and Pennings, 2013) and impact the metabolic rate of reproducing beetles.

The objectives of this study were to examine the energetics of reproduction and prehatching parental care in burying beetles. First, we examined how resting metabolic rate (RMR) changed over the course of sexual maturation. We measured the RMR of newly eclosed beetles until sexual maturation at 21 days post eclosion. In Monarch butterflies (*Danaus plexippus*), males and females exhibit considerable development of reproductive tissues and accessory glands after eclosion, but then undergo reproductive diapause after these tissues are formed (Tatar and Yin, 2001). Therefore, we predicted that newly eclosed individual's RMR would be greatest close to eclosion and decrease as they become sexually mature. Secondly, we determined the relationship between carcass size with the energetic costs related to prehatching parental care (carcass preparation) for reproducing burying beetles. We predicted that the overall energetic cost during carcass preparation will increase with carcass size because larger carcasses require more time to prepare (Scott and Traniello, 1990; Scott, 1998). Furthermore, we predicted that there will be a plateau in metabolic effort on the largest carcasses as beetles specialize on specific carcass sizes and they work close to maximal capacity when preparing carcasses (Fetherston et al., 1990; Rauter and Moore, 2004; Creighton et al., 2015).

## 4.4 Materials and Methods

### 4.4.1 Field Sample Collection and Establishment of Laboratory Population

We collected *N. orbicollis* from a bottomland forest located at Purdue University's Martell Forest, Tippecanoe County, Indiana (40.455°N -86.925°W) in May of 2019. We captured all beetles using pitfall traps baited with aged chicken. We used the wild caught *N. orbicollis* to generate the laboratory population used in our experiments. We housed individual wild-caught burying beetles in small plastic containers (15 × 11 × 7 cm) with a moistened paper towel at 21°C and maintained the beetles on a diet of chicken liver fed *ad libidum* with a 14:10 LD cycle. We selected these conditions because they replicated the natural temperature and light/dark pattern consistent with the beetles' natural environment during their summer breeding season (Cook et al., 2019). To establish the laboratory population used for experiments, we placed a wild-caught male and female pair in a 29 × 18 × 11 cm container, two-thirds filled with soil, and provided them with a mouse carcass (30 g). We allowed beetles to breed and generate the laboratory population. We removed the wild-caught males when larvae first appeared on the carcass, and then removed

wild-caught females when larvae dispersed from the carcass. We left first-generation larvae undisturbed until eclosion (approximately 28-30 days).

### 4.4.2 Resting Metabolic Rate

On the day beetles eclosed, we randomly selected an individual male and female from one of 10 laboratory lines (for a total of 10 males and 10 females) at 15:30 h (when nonreproducing beetles are inactive). We then transferred beetles individually into a sealable 15 mL canonical polypropylene centrifuge tube, each fitted with a three-way stopcock valve and a 15 mL syringe to act as a constant volume respirometry chamber. Before placing beetles into centrifuge tubes, we purged tubes with air that was first scrubbed of $H_2O$ using a Dririte column (W.A. Hammond Drierite Co. LTD, Xenia, Ohio, USA). We placed the centrifuge tubes containing individual beetles in an environmental chamber at 21°C under red light to replicate the conditions in the ground (Trumbo and Rauter, 2014). After 30 min, we removed 12 mL of air from the sealed centrifuge tube and injected it into a flow-through respirometry system at a rate of 150 $mL \cdot min^{-1}$ entering the gas analyzer (Lighton, 2008; Field Metabolic System (FMS); Stable Systems International, Las Vegas, NV, USA) that monitored and recorded the percent $O_2$ and percent $CO_2$ every 0.5 s. We connected a personal computer running Expedata software (v. 1.8.4, Stable Systems International, Las Vegas, NV, USA) to the FMS to record the percent $O_2$ and percent $CO_2$ measurements. We used dried atmospheric air to calibrate the gas analyzer before each trial. Three times a week we used pure $N_2$ gas to test the system for leaks (Williams, 1983; Flaherty et al., 2014). For 10 min prior and 10 min following the injection of samples into the FMS, we collected baseline percent $O_2$ and $CO_2$ samples to correct for the inherent drift in the analyzer. Based on Lighton (2008), we calculated the rate of $O_2$ consumption ($VO_2$) and $CO_2$ production ($VCO_2$) using the following equations:

(1) $$VO_2 = (F_iO_2 - F_eO_2) \times flow\ rate$$

(2) $$VCO_2 = (F_eCO_2 - F_iCO_2) \times flow\ rate$$

Where $F_i$ is the fractional concentration of incurrent air entering the animal chamber and $F_e$ is the fractional concentration of excurrent air leaving the animal chamber. In addition, we recorded

individual's pronotum width (mm) before metabolic measurements, additionally before and after each metabolic measurement we weighed beetles to the nearest 0.001 mg and used the mean of the two mass measurements to calculate the mass-specific rate of $O_2$ consumption ($VO_2$; Suarez et al., 1996; Suarez, 2000) and $CO_2$ production ($VCO_2$; Trumbo and Rauter, 2014). We converted the percent $O_2$ and percent $CO_2$ measurements to the mass-specific rate of oxygen consumption ($VO_2$ measured as mL $O_2 \cdot mg^{-1} \cdot s^{-1}$; (Lighton, 1991; Suarez et al., 1996; Bastías et al., 2019) and carbon dioxide production ($VCO_2$ measured as mL $CO_2 \cdot mg^{-1} \cdot s^{-1}$; (Lighton, 1991; Beaupre and Zaidan III, 2001; Doubell et al., 2017). After each RMR trial, we transferred individuals into a small plastic container (15 × 11 × 7 cm) with a moistened paper towel at 21°C and maintained the beetles on a diet of chicken liver fed twice weekly with a 14:10 LD cycle. We evaluated RMR daily using these methods beginning with the date of eclosion and continuing until 21 days post eclosion.

### 4.4.3 Metabolic Rate and Carcass Quality

We placed one of two commercially available thawed mouse carcass (20 g fresh or 120 g fresh) in a flow-through breeding chamber (33 × 23 × 12 cm) filled 1/4 with topsoil. We placed breeding chambers containing mouse carcasses in an environmental chamber at 21°C for 24 h before recording initial percent $O_2$ and percent $CO_2$ measurements of the breeding chamber. Then using beetles that were not previously used for RMR measurements, we recorded pronotum widths (mm) of males and females and weighed them to the nearest 0.001 mg before establishing the sexually mature pairs on either 20 g or 120 g carcasses. The range of carcass sizes encompasses the preferred range of carrion for the American burying beetle (Trumbo, 1992). We placed the breeding chambers with beetle pairs and a carcass in an environmental chamber at 21°C under red light. After 1 h, we attached the breeding chamber to a flow-through respirometry system at a rate of 350 $mL \cdot min^{-1}$ (the approximate volume of the breeding chamber head space) before entering the FMS gas analyzer (Lighton, 2008) that monitored and recorded the percent $O_2$ and percent $CO_2$ every 0.5 s for a total of 5.0 min. We used Expedata to record the percent $O_2$ and percent $CO_2$ measurements. As previously described, we used calibrated the gas analyzer with dried atmospheric air prior to each experiment, and tested the system for leaks using pure $N_2$ gas several times a week (Williams, 1983; Flaherty et al., 2014). For 10 min prior and 10 min following the connection of breeding chambers to the FMS, we collected baseline percent $O_2$ and $CO_2$ samples

to correct for drift in the analyzer. We used Expedata, equations modified from Withers (1977) and Fedak et al. (1981), and a respiratory quotient of 0.054 that we calculated from our RMR data. Based on (Lighton, 2008), we calculated the rate of $O_2$ consumption ($VO_2$) and $CO_2$ production ($VCO_2$) using the following equations:

$$(1)\quad VO_2 = [STP \times (F_iO_2 - F_eO_2) \times FR]/[1 - F_iO_2 + RQ \times (F_iO_2 - F_eO_2)]$$

$$(2)\quad VCO_2 = [STP \times (F_iCO_2 - F_eCO_2) \times FR]/[1 - F_iCO_2 + RQ \times (F_iCO_2 - F_eCO_2)],$$

where $F_i$ is incurrent air, $F_e$ is excurrent air, $FR$ is flow rate ($mL \cdot s^{-1}$), and $RQ$ is respiratory quotient (Lighton, 2008). After each measurement, we removed the male and female pair and transferred them into a small plastic container (15 × 11 × 7 cm) with a moistened paper towel at 21°C. We left the breeding chamber in the environmental chamber for one hour before taking additional measurements of the breeding chamber without male and females present, using the previously methods. After we recorded the breeding chamber (minus the beetle pair) measurements, we placed the male and female pair back into the breeding chamber. We repeated these methods every 24 h until larvae appeared on the carcass. For each day, we subtracted the average $VCO_2$ of the breeding chamber with soil and the mouse carcass from the average $VCO_2$ of the breeding chamber with soil, the mouse carcass, and the breeding pair of beetles and multiplied this value by 86,400 $s \cdot day^{-1}$ to calculate the daily average metabolic rate of carcass preparation for each breeding pair ($MR_{CP}$).

In the case of respirometry data collection that is time-dependent, the flow rate of air should be high enough from the chamber to the gas analyzer to reduce lag between the percent $O_2$ and $CO_2$ in the chamber and the recorded measurement by the gas analyzer (Lighton and Halsey, 2011; Flaherty et al., 2014). We ensured that the quantity of $O_2$ and $CO_2$ in the chamber was accurately reflected by allowing the measurements to stabilize before collecting data (Lighton and Halsey, 2011). Additionally, we corrected for a lag in response between the chamber and the gas analyzer using a $z$ transformation (Lighton, 2008; Lighton and Halsey, 2011). Therefore, our $VCO_2$ estimates should not be influenced by the system's lagged response (Flaherty et al., 2014).

### 4.4.4 Statistical Analysis

We performed all statistical computations in program R (version 3.6.1, Core Team 2019), using the 'nlme' library for the linear mixed-effects models. To compare male and female RMR, we calculated the mean $VO_2$ and $VCO_2$ value for each animal for each day and used a repeated mixed-measures analysis of variance (ANOVA) model in which the day of measurement, beetle sex, mass, and pronotum width were fixed factors and the interaction between the day of measurement and the identity of individual beetles were random factors (Zar, 2014). To compare the effects of carcass size on $MR_{CP}$, we calculated the mean $VO_2$ and $VCO_2$ value of each breeding pair for each day using a repeated mixed-measures ANOVA model in which carcass size, day of measurements, male and female mass and pronotum width were fixed factors, and time before larvae appeared on the carcass as a random factor (Zar, 2014).

## 4.5 Results

### 4.5.1 Resting Metabolic Rate

The overall observed RMR among all beetles and days was 1.611 ± 0.012 mL $O_2 \cdot g^{-1} \cdot h^{-1}$ and 1.315 ± 0.013 mL $CO_2 \cdot g^{-1} \cdot h^{-1}$, mean ± SE; n=20. There was no difference between the male and females in measured $O_2$ consumption (males: 1.596 ± 0.014 mL $O_2 \cdot g^{-1} \cdot h^{-1}$; females: 1.626 ± 0.011 mL $O_2 \cdot g^{-1} \cdot h^{-1}$; $F_{[1,2]} = 0.09$, $p = 0.77$) or $CO_2$ production (males: 1.266 ± 0.349 mL $CO_2 \cdot g^{-1} \cdot h^{-1}$; females: 1.365 ± 0.326 mL $CO_2 \cdot g^{-1} \cdot h^{-1}$; $F_{[1,2]} = 0.03$, $p = 0.87$). Consumption of $O_2$ decreased over time after eclosion (Fig. 1A; $F_{[1,178]} = 28.65$, $p < 0.001$). Similarly, production of $CO_2$ decreased over time after eclosion (Fig. 1B; $F_{[1,178]} = 23.62$, $p < 0.001$). Average overall beetle mass was 285.0 ± 0.3 mg, and it significantly influenced observed $VCO_2$ ($F_{[176,178]} = 1.31$, $p = 0.030$), with larger beetles producing more $CO_2$ daily. However, beetle size, as measured by pronotum width did not significantly influence observed $VO_2$ ($F_{[8,110]} = 0.35$, $p = 0.921$) or observed $VCO_2$ ($F_{[8,110]} = 1.30$, $p = 0.343$).

### 4.5.2 Metabolic Rate and Carcass Quality

The overall observed $MR_{CP}$ across all days of the trials was 5.306 ± 2.72 mL $O_2 \cdot day^{-1}$ and 1.09 ± 0.14 mL $CO_2 \cdot day^{-1}$, mean ± SE; n=195. Time before larvae appeared on a carcass was the only significant factor that influenced observed $VO_2$ ($F_{[1,29]} = 1.97$, $p = 0.047$; Table 1; Fig. 2A).

However, the number of days male and female pairs were preserving carcasses significantly influenced observed $VCO_2$ ($F_{[1,29]} = 2.50$, $p = 0.012$; Fig. 2B). The average observed $O_2$ consumed by carcasses without beetles for 20 g carcasses was significantly different from 120 g carcasses ($VO_2 = 293 \pm 0.53$ mL $O_2 \cdot carcass^{-1}$; $VO_2 = 960 \pm 0.69$ mL $O_2 \cdot carcass^{-1}$; $F_{[1,29]} = 2.95$, $p < 0.01$ respectively). The average $CO_2$ production of carcasses without beetles for 20 g carcasses was ($VCO_2 = 45.55 \pm 1.26$ mL $CO_2 \cdot carcass^{-1}$), and was greater for 120 g carcasses ($VCO_2 = 183.22 \pm 4.33$ mL $CO_2 \cdot carcass^{-1}$; $F_{[1,192]} = 8.33$, $p < 0.01$). The number of days male and female pairs were preserving carcasses did not influence carcass $O_2$ production ($F_{[1,140]} = 1.57$, $p = 0.13$; Fig. 3A). However, the number of days male and female pairs were preserving carcasses significantly influenced carcass $CO_2$ production ($F_{[1,140]} = 15.45$, $p < 0.01$; Fig. 3B). The overall accumulated $VO_2$ for breeding pairs during carcass preparation on 20 g carcasses was ($VO_2 = 145.84 \pm 0.53$ mL $O_2 \cdot carcass^{-1}$) and was significantly different from 120 g carcasses ($VO_2 = 379.55 \pm 0.69$ mL $O_2 \cdot carcass^{-1}$; $F_{[1,195]} = 2.95$, $p < 0.01$; Fig. 4A). The overall accumulated $VCO_2$ for breeding pairs during carcass preparation on 20 g carcasses was ($42.71 \pm 0.13$ mL $CO_2 \cdot carcass^{-1}$), and was greater for 120 g carcasses ($VCO_2 = 170.55 \pm 0.20$ mL $CO_2 \cdot carcass^{-1}$; $F_{[1,195]} = 6.28$, $p = 0.012$; Fig. 4B). However, there was no significant difference between $MR_{CP}$ based on male and female mass, male and female body size, or the carcass size (Table 1).

### 4.6 Discussion

This study demonstrates that the time in which beetles become sexually mature is a time of elevated metabolic rates in *N. orbicollis* and provides new information on metabolic trade-offs associated with sexual development (Trumbo and Rauter, 2014). As predicted, the RMR of newly eclosed beetles was greatest after emerging from the soil. This high metabolic activity soon after emergence may coincide with an increase and eventual plateau of juvenile hormone that coincides with an increase in developing reproductive tissue size, before a suitable reproductive resource is secured (Trumbo, 1997). This is similar to findings in insects where adult reproductive diapause is delineated by a period of diapause in oogenesis in females and the accessory gland development in males, along with a change in behaviors and somatic metabolic processes in both sexes (Herman, 1981; Pener, 1992; Tatar and Yin, 2001). As metabolic processes influence how organisms allocate energy to reproduction, self-maintenance, and growth (Gillooly et al., 2001; Brown et al., 2004),

variation in metabolic rate is expected to change the shape of the relationship among life history traits (Sibly and Calow, 1987; Clarke, 1993). For example, in insects a prolonged elevated RMR can inhibit immune responses (Ardia et al., 2012) and increase oxidative stress (Lalouette et al., 2011), which may result in a change in the energetic allocation between competing functions such as maintenance and reproduction (Lann et al., 2011).

Similar to our RMR results, we demonstrated that carcass preparation during prehatching parental care increases metabolic activity, providing insights into trade-offs associated with the cost of prehatching parental care (De Gasperin et al., 2016; Trumbo, 2017). The metabolic cost of reproduction is hypothesized to be a byproduct of an general increase in metabolic intensity (i.e. mass-specific metabolic rate) of parental tissue (Angilletta and Sears, 2000). In our study, the greatest metabolic cost for prehatching carcass preparation occurred with the larger carcass treatment. However, metabolic cost associated with carcass preparation appear to be cumulative because they were only significantly different between treatments based on the number of days beetles were preparing their carcass (Table 1). Many studies use resource manipulation to separate the cost of parental care in insects (Boggs, 1981; Rauter and Moore, 2004; Creighton et al., 2009; Trumbo and Rauter, 2014; Capodeanu-Nägler et al., 2016), however few evaluate the cost of prehatching parental care (De Gasperin and Kilner, 2015; De Gasperin et al., 2016; Trumbo, 2017).

Previous studies in burying beetles evaluating prehatching parental care evaluated cost associated with parental longevity (De Gasperin et al., 2016; Trumbo, 2017). De Gasperin et al. (2016) determined that the roundness of a carcass after preparation influenced beetle lifespan, where females with rounder carcass nests had longer lifespans after reproduction. However, measures of offspring success or offspring performance was not associated with the roundness of carcass nests (De Gasperin et al., 2016). Trumbo (2017) evaluated carcass quality in terms of the freshness of the reproductive resource. In the study, offspring reared on aged carcasses had lower average mass, however the parent's longevity and future reproduction were not affected. This suggest that in a challenging environment, parents are able to alleviate the cost of current and future reproduction attempts (Trumbo, 2017). Trumbo and Rauter (2014), determined that there was an absence of a significant longevity cost for prehatching care in terms of egg production. However, our study suggest that the cost associated with prehatching parental care is in relationship to the length of carcass preparation.

The effects of prehatching parental care is widely studied across a variety of taxa (Simon, 1983; Eggert et al., 1998; Schwagmeyer et al., 1999; Villuendas and Sarzo, 2003; Liker and Székely, 2005; Liker et al., 2015; Takahashi et al., 2017). In a study evaluating prehatching parental care in the kestrel, *Falco tinnunculus*, researchers determined that the daily energy expenditure of parents increased as resource quantity and quality decreased (Masman et al., 1989). We observed similar results, in that breeding pairs' cumulative metabolic rate was greater on larger carcasses, which constitute a greater challenge when preserving carcasses (Trumbo and Fernandez, 1995; Rozen et al., 2008; Trumbo, 2017). In these studies, aged carcasses resulted in lower average larval body mass, lower brood sizes, and fewer surviving offspring. In our experiment, we provided the same age carcass to breeding pairs. However, the time before larvae arrived on a carcass was significantly different between treatments ($F_{[1,28]} = 89.60$, $p = <0.001$). For example, on 20 g carcasses average larval arrival time was (4.6 ± 0.2 days), whereas on 120g carcasses average larval arrival was (7.8 ± 0.3 days). For their experiment, Rozen et al. (2008) provided mated females with either a freshly thawed mouse, or a carcass that was aged for 7 days to enable the progression through putrefaction due to microbial growth. Therefore, larger carcasses may be more similar to aged carcasses in that they take more time to prepare, because of their size (Trumbo, 1994; Scott, 1998), and this may result in higher rates of putrefaction and increased microbial growth on the resource.

In our study we did not evaluate measures of larval performance, parental fecundity or lifespan. However, researchers evaluated the cost-of-reproduction (Williams, 1966) and the terminal-investment hypothesis (Clutton-Brock, 1984) in *N. orbicollis* (Creighton et al., 2009). In their study, they determined that female lifespan was influenced by brood size and carcass size. Female *N. orbicollis* exhibited shorter lifespans when they raised larger broods and when they used larger carcasses for reproduction over their lifespan (Creighton et al., 2009). Our results complement this study evaluating cost associated with parental care by quantifying cost before larvae arrive on the prepared carcass. Paired with previous research on the reproductive benefits of parental care in *Nicrophorus* spp. beetles (Trumbo, 1990; Trumbo, 1991; Trumbo and Fernandez, 1995; Creighton, 2005; Creighton et al., 2009; Ward et al., 2009; Trumbo and Rauter, 2014; De Gasperin and Kilner, 2015; De Gasperin et al., 2016; Trumbo, 2017), our study provides new insight into how parents accumulate metabolic cost during prehatching parental care when presented with resources that vary in quality.

The results of the present study indicate that energetic cost associated with prehatching parental care is magnified by the time it takes beetles to preserve a carcass and not the size of the resource. Furthermore, larger carcasses may represent a lower quality resource if they are above an optimal size for parents. The co-occurrence of heightened metabolic rates and elevated parental activity during prehatching parental care in *N. orbicollis* is consistent with previous research focusing on posthatching parental care (Trumbo and Rauter, 2014). However, the energetic cost of parental care and reproduction has important implications for studies of intraspecific variation in reproductive effort, and an enhanced understanding of factors that influence the metabolic cost associated with reproduction will improve efforts to understand intraspecific variation in life history (Angilletta and Sears, 2000). Further research is needed to determine the relationship between metabolic rate, optimal carcass size, larval performance, and parental performance on longevity and the cost associated with parental care in burying beetles. Additionally, *Nicrophorus* spp. beetles exhibit niche variation in respect to spatial and temporal patterns as well as carcass size (Scott, 1998; Hopwood et al., 2016), and provide opportunities to further evaluate these trade-offs.

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## 4.8 References

Anderson, R. S. (1982). On the decreasing abundance of *Nicrophorus americanus* Olivier (Coleoptera: Silphidae) in eastern North America. *Coleopts. Bull.* **36**, 362–365.

Angilletta, M. J. and Sears, M. W. (2000). The metabolic cost of reproduction in an oviparous lizard. *Functional Ecology* **14**, 39–45.

Ardia, D. R., Gantz, J. E., Brent C., Schneider and Strebel, S. (2012). Costs of immunity in insects: an induced immune response increases metabolic rate and decreases antimicrobial activity: Energetic costs of immunity. *Functional Ecology* **26**, 732–739.

Auer, S. K., Salin, K., Anderson, G. J. and Metcalfe, N. B. (2016). Flexibility in metabolic rate and activity level determines individual variation in overwinter performance. *Oecologia* **182**, 703–712.

Bastías, D. A., Martínez-Ghersa, M. A., Newman, J. A., Card, S. D., Mace, W. J. and Gundel, P. E. (2019). *Sipha maydis* sensitivity to defences of *Lolium multiflorum* and its endophytic fungus *Epichloë* occultans. *PeerJ* **7**, e8257.

Beaupre, S. J. and Zaidan III, F. (2001). Scaling of $CO_2$ Production in the Timber Rattlesnake ( *Crotalus horridus* ), with Comments on Cost of Growth in Neonates and Comparative Patterns. *Physiological and Biochemical Zoology* **74**, 757–768.

Birnie-Gauvin, K., Walton, S., Palme, C., Manouchehri, B., Venne, S., Lennox, R., Chapman, J., Bennett, J. and Cooke, S. (2017). Conservation physiology can inform threat assessment and recovery planning processes for threatened species. *Endang. Species. Res.* **32**, 507–513.

Biro, P. A. and Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology & Evolution* **25**, 653–659.

Boggs, C. L. (1981). Nutritional and Life-History Determinants of Resource Allocation in Holometabolous Insects. *The American Naturalist* **117**, 692–709.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. and West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789.

Calow, P. (1979). The Cost of Reproduction – a Physiological Approach. *Biological Reviews* **54**, 23–40.

Capodeanu-Nägler, A., Keppner, E. M., Vogel, H., Ayasse, M., Eggert, A.-K., Sakaluk, S. K. and Steiger, S. (2016). From facultative to obligatory parental care: Interspecific variation in offspring dependency on post-hatching care in burying beetles. *Sci Rep* **6**, 29323.

Clarke, A. (1993). Seasonal Acclimatization and Latitudinal Compensation in Metabolism: Do They Exist? *Functional Ecology* **7**, 139.

Clutton-Brock, T. H**.** (1984). Reproductive Effort and Terminal Investment in Iteroparous Animals. *The American Naturalist* **123**, 212–229.

Cook, L. M., Smith, A. N., Meyers, P. J., Creighton, J. C. and Belk, M. C. (2019). Evidence for Differential Diel Activity Patterns in Two Co-Occurring Species of Burying Beetles (Coleoptera: Silphidae: Nicrophorinae). *Western North American Naturalist* **79**, 270.

Creighton, J. C. (2005). Population density, body size, and phenotypic plasticity of brood size in a burying beetle. *Behav. Ecol.* **16**, 1031–1036.

Creighton, J. C., Vaughn, C. C. and Chapman, B. R. (1993). Habitat preference of the endangered American burying beetle (*Nicrophorus americanus*) in Oklahoma. *Sowthwest Nat.* **38**, 275.

Creighton, J. C., Heflin, N. D. and Belk, M. C. (2009). Cost of reproduction, resource quality, and terminal investment in a burying beetle. *Am. Nat.* **174**, 673–684.

Creighton, J. C., Smith, A. N., Komendat, A. and Belk, M. C. (2015). Dynamics of biparental care in a burying beetle: experimental handicapping results in partner compensation. *Behav. Ecol. Sociobiol.* **69**, 265-271.

Dale, S. (2016). Cost of reproduction: a comparison of survival rates of breeding and non-breeding male ortolan buntings. *Journal of Avian Biology* **47**, 583–588.

De Gasperin, O. and Kilner, R. M. (2015). Interspecific interactions change the outcome of sexual conflict over prehatching parental investment in the burying beetle Nicrophorus vespilloides. *Ecology and Evolution* **5**, 5552–5560.

De Gasperin, O., Duarte, A., Troscianko, J. and Kilner, R. M. (2016). Fitness costs associated with building and maintaining the burying beetle's carrion nest. *Sci Rep* **6**, 35293.

Doubell, M., Grant, P. B. C., Esterhuizen, N., Bazelet, C. S., Addison, P. and Terblanche, J. S**.** (2017). The metabolic costs of sexual signalling in the chirping katydid *Plangia graminea* (Serville) (Orthoptera: Tettigoniidae) are context dependent: cumulative costs add up fast. *Journal of Experimental Biol* **220**, 4440–4449.

Eggert, A.-K., Reinking, M. and Müller, J. K. (1998). Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour* **55**, 97–107.

Fedak, M. A., Rome, L. and Seeherman, H. J. (1981). One-step N2-dilution technique for calibrating open-circuit VO2 measuring systems. *Journal of Applied Physiology* **51**, 772–776.

Fedorka, K. M., Zuk, M. and Mousseau, T. A. (2004). Immune Suppression and the Cost of Reproduction in the Ground Cricket, Allonemobius Socius. *Evolution* **58**, 2478–2485.

Fetherston, I. A., Scott, M. P. and Traniello, J. F. A. (1990). Parental Care in Burying Beetles: The Organization of Male and Female Brood-care Behavior. *Ethology* **85**, 177–190.

Flaherty, E. A., Ben-David, M. and Pauli, J. N. (2014). A comparison of locomotor performance of the semiarboreal Pacific marten ( *Martes caurina* ) and semiaquatic mustelids. *Canadian Journal of Zoolology*, **92**, 259–266.

Forsman, A. (2001). Clutch size versus clutch interval: life history strategies in the colour-polymorphic pygmy grasshopper Tetrix subulata. *Oecologia* **129**, 357–366.

Foster, S. J. and Vincent, A. C. J. (2004). Life history and ecology of seahorses: implications for conservation and management. *Journal of Fish Biology* **65**, 1–61.

Fowler, M. A. and Williams, T. D. (2017). A Physiological Signature of the Cost of Reproduction Associated with Parental Care. *The American Naturalist* **190**, 762–773.

Fox, C. W. and Mousseau, T. A. (1996). Larval host plant affects fitness consequences of egg size variation in the seed beetle Stator limbatus. *Oecologia* **107**, 541–548.

Friedlaender, A. S., Johnston, D. W., Fraser, W. R., Burns, J., Patrick N., H. and Costa, D. P. (2011). Ecological niche modeling of sympatric krill predators around Marguerite Bay, Western Antarctic Peninsula. *Deep Sea Research Part II: Topical Studies in Oceanography* **58**, 1729–1740.

Getz, W. M. (2011). Biomass transformation webs provide a unified approach to consumer–resource modelling. *Ecology Letters* **14**, 113–124.

Gilbert, J. D. J. and Manica, A. (2010). Parental Care Trade-Offs and Life-History Relationships in Insects. *The American Naturalist* **176**, 212–226.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. and Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science* **293**, 2248–2251.

Glazier, D. S. (2015). Is metabolic rate a universal 'pacemaker' for biological processes? *Biological Reviews* **90**, 377–407.

Harshman, L. G. and Zera, A. J. (2007). The cost of reproduction: the devil in the details. *Trends in Ecology & Evolution* **22**, 80–86.

Herman, W. S. (1981). Studies on the adult reproductive diapause of the monarch butterfly, *Danaus plexippus* . *The Biological Bulletin* **160**, 89–106.

Ho, C.-K. and Pennings, S. C. (2013). Preference and Performance in Plant–Herbivore Interactions across Latitude–A Study in U.S. Atlantic Salt Marshes. *PLoS One* **8**,.

Hopwood, P. E., Moore, A. J., Tregenza, T. and Royle, N. J. (2016). Niche variation and the maintenance of variation in body size in a burying beetle. *Ecological Entomology* **41**, 96–104.

Jervis, M. A., Ellers, J. and Harvey, J. A. (2008). Resource Acquisition, Allocation, and Utilization in Parasitoid Reproductive Strategies. *Annual Review of Entomology* **53**, 361–385.

Kearney, M., Simpson, S. J., Raubenheimer, D. and Helmuth, B. (2010). Modelling the ecological niche from functional traits. *Phil. Trans. R. Soc. B* **365**, 3469–3483.

Lalouette, L., Williams, C. M., Hervant, F., Sinclair, B. J. and Renault, D. (2011). Metabolic rate and oxidative stress in insects exposed to low temperature thermal fluctuations. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **158**, 229–234.

Lann, C. L., Wardziak, T., Baaren, J. van and Alphen, J. J. M. van (2011). Thermal plasticity of metabolic rates linked to life-history traits and foraging behaviour in a parasitic wasp. *Functional Ecology* **25**, 641–651.

Lercari, D., Horta, S., Martínez, G., Calliari, D. and Bergamino, L. (2015). A food web analysis of the Río de la Plata estuary and adjacent shelf ecosystem: trophic structure, biomass flows, and the role of fisheries. *Hydrobiologia* **742**, 39–58.

Lighton, J. R. B. (1991). Ventilation in Namib desert tenebrionid beetles: Mass scaling and evidence of a novel quantized flutter-phase. *Journal of Experimental Biology* **159**, 249–268.

Lighton, J. R. B. (2008). *Measuring Metabolic Rates: A Manual for Scientists*. 2nd ed. Oxford University Press.

Lighton, J. R. B. and Halsey, L. G. (2011). Flow-through respirometry applied to chamber systems: Pros and cons, hints and tips. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **158**, 265–275.

Liker, A. and Székely, T. (2005). Mortality Costs of Sexual Selection and Parental Care in Natural Populations of Birds. *Evolution* **59**, 890–897.

Liker, A., Freckleton, R. P., Remeš, V. and Székely, T. (2015). Sex differences in parental care: Gametic investment, sexual selection, and social environment. *Evolution* **69**, 2862–2875.

Llandres, A. L., Marques, G. M., Maino, J. L., Kooijman, S. a. L. M., Kearney, M. R. and Casas, J. (2015). A dynamic energy budget for the whole life-cycle of holometabolous insects. *Ecological Monographs* **85**, 353–371.

Lomolino, M. V. and Creighton, J. C. (1996). Habitat selection, breeding success and conservation of the endangered American burying beetle *Nicrophorus americanus*. *Biol. Conserv.* **77**, 235–241.

Low, M., Arlt, D., Pärt, T. and Öberg, M. (2015). Delayed timing of breeding as a cost of reproduction. *Journal of Avian Biology* **46**, 325–331.

Macario, P., Pichorim, M., Doherty, P. F., Toledo-Lima, G. S., Oliveira-Júnior, T. M., Câmara, T. P. F., Melo, S. M., Silveira, J. L. S., Araújo, J. C. and França, L. F. (2017). Apparent survival and cost of reproduction for White-lined Tanager (Tachyphonus rufus, Thraupidae) in the northern Atlantic Rainforest, Brazil. *PLoS One* **12**,.

Magnhagen, C. (1991). Predation risk as a cost of reproduction. *Trends Ecol. Evol.* **6**, 183–185.

Masman, D., Dijkstra, C., Daan, S. and Bult, A. (1989). Energetic limitation of avian parental effort: Field experiments in the kestrel (Falco tinnunculus). *Journal of Evolutionary Biology* **2**, 435–455.

Mckenna-Foster, A., Perrotti, L., Blyth, J., LoPresti, E. and Kennedy, R. S. (2016). Measuring success of a reintroduced population of the American burying beetle (*Nicrophorus americanus* Olivier) to Nantucket Island, MA. *J. Insect. Conserv.* **20**, 895–904.

Miller, C. J., Bates, S. T., Gielda, L. M. and Creighton, J. C. (2019). Examining transmission of gut bacteria to preserved carcass via anal secretions in Nicrophorus defodiens. *PLoS ONE* **14**, e0225711.

Pärt, T. (2001). Experimental evidence of environmental effects on age–specific reproductive success: the importance of resource quality. *Proc. R. Soc. Lond. B* **268**, 2267–2271.

Pener, M. P. (1992). Environmental Cues, Endocrine Factors, and Reproductive Diapause in Male Insects. *Chronobiology International* **9**, 102–113.

Pontzer, H., Raichlen, D. A., Gordon, A. D., Schroepfer-Walker, K. K., Hare, B., O'Neill, M. C., Muldoon, K. M., Dunsworth, H. M., Wood, B. M., Isler, K., et al. (2014). Primate energy expenditure and life history. *Proceedings of the National Academy of Sciences* **111**, 1433–1437.

Price, C. A., Weitz, J. S., Savage, V. M., Stegen, J., Clarke, A., Coomes, D. A., Dodds, P. S., Etienne, R. S., Kerkhoff, A. J., McCulloh, K., et al. (2012). Testing the metabolic theory of ecology. *Ecology Letters* **15**, 1465–1474.

Raubenheimer, D., Simpson, S. J. and Tait, A. H. (2012). Match and mismatch: conservation physiology, nutritional ecology and the timescales of biological adaptation. *Phil. Trans. R. Soc. B* **367**, 1628–1646.

Rauter, C. M. and Moore, A. J. (2004). Time constraints and trade-offs among parental care behaviours: effects of brood size, sex and loss of mate. *Animal Behaviour* **68**, 695–702.

Rozen, D. E., Engelmoer, D. J. P. and Smiseth, P. T. (2008). Antimicrobial strategies in burying beetles breeding on carrion. *Proceedings of the National Academy of Sciences* **105**, 17890–17895.

Schaub, M. and von Hirschheydt, J. (2009). Effect of current reproduction on apparent survival, breeding dispersal, and future reproduction in barn swallows assessed by multistate capture–recapture models. *Journal of Animal Ecology* **78**, 625–635.

Schnell, G. D., Hiott, A. E., Creighton, J. C., Smyth, V. L. and Komendat, A. (2008). Factors affecting overwinter survival of the American burying beetle, *Nicrophorus americanus* (Coleoptera: Silphidae). *J. Insect Conserv.* **12**, 483–492.

Schwagmeyer, P. L., St. Clair, R. C., Moodie, J. D., Lamey, T. C. and Schnell, G. D. (1999). Species Differences in Male Parental Care in Birds: A Reexamination of Correlates with Paternity. *The Auk* **116**, 487–503.

Scott, M. P. (1998). The ecology and behavior of burying beetles. *Annu. Rev. Entomol.* **43**, 595–618.

Scott, M. P. and Traniello, J. F. A. (1990). Behavioural and ecological correlates of male and female parental care and reproductive success in burying beetles (Nicrophorus spp.). *Animal Behaviour* **39**, 274–283.

Sibly, R. and Calow, P. (1987). Ecological Compensation--a Complication for Testing Life-history Theory. *Journal of Theoretical Biology* **125**, 177–186.

Sikes, D. S. and Venables, C. (2013). Molecular phylogeny of the burying beetles (Coleoptera: Silphidae: Nicrophorinae). *Mol. Phylogenet. Evol.* **69**, 552-565.

Simon, M. P. (1983). The ecology of parental care in a terrestrial breeding frog from New Guinea. *Behav Ecol Sociobiol* **14**, 61–67.

Suarez, R. K. (2000). Energy Metabolism during Insect Flight: Biochemical Design and Physiological Performance. *Physiological and Biochemical Zoology* **73**, 765–771.

Suarez, R. K., Lighton, J. R., Joos, B., Roberts, S. P. and Harrison, J. F. (1996). Energy metabolism, enzymatic flux capacities, and metabolic flux rates in flying honeybees. *Proceedings of the National Academy of Sciences* **93**, 12616–12620.

Takahashi, M. K., Okada, S. and Fukuda, Y. (2017). From embryos to larvae: seven-month-long paternal care by male Japanese giant salamander. *Journal of Zoology* **302**, 24–31.

Tatar, M. and Yin, C.-M. (2001). Slow aging during insect reproductive diapause: why butterflies, grasshoppers and flies are like worms. *Experimental Gerontology* 16.

Tomlinson, S., Arnall, S. G., Munn, A., Bradshaw, S. D., Maloney, S. K., Dixon, K. W. and Didham, R. K. (2014). Applications and implications of ecological energetics. *Trends in Ecology & Evolution* **29**, 280–290.

Trumbo, S. T. (1990). Regulation of brood size in a burying beetle,Nicrophorus tomentosus (Silphidae). *J Insect Behav* **3**, 491–500.

Trumbo, S. T. (1991). Reproductive Benefits and the Duration of Paternal Care in a Biparental Burying Beetle, Necrophorus Orbicollis. *Behav* **117**, 82–105.

Trumbo, S. T. (1992). Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (Nicrophorus). *Ecological Entomology* **17**, 289–298.

Trumbo, S. T. (1994). Interspecific Competition, Brood Parasitism, and the Evolution of Biparental Cooperation in Burying Beetles. *Oikos* **69**, 241.

Trumbo, S. T. (1997). Juvenile hormone-mediated reproduction in burying beetles: From behavior to physiology. *Archives of Insect Biochemistry and Physiology* **35**, 479–490.

Trumbo, S. T. (2009). Age-related reproductive performance in the parental burying beetle, Nicrophorus orbicollis. *Behavioral Ecology* **20**, 951–956.

Trumbo, S. T. (2017). Feeding upon and preserving a carcass: the function of prehatch parental care in a burying beetle. *Animal Behaviour* **130**, 241–249.

Trumbo, S. T. and Fernandez, A. G. (1995). Regulation of brood size by male parents and cues employed to assess resource size by burying beetles. *Ethology Ecology & Evolution* **7**, 313–322.

Trumbo, S. T. and Rauter, C. M. (2014). Juvenile hormone, metabolic rate, body mass and longevity costs in parenting burying beetles. *Animal Behaviour* **92**, 203–211.

Trumbo, S. T. and Robinson, G. E. (2004). Nutrition, hormones and life history in burying beetles. *Journal of Insect Physiology* **50**, 383–391.

Trumbo, S. T., Sikes, D. S. and Philbrick, P. K. B. (2016). Parental care and competition with microbes in carrion beetles: a study of ecological adaptation. *Animal Behaviour* **118**, 47–54.

van Noordwijk, A. J. and de Jong, G. (1986). Acquisition and Allocation of Resources: Their Influence on Variation in Life History Tactics. *The American Naturalist* **128**, 137–142.

Villuendas, E. and Sarzo, B. (2003). Growth of Audouin's gull chicks: the role of prehatch and posthatch factors. *Sci. Mar.* **67**, 113–116.

Ward, R. J. S., Cotter, S. C. and Kilner, R. M. (2009). Current brood size and residual reproductive value predict offspring desertion in the burying beetle Nicrophorus vespilloides. *Behavioral Ecology* **20**, 1274–1281.

Williams, G. C. (1966). Natural Selection, the Costs of Reproduction, and a Refinement of Lack's Principle. *The American Naturalist* **100**, 687–690.

Williams, T. M. (1983). Locomotion in the North American mink, a semi-aquatic mammal. II. THe effect of an elongate body on running energetics and gait patterns. *Journal of Experimental Biology* **105**, 283-295.

Wilson, D. S., Knollenberg, W. G. and Fudge, J. (1984). Species packing and temperature dependent competition among burying beetles (Silphidae, Nicrophorus). *Ecological Entomology* **9**, 205–216.

Withers, P. C. (1977). Measurement of VO2, VCO2, and evaporative water loss with a flow-through mask. *Journal of Applied Physiology* **42**, 120–123.

Zar, J. H. (2014). *Biostatistical analysis*. 5th ed. London, U.K.: Pearson Education Limited.

**Table 4.1.** Fixed Effects ANOVA Table for Metabolic Rate

ANOVA table for fixed effects on metabolic rate and carcass quality experiment. Bold values are statistically significant.

| Response Variable | df num/den | *F*-value | *p*-value |
|---|---|---|---|
| Time (days)-$O_2$ | 9/140 | 1.61 | 0.117 |
| Days before larvae arrive on carcass-$O_2$ | 9/140 | 1.97 | **0.047** |
| Carcass size-$O_2$ | 1/29 | 1.73 | 0.199 |
| Female mass (mg) -$O_2$ | 1/140 | 2.62 | 0.108 |
| Female pronotum width (mm) -$O_2$ | 1/140 | 0.00 | 0.957 |
| Male mass (mg) -$O_2$ | 1/140 | 3.21 | 0.075 |
| Male pronotum width (mm) -$O_2$ | 1/140 | 0.01 | 0.941 |
| Time (days)-$CO_2$ | 9/140 | 2.47 | **= 0.012** |
| Days before larvae arrive on carcass-$CO_2$ | 9/140 | 0.64 | 0.764 |
| Carcass size-$CO_2$ | 1/29 | 0.01 | 0.924 |
| Female mass (mg) -$CO_2$ | 1/140 | 0.02 | 0.894 |
| Female pronotum width (mm) -$CO_2$ | 1/140 | 0.09 | 0.762 |
| Male mass (mg) -$CO_2$ | 1/140 | 2.05 | 0.155 |
| Male pronotum width (mm) -$CO_2$ | 1/140 | 0.60 | 0.441 |

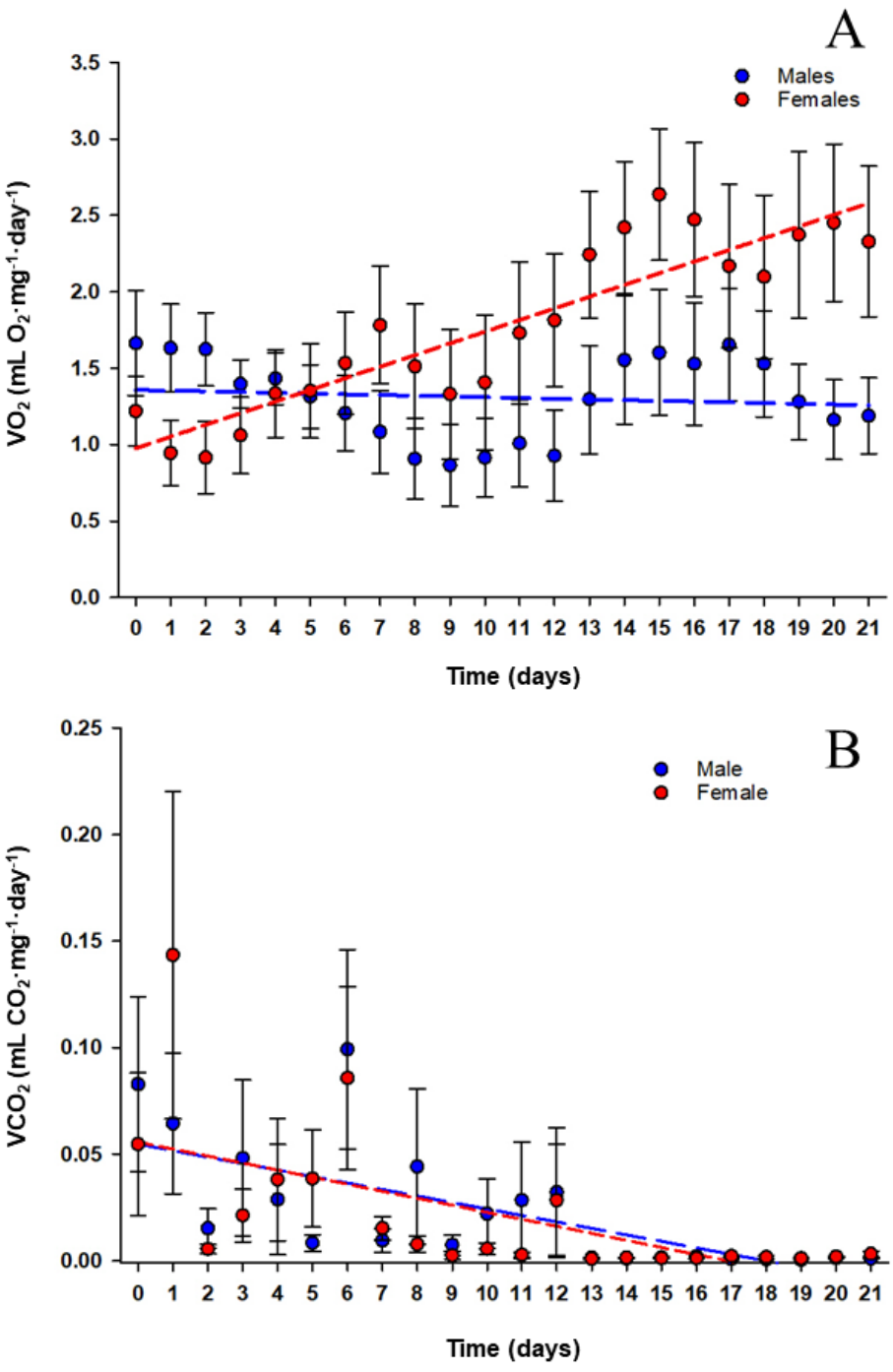


**Figure 4.1.** Resting Metabolic Rate

Measured resting metabolic rate from eclosion to sexual maturity [calculated as $VO_2$ (A) and $VCO_2$ (B)], ± SE, for male and female *N. orbicollis* from a captive population at the Purdue University.

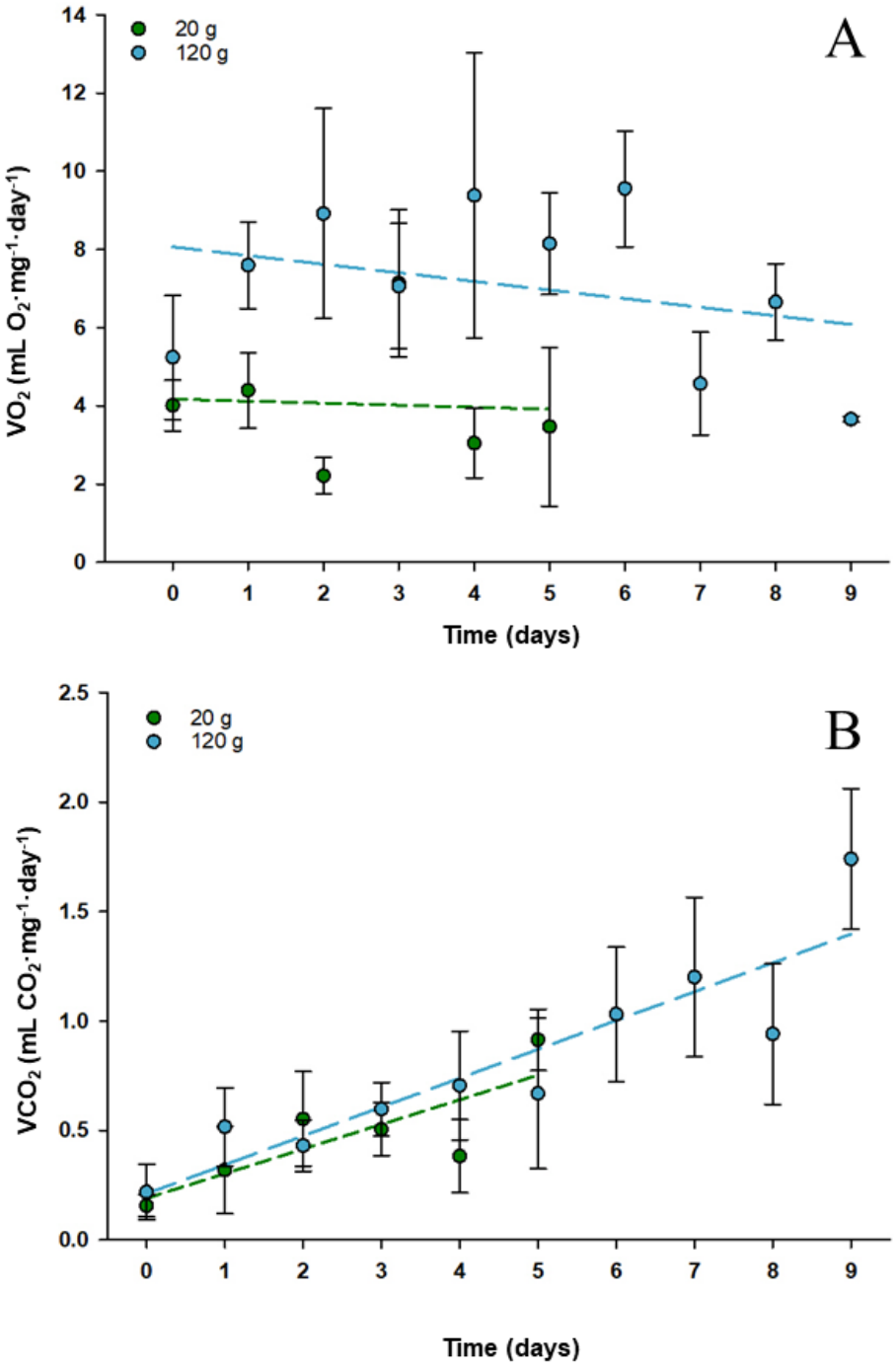


**Figure 4.2.** Metabolic Rate of Carcass Preparation

Measured metabolic rate of carcass preparation [calculated as $VO_2$ (A) and $VCO_2$ (B)], ± SE, for breeding pairs of *N. orbicollis* on 20 g or 120 g mouse carcasses.

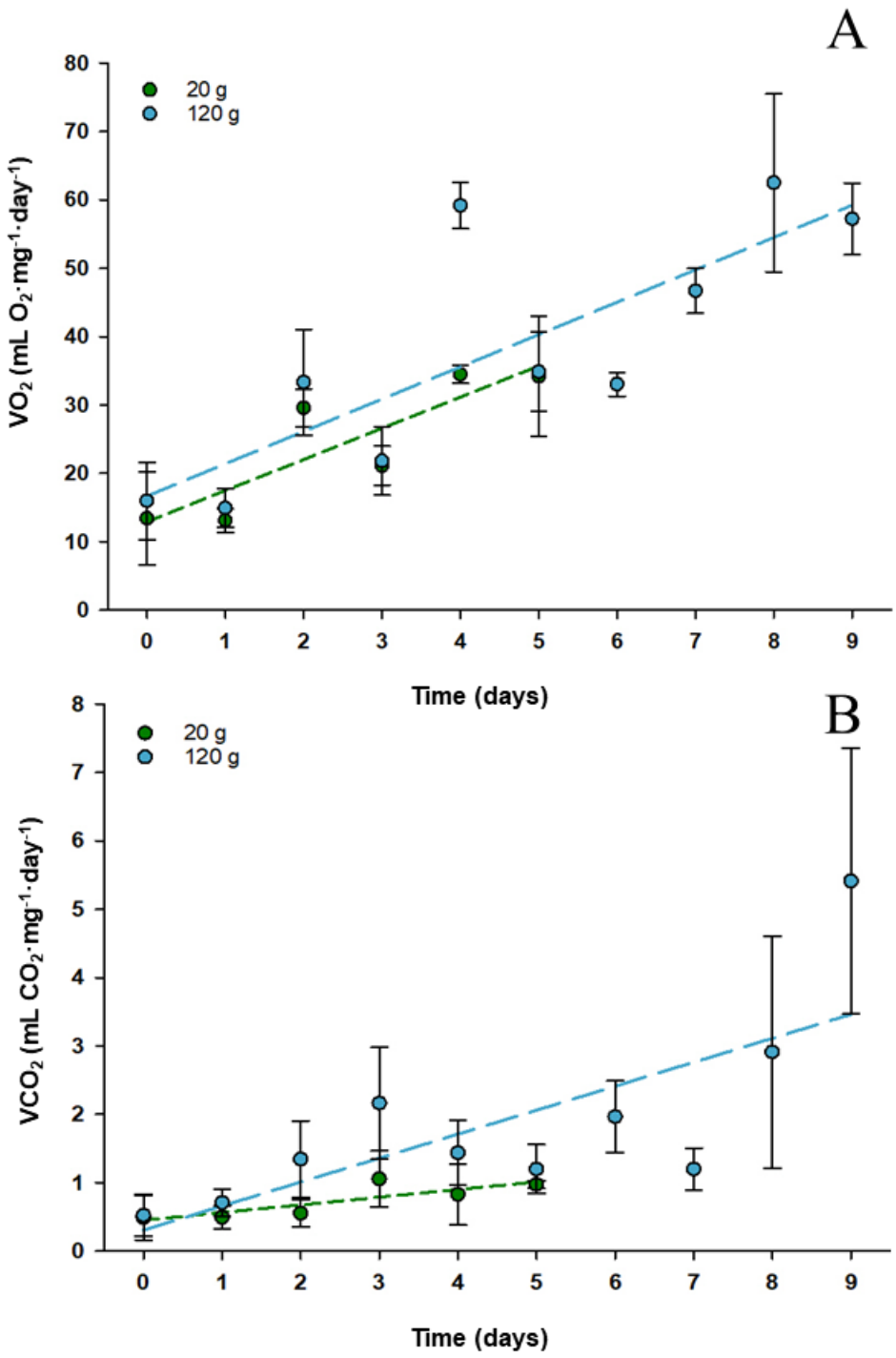


**Figure 4.3.** Metabolic Activity of Breeding Chamber

Measured metabolic activity of breeding chamber without beetles [calculated as $VO_2$ (A) and $VCO_2$ (B)], ± SE, for either 20 g or 120 g mouse carcass treatments.

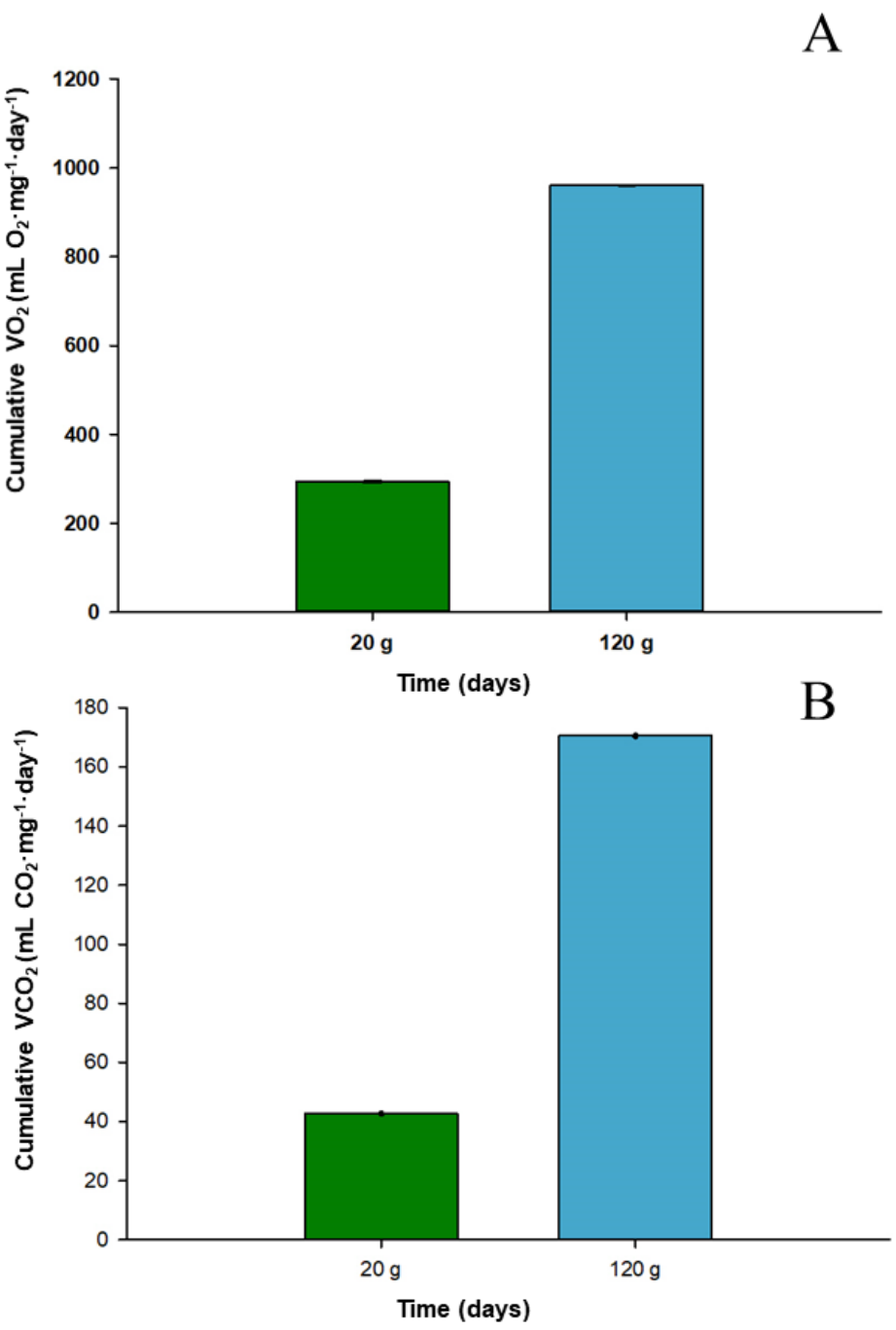


**Figure 4.4.** Cumulative Metabolic Rate of Carcass Preparation

Measured cumulative metabolic rate of carcass preparation [calculated as $VO_2$ (A) and $VCO_2$ (B)], ± SE, for breeding pairs of *N. orbicollis* on 20 g or 120 g mouse carcasses.

# CHAPTER 5. ESTIMATING POPULATION ABUNDANCE OF BURYING BEETLES USING PHOTO-IDENTIFICATION AND MARK-RECAPTURE METHODS

## 5.1 Abstract

Successful conservation and management of protected wildlife populations requires reliable population abundance data. Traditional capture-mark-recapture methods can be costly, time consuming, and invasive. Photographic mark-recapture (PMR) is a cost-effective, minimally invasive way to study population dynamics in species with distinct markings or color patterns. We tested the feasibility and the application of PMR using the software Hotspotter to identify *Nicrophorus* spp. from digital images of naturally occurring spot patterns on their elytra. We conducted a laboratory study evaluating the identification success of Hotspotter on *Nicrophorus americanus* Olivier and *Nicrophorus orbicollis* Say before implementation of a mark-recapture study *in situ*. We compared the performance of Hotspotter using both 'high-quality' and 'low-quality' photographs. For high-quality photographs, Hotspotter had a false rejection rate of 2.7-3.0 % for laboratory-reared individuals and 3.9 % for wild-caught individuals. For low-quality photographs, the false rejection rate was much higher, 48.8-53.3 % for laboratory-reared individuals and 28.3 % for wild-caught individuals. We subsequently analyzed encounter histories of wild-caught individuals with closed population models in Program MARK to estimate population abundance. In our study, we demonstrated the utility of using PMR in estimating population abundance for *Nicrophorus* spp. based on elytral spot patterns.

## 5.2 Introduction

Successful monitoring of wildlife populations requires reliable estimates of abundance, dispersal, and population demographics (Morris and Doak 2002, De Gasperis et al. 2017, Croose et al. 2019). It is often problematic to collect accurate data for wildlife populations, especially if they are cryptic, elusive, nocturnal, occur at low densities, or are species of conservation concern, and estimating abundance of these populations constitutes a management and conservation challenge in ecology and conservation biology (Wilson and Delahay 2001, Croose et al. 2019, Ruzzante et al. 2019). Evaluating animal population dynamics requires recognizing and following individual animals

through space and time (Bolger et al. 2012). Traditionally, individual recognition is accomplished by capturing animals and uniquely marking them (Sutherland 2006, Bolger et al. 2012, Morrison et al. 2016), which can be physically invasive, and can influence the behavior, development, or persistence of wildlife (McMahon et al. 2005, Scherer et al. 2005, Butler et al. 2012). Additionally, these methods do not always scale well to large populations or to populations with low densities and can be expensive (Elbin and Burger 1994, Crall et al. 2013, Recio et al. 2019).

Many direct and indirect methods are used to survey wildlife and estimate population abundances (Seber 1986, Wilson and Delahay 2001, Acevedo et al. 2010, Ruzzante et al. 2019). However, the most commonly used observational animal survey methods do not observe all individuals within a population (Pierce et al. 2012). Mark-recapture models are analytical methods used to estimate population abundance from known marked individuals within a population (Otis et al. 1978, White et al. 1982, White and Burnham 1999, summarized by Williams et al. 2002). With mark-recapture models, researchers use marked animals within a population to estimate detection probabilities that can then be used to estimate population abundance (White et al. 1982). Mark-recapture surveys must meet the assumptions that: 1) marked and unmarked individuals have the same probability of capture; 2) all marked individuals are identifiable (marks are not lost or overlooked by observers), 3) marks have no effect on the probability of survival or the behavior of marked individuals, 4) for closed population models, the population is closed to additions (immigration and birth) and deletions (emigration and death) between marking and recapture (Otis et al. 1978, White et al. 1982).

An alternative approach to traditional capture-based marking methods for mark-recapture studies is the application of digital photography and image analysis tools to develop photographic mark-recapture (PMR) techniques. PMR can be used in studies evaluating a wide variety of species with natural patterns or marks, reducing the need to physically mark individuals (Bolger et al. 2012, Crall et al. 2013, Morrison et al. 2016). Photographic identification is minimally invasive, can provide a variety of information (e.g., cameras provide data on locations, temperature, and diurnal and nocturnal activity patterns), is relatively inexpensive, and archives information on individuals for long-term monitoring of sensitive species (Morrison et al. 2011, 2016, Crall et al. 2013). Computer-assisted photo identification has been used to identify individuals of a diverse set of taxa, including mammals (Bolger et al. 2012, Crall et al. 2013, Gilman et al. 2016), amphibians (Bendik et al. 2013, Morrison et al. 2016, Kim et al. 2017), fish (Crall et al. 2013), birds (Burghardt

2008, Sherley et al. 2010), reptiles (Treilibs et al. 2016, Suriyamongkol and Mali 2018), and insects (Caci et al. 2013, De Gasperis et al. 2017). Conditions for PMR require that: 1) researchers capture photographs of individuals that are either free-ranging or live-captured, with remotely-triggered or hand-held cameras; 2) individuals have unique marks or patterns on their body that allow observers to differentiate among individuals; and 3) individual's patterns are stable over the length of the study period and are photographable across a range of environmental conditions (Bolger et al. 2012).

Early uses of PMR employed hard copy photographs that were manually compared by researchers to determine whether new images were individuals new to the study or resightings (recaptures) of previously 'marked' individuals (Friday et al. 2000, Bolger et al. 2012). This procedure was particularly successful for small populations of marine mammals and mammalian terrestrial predators (Rugh et al. 1992, Karanth and Nichols 1998, Friday et al. 2000). However, this is not always possible with large populations, and as the number of images increases successful identification decreases (Gamble et al. 2008, Bolger et al. 2012, Crall et al. 2013). There have been several attempts to use computers to semi-automate the matching process in studies evaluating large populations (Adams et al. 2006, Town et al. 2013). Recently, advances in systems with image analysis algorithms allow researchers to detect, store, and compare information on natural markings from digital images (Sherley et al. 2010, Bolger et al. 2012, Crall et al. 2013). These advanced systems (e.g., Wild-ID and HotSpotter) typically include: a database of acquired images, a landmark or pattern extraction method that reduces the amount of information from images, and an algorithm that compares the pattern information from new images to images from the photograph library and returns a score signifying the closeness of the match (Bolger et al. 2012, Crall et al. 2013). These software systems allow researchers to examine a reduced number of the potential matching images to reject false-positives or confirm true-positive matches, compile individuals into histories of encounters, and then analyze them using mark-recapture methods (Otis et al. 1978, White and Burnham 1999).

Using photographic mark-recapture methods can be useful in assessing population dynamics if they provide capture histories and can be evaluated in a cost-effective manner using mark-recapture modelling (Bolger et al. 2012). Additionally, many mark-recapture analytical methods frequently require large amounts of data to produce sufficient sample sizes, which is often cost prohibitive (McClintock and White 2009). Compared to traditional mark-recapture methods,

photographic mark-recapture allows for larger sample sizes when applied to species with natural markings unique to individuals (Bolger et al. 2012, Crall et al. 2013). Additionally, PMR reduces match or mismatch decisions that traditionally relied on the ability of an individual observer to differentiate between images (Morrison et al. 2011, 2016). Individual photographic identification software can result in higher recapture rates, and increase the power of demographic estimates and estimation of a greater number of population parameters because the processing of a greater number of images requires less effort than manual inspection of images (Morrison et al. 2016).

Using computer-assisted photo identification systems paired with PMR can result in fabundance (Yoshizaki et al. 2009, Marshall and Pierce 2012). If markings vary temporally or are relatively constant across individuals, misidentification errors are possible, and mark-recapture assumptions are violated (Morrison et al. 2011). In addition, the inability of PMR to correctly match multiple photographs for the same individual makes it vulnerable to errors. Common in biometric recognition, this "false reject error" (FRR), is a type I error (Jain 2007). Even moderate FRR levels may result in an overestimate of abundance leading to an underestimate of survival within a mark-recapture framework. Additionally, if an individual's "unique" pattern changes over time, false acceptance errors (i.e., falsely matching photographs of two different individuals) can increase, leading to an underestimate of abundance (Yoshizaki et al. 2009). Therefore, determining misidentification error is a necessary when evaluating success of identification software (Bendik et al. 2013, Morrison et al. 2016, De Gasperis et al. 2017).

Our study used beetles from the genus *Nicrophorus* (Coleoptera: Silphidae: Nicrophorinae) to evaluate whether machine-learning individual-identification software can be used in combination with photographic mark-recapture to estimate population abundance *in situ*. Beetles from the genus *Nicrophorus* are fossorial species that bury and prepare small vertebrate carcasses for use as food by their young (Lomolino et al. 1995, Creighton 2005). The American burying beetle (*Nicrophorus americanus* Olivier) once occurred in 35 states, the District of Columbia, and three Canadian provinces in eastern and central North America (Backlund et al. 2008). It has disappeared from most of its former range, and has since been listed as Federally Endangered (Federal Register 1989). The U.S. Fish and Wildlife Service (USFWS) established a recovery plan with three criteria to delist the species including: 1) the discovery of existing populations or successful re-introduction of ABBs throughout the historical range, 2) that for a minimum of five

years these populations be self-supporting, and 3) that a minimum of 500 adults are present in these populations each year (USFWS 1991).

In order to accurately estimate population abundance among extant and reintroduced populations, researchers need a method of trapping and marking individual beetles that is retained by the individual and does not influence behavior or reduce survivorship (Butler et al. 2012). Several marking techniques are used in burying beetle demographic studies including temporary marks (enamel paint or bee tags) and permanent marks (elytron-clipping or elytron-cauterizing; Kozol et al. 1988, Lomolino and Creighton 1996, Butler et al. 2012). For temporary marks, average retention rates ranged from seven to 12.8 days (Butler et al. 2012), which could bias population estimate results if study timelines are greater than mark retention, which would then violate the assumptions for using mark-recapture. It is recommended to use a combination of temporary and permanent marks for burying beetle demographic studies. However, permanent marking techniques (i.e., elytron-clipping) change stridulatory characteristics and reduce reproductive success (Hall et al. 2015). These findings highlight the need for a replacement marking technique that is non-invasive to ensure conservation of this endangered species.

Our study used laboratory-reared and wild-caught individuals to investigate the efficacy of using elytra spot patterns as a non-invasive mark to individually identify both *N. americanus* and *Nicrophorus orbicollis* Say to potentially incorporate a machine-learning individual identification software (Hotspotter; Crall et al. 2013) into reintroduction programs and long-term population monitoring. Our goals were to 1) evaluate the advantages and limitations of using machine-learning individual identification software in *Nicrophorus* spp.; 2) determine the accuracy of automated photo matching using both "high" and "low-quality" photographs; and 3) confirm field applications by using PMR in coordination with mark-recapture modeling to estimate the population size of local burying beetles.

## 5.3 Materials and Methods

### 5.3.1 Laboratory Population Data Collection

We photographed individuals of known identity from three captive breeding populations at Purdue University in West Lafayette Indiana, Purdue University Northwest in Hammond Indiana, and Roger Williams Park Zoo in Providence Rhode Island during the summer of 2018 and 2019.

Photographs from Purdue University included 40 individual *N. orbicollis*. Photographs from Purdue University Northwest and Roger Williams Park Zoo included 35 individual *N. americanus* (n = 20 and n = 15 respectively). Because beetles were part of laboratory populations we assured that all beetles were unique (e.g., a single container housed an individual beetle of known identification). To standardize the distance and resolution of all images, we photographed each beetle from a standard distance of 12 cm. We captured multiple photographs (minimum of 5 per individual) using a camera phone (Samsung Galaxy S5, Samsung Electronics, Suwon, South Korea) of the dorsal region of each individual. Pictures were taken without the flash setting to reduce glare and while the beetle was in hand to minimize movement. We used these photographs to build a reference database in HotSpotter to test the accuracy and efficiency in identifying individual beetles. Before conducting analyses, we standardized the orientation and extent of images. We rotated images to orient the anterior region of the beetle to the right. We included both low-quality photos (ones with glare and lower resolution) and high-quality photos (ones with less glare and high resolution) to test the ability of HotSpotter to identify correctly individual beetles (Fig. 1). For *N. orbicollis*, we restricted images to include the dorsal region, from the anterior of the elytra to the posterior of the elytra, to focus on the spot patterns of beetles (Fig. 1). For *N. americanus*, we restricted images to include the dorsal region, from the anterior of the pronotum to the posterior of the elytra, to include the spot pattern that is present in *N. americanus* but absent in other *Nicrophorus* species (Fig. 1).

### 5.3.2 Performance of HotSpotter Software

We evaluated the ability of a free and open-source wildlife identification software package: HotSpotter (ver. 1.0; Crall et al. 2013) to individually identify *Nicrophorus* beetles. HotSpotter has successfully identified unique patterns in zebras (*Equus grevyi*, *E. quagga*, and *E. zebra zebra*; Lea et al. 2018), giraffes (*Giraffa camelopardalis*), jaguars (*Panthera onca*), lionfish (*Pterois volitans*; Crall et al. 2013), *Nautilus* spp. (Barord et al. 2014), and leopards (*Panthera pardus*; Balme et al. 2019). On average HotSpotter correctly identifies approximately 98% of individuals among the first five identities proposed by the software (Crall et al. 2013), outperforming another open-source wildlife identification software (Wild-ID) developed by Bolger et al. (2012). HotSpotter uses viewpoint invariant descriptors and a scoring mechanism that emphasizes the most distinctive key points and descriptors. First, HotSpotter uses an algorithm that employs a scale

invariant feature transformation (SIFT) operator, similar to the algorithm used by Wild-ID's SIFT (Lowe 2004). This algorithm identifies "key points" or "landmarks" within the patterns of photographed individuals in all photographs in the database that are invariant to rotation and scale. Second, HotSpotter uses an additional algorithm that relates all pattern descriptors from individuals (not photos) in the database (called the "local naïve Bayes nearest-neighbor" algorithm). HotSpotter's final scoring uses a combination of both algorithms and provides similarity scores that are rankable, with higher scores indicating more likely matches.

Because the individual identities of beetles were already known within our database, we followed the protocol used by (Morrison et al. 2016) and assumed HotSpotter correctly identified matching pairs if they were within the top 20 highest-ranking candidate matches. Similarly, if matching pairs were outside of the top 20 high-scoring candidate matches, we regarded them as false rejections and reported error rates in terms of the FRR (Jain 2007, Bolger et al. 2012, Bendik et al. 2013, Morrison et al. 2016).

Our test data set contained many pictures of individuals with multiple matching images, and these would be over-represented in the estimate of misidentification probability. Therefore, we calculated misidentification from a subset of two randomly selected images per individual, and repeated this random process 1000 times. We reported the mean misidentification probability across all iterations for the entire data set (Bendik et al. 2013, Morrison et al. 2016). We performed all statistical computations in program R (Version 3.6.1, Core Team 2019).

### 5.3.3 Field Site

We performed all fieldwork at one site within a section of bottomland forest located at Purdue University's Martell Forest, Tippecanoe County, Indiana (Lat. 40.455°N, Long. -86.925°W) in July of 2019. The study site is predominately a mixed hardwood forest dominated by tree genera including oak (*Quercus* spp.), hickory (*Carya* spp.), and maple (*Acer* spp.). We selected the time and site of trapping based on the reproductive period of *N. orbicollis* and their habitat preference (Scott 1998).

### 5.3.4 Population Size Estimates from the MARK Closed Population Estimator

We collected beetles from 16–19 July 2019 using a single transect of five pitfall traps spaced 10 m apart and baited with aged chicken. We marked individual wild-caught beetles with oil-based paint markers applied to the pronotum. To ensure that wild-caught beetles would not lose marks, we tested how long laboratory beetles retained them. On average, laboratory beetles retained marks for 4.88 days ± 1.03 SD, a timeframe beyond our study design. We released beetles back into the study site daily within 2 hr of collection after marking each individual with a unique color pattern and capturing multiple pictures. We used Program MARK, version 9.x (White and Burnham 1999) for estimating population abundance for individuals collected on all three nights. We used the MARK closed population estimator to estimate population abundance from non-invasive photographic data (Bolger et al. 2012). We analyzed data as conventional mark-release-recapture (MRR; Hagler and Jackson 2001) data using the closed-capture models of MARK (White and Burnham 1999). We considered four models in our analysis [$M_h$ (heterogeneous capture probability), $M_t$ (capture probability varies with time), $M_b$ (capture probability varies with animal behavior), and $M_o$ (constant capture probability); Table 2)]. The estimate from the best approximating model of the candidate set was the most parsimonious, minimized estimate bias, and optimized precision based on the lowest Akaike's Information Criterion corrected for small sample size ($AIC_c$; Burnham et al. 2011).

## 5.4 Results

### 5.4.1 Performance of HotSpotter Software

Spot patterns for adult *Nicrophorus* beetles were uniquely identifiable and persisted through adulthood (Fig. 1). For laboratory and wild-caught populations, HotSpotter produced high matching success (reported as mean misidentification probability), particularly in the high-quality datasets (Table 1). The overall FRR for all photographs of laboratory-reared *N. americanus* was 16.0%. The quality of photographs influenced the FRR for laboratory-reared *N. americanus* with low-quality photographs resulting in an FRR of 53.3% and high-quality photographs resulting in an FRR of 3.0% (Table 1). The overall FRR for all photographs of laboratory-reared *N. orbicollis* was 17.4%. Similar to laboratory-reared *N. americanus*, the quality of photographs influenced the

FRR for laboratory-reared *N. orbicollis*. Low-quality photographs resulted in an FRR of 48.8% and high-quality photographs resulted in an FRR of 2.7% (Table 1).

The overall FRR for all photographs of wild-caught *N. orbicollis* was 2.6%, and 11.1% for photographs of recaptured individuals (Table 1). Similar to photographs of laboratory-reared beetles, the quality of photographs influenced the FRR of wild-caught beetles. Low-quality photographs resulted in an FRR of 28.3%, whereas high-quality photographs resulted in an FRR of 3.9% in all wild-caught individuals (Table 1).

### 5.4.2 Population Size Estimates from the MARK Closed Population Estimator

For mark-recapture analysis, we collected 518 suitable images from *N. orbicollis* at Martell Forest. After processing with HotSpotter, these images represented 98 unique individuals. We used the encounter histories of all 98 individuals for mark-recapture analysis.

The top-ranked model in our dataset, as indicated by the lowest $AIC_c$, was the $M_o$ model that accounted for constant capture and recapture probabilities (Table 2). To account for model selection uncertainty, we computed model averaged parameter estimates (Table 3; Burnham and Anderson 2002). The population abundance estimated for the top-ranked model was 343 individuals (± 89 SE; Table 4).

## 5.5 Discussion

### 5.5.1 Performance of HotSpotter Software

The use of machine-learning software for photographic-identification in *Nicrophorus* spp. has several advantages over traditional marking approaches. Compared to traditional mark-recapture techniques, computer-assisted machine-learning PMR is less invasive, it requires less equipment and therefore is less expensive, it is relatively rapid, and little experience is required to produce quality photographs (Bendik et al. 2013). As demonstrated by our study, elytron spot patterns are individually unique in burying beetles. Furthermore, spot patterns provide a appropriate basis for beetle identification, while having less potential for animal stress or negative survival effects (McMahon et al. 2005, Scherer et al. 2005, Hall et al. 2015). False rejection rates increased when using score-based image matching with low-quality photographs (Table 1). Poor photo quality and visual inspection for more image pairs increases the need to manually inspect images and the

benefits of individual identification using semi-automation via score-sorting decreases (Bendik et al. 2013). However, with high-quality photographs, the machine-learning identification PMR scheme using Hotspotter displayed high success for identifying individual burying beetles with greater precision than traditional methods (Table 1; Butler et al. 2012). Compared to other studies, our error rates, FRR and mean misidentification probability, were relatively low when using high-quality photographs versus low-quality photographs (Gope et al. 2005, Foster et al. 2007, Bendik et al. 2013, Morrison et al. 2016). In these studies, FRR ranged from 0.0076–0.675, whereas our FRR ranged from 0.027–0.039. If patterns can be localized and described by computer-assisted identification software, then modifications to the SIFT algorithm and local naïve Bayes Nearest-Neighbor algorithm in Hotspotter may reduce error rates (Morrison et al. 2016). Given the low FRR we observed with Hotspotter, we did not test other software platforms. However, the relatively high matching successes obtained with the Hotspotter software in our study may not hold for other types of spot patterns in different species (Bolger et al. 2012, Morrison et al. 2016).

Similar to Bendik et al. (2013), our results suggested that computer-assisted individual photo identification in which matches are distinguished based on a relative score is appropriate for data sets with high-quality and large quantities of photographs. Although computer-assisted individual-identification has promise to save time compared to manual photo matching (Morrison et al. 2011), it can still be a labor-intensive process for studies that include large libraries of photographs (Morrison et al. 2016). Furthermore, PMR using Hotspotter has the potential to obtain estimates of population abundance rapidly (over several days of surveying), because it does not require extensive training or expensive equipment (Bendik et al. 2013, Crall et al. 2013). In our study, Hotspotter demonstrated utility in estimating demographic parameters of *Nicrophorus* populations.

Furthermore, as demonstrated by our results and previous PMR studies (Stevick et al. 2001, Davies et al. 2012, Morrison et al. 2016), researchers need to assure that they are collecting high-quality photographs because image quality can significantly influence matching success. Several studies and reviews have focused on camera features that ensure high quality photographs for wildlife studies (Meek and Pittet 2012, Rovero et al. 2013, Meek et al. 2014, Trolliet et al. 2014). For *Nicrophorus* spp. we recommend researchers use a camera with ≥ 16 megapixel resolution, capture pictures of beetles in hand to reduce movement that results in blurry imagining, photograph

beetles from a standardized distance of 12 cm, and reduce image glare by using natural lighting instead of the camera's flash.

### 5.5.2 Population Size Estimates from the MARK Closed Population Estimator

Traditionally, mark-recapture studies of burying beetles rely on a combination of temporary and permanent marks (Lomolino and Creighton 1996, Bedick et al. 2006, Raithel et al. 2006, Backlund et al. 2008). Our study incorporated PMR with MARK to estimate the abundance of *N. orbicollis* at our sampling site. Recapture rates and standard errors were reasonable compared to other mark-recapture studies with insects (López-Pantoja et al. 2008, Torres-Vila et al. 2012, De Gasperis et al. 2016), despite the rather limited sampling effort in this preliminary study (our sampling occurred over only three days). Despite this limitation, we produced data on the capture history of 98 individual beetles and produced estimates of abundance that are problematic to acquire in a rare or evasive species (Karanth and Nichols 1998, Karanth et al. 2006).

Our capture-history data fit best with the closed capture-recapture null model ($M_o$; Table 2). Our models' estimates of beetle abundance have relatively narrow variances and averaged 269 individuals (Table 4). The estimates are not inclusive of the entire population of *N. orbicollis* at our study site, but rather represent the total number of beetles present within the estimated capture distance (0.8 km; Perrotti and Mckenna-Foster 2019). This noninvasive and efficient sampling approach is appropriate to use in studies evaluating burying beetle population dynamics across populations, but needs testing over a longer sampling period that mirrors ongoing species monitoring efforts (Backlund et al. 2008, Perrotti and Mckenna-Foster 2019). Furthermore, incorporating mark-recapture techniques into studies estimating demographic factors such as survival requires multiple trapping sessions (Borchers and Efford 2008, Fletcher and Efford 2009). For our study, as well as previous studies that employed temporary marks in *Nicrophorus* beetles (Kozol et al. 1988, Lomolino and Creighton 1996, Butler et al. 2012), average temporary mark retention was between five and 12 days. Under these conditions, it would be difficult to design a mark-recapture experiment that spans multiple trapping sessions, however PMR using permanent markings facilitates a study design that covers multiple trapping sessions and allows researchers to estimate demographic factors (Morrison et al. 2011, Bolger et al. 2012). Furthermore, it is

critical that researchers consider these PMR specific limitations and exercise caution when using PMR to ensure that mark-recapture assumptions are not violated.

### 5.5.3 Conservation Implications

A fast, accurate, inexpensive, and comparatively non-invasive method for monitoring/tracking endangered or threatened species is necessary for conservation and species management because it provides efficient and reliable population estimates for monitoring species (Jackson et al. 2006, Bendik et al. 2013, Crall et al. 2013, Morrison et al. 2016). In burying beetles, there are many studies focused on the understanding of population dynamics and dispersal (Lomolino et al. 1995, Lomolino and Creighton 1996, Raithel et al. 2006, Backlund et al. 2008, Schnell et al. 2008), but few with reintroduced populations (Mckenna-Foster et al. 2016, Perrotti and Mckenna-Foster 2019). More demographic studies among both extant and reintroduced populations are needed to meet USFWS recovery goals. Our results demonstrate that it is possible to identify individual beetles and obtain estimates of population abundance in a system where traditional mark-recapture techniques may bias results or be detrimental to fitness or survival of individuals within the population (Butler et al. 2012, Hall et al. 2015). However, when monitoring population trends, there are several considerations (Tikkamäki and Komonen 2011). The location of release sites for beetles and weather conditions influences daily variation in trapping success and flight behavior (Raithel et al. 2006). Additionally, long-term population dynamics of burying beetles are poorly understood (Lomolino et al. 1995, Raithel et al. 2006, Schnell et al. 2008, Perrotti and Mckenna-Foster 2019), and thus PMR should be conducted over many years and at different field sites. The advantages of computer-assisted photographic-identification over traditional methods, as demonstrated here, may provide researchers with much needed population abundance information for burying beetles that will ultimately help guide conservation efforts and sound management practices.

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## 5.7 References

Acevedo, P., Ferreres, J., Jaroso, R., Durán, M., Escudero, M.A., Marco, J., *et al.* (2010) Estimating roe deer abundance from pellet group counts in Spain: An assessment of methods suitable for Mediterranean woodlands. *Ecological Indicators*, **10**, 1226–1230.

Adams, J.D., Speakman, T., Zolman, E. & Schwacke, L.H. (2006) Automating image matching, cataloging, and analysis for photo-identification research. *Aquatic Mammals*, **32**, 374–384.

Backlund, D.C., Marrone, G.M., Williams, C.K. & Tilmon, K. (2008) Population estimate of the endangered American burying beetle, *Nicrophorus americanus* Olivier (Coleoptera: Silphidae) in South Dakota. *The Coleopterists Bulletin*, **62**, 9–15.

Balme, G., Rogan, M., Thomas, L., Pitman, R., Mann, G., Whittington-Jones, G., *et al.* (2019) Big cats at large: Density, structure, and spatio-temporal patterns of a leopard population free of anthropogenic mortality. *Population Ecology*, **61**, 256–267.

Barord, G.J., Dooley, F., Dunstan, A., Ilano, A., Keister, K.N., Neumeister, H., *et al.* (2014) Comparative population assessments of Nautilus sp. in the Philippines, Australia, Fiji, and American Samoa using baited remote underwater video systems. *PLoS ONE*, **9**, e100799.

Bedick, J.C., Hoback, W.W. & Albrecht, M.C. (2006) High water-loss rates and rapid dehydration in the burying beetle, *Nicrophorus marginatus*. *Physiological Entomology*, **31**, 23–29.

Bendik, N.F., Morrison, T.A., Gluesenkamp, A.G., Sanders, M.S. & O'Donnell, L.J. (2013) Computer-assisted photo identification outperforms visible implant elastomers in an endangered salamander, *Eurycea tonkawae*. *PLoS ONE*, **8**, e59424.

Bolger, D.T., Morrison, T.A., Vance, B., Lee, D. & Farid, H. (2012) A computer-assisted system for photographic mark–recapture analysis. *Methods in Ecology and Evolution*, **3**, 813–822.

Borchers, D.L. & Efford, M.G. (2008) Spatially explicit maximum likelihood methods for capture–recapture studies. *Biometrics*, **64**, 377–385.

Burghardt, T. (2008) *Visual animal biometrics automatic detection and individual identification by coat pattern* (Doctor of Philosophy).

Burnham, K.P. & Anderson, D.R. (2002) *Model selection and multimodel inference: a practical information-theoretic approach*. 2nd edn. Springer-Verlag, New York.

Burnham, K.P., Anderson, D.R. & Huyvaert, K.P. (2011) AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral Ecology and Sociobiology*, **65**, 23–35.

Butler, S.R., Jurzenski, J. & Hoback, W.W. (2012) Evaluation of marking techniques, mark retention, and mortality due to marking of burying beetles (Coleoptera: Silphidae). *The Coleopterists Bulletin*, **66**, 149–154.

Caci, G., Biscaccianti, A.B., Cistrone, L., Bosso, L., Garonna, A.P. & Russo, D. (2013) Spotting the right spot: computer-aided individual identification of the threatened cerambycid beetle *Rosalia alpina*. *Journal of Insect Conservation*, **17**, 787–795.

Crall, J.P., Stewart, C.V., Berger-Wolf, T.Y., Rubenstein, D.I. & Sundaresan, S.R. (2013) HotSpotter — Patterned species instance recognition. Presented at the 2013 IEEE Workshop on Applications of Computer Vision (WACV), pp. 230–237.

Creighton, J.C. (2005) Population density, body size, and phenotypic plasticity of brood size in a burying beetle. *Behavioral Ecology*, **16**, 1031–1036.

Croose, E., Birks, J.D.S., Martin, J., Ventress, G., MacPherson, J. & O'Reilly, C. (2019) Comparing the efficacy and cost-effectiveness of sampling methods for estimating population abundance and density of a recovering carnivore: the European pine marten (*Martes martes*). *European Journal of Wildlife Research*, **65**, 37.

Davies, T.K., Stevens, G., Meekan, M.G., Struve, J. & Rowcliffe, J.M. (2012) Can citizen science monitor whale-shark aggregations? Investigating bias in mark–recapture modelling using identification photographs sourced from the public. *Wildlife Research*, **39**, 696.

De Gasperis, S.R., Carpaneto, G.M., Nigro, G., Antonini, G., Chiari, S., Cini, A., *et al.* (2017) Computer-aided photographic identification of *Rosalia alpina* (Coleoptera:

Cerambycidae) applied to a mark-recapture study. *Insect Conservation and Diversity*, **10**, 54–63.

De Gasperis, S.R., Passacantilli, C., Redolfi De Zan, L. & Carpaneto, G.M. (2016) Overwintering ability and habitat preference of *Morimus asper*: a two-year mark-recapture study with implications for conservation and forest management. *Journal of Insect Conservation*, **20**, 821–835.

Elbin, S.B. & Burger, J. (1994) In my experience: implantable microchips for individual identification in wild and captive populations. *Wildlife Society Bulletin*, **22**, 677–683.

Federal Register. (1989) *Endangered and threatened wildlife and plants: determination of endangered status for the American burying beetle. Federal Register 50, CFR Part 17, 54, 133, 29652-29655, July 13, 1989.*

Fletcher, D. & Efford, M.G. (2009) Effect of senescence on estimation of survival probability when age is unknown. In *Modeling Demographic Processes In Marked Populations*. Springer US, Boston, MA, pp. 1037–1053.

Foster, G., Krijger, H. & Bangay, S. (2007) Zebra fingerprints: towards a computer-aided identification system for individual zebra. *African Journal of Ecology*, **45**, 225–227.

Friday, N., Smith, T.D., Stevick, P.T. & Allen, J. (2000) Measurement of photographic quality and individual distinctiveness for the photographic identification of humpback whales, *Megaptera novaeangliae*. *Marine Mammal Science*, **16**, 355–374.

Gamble, L., Ravela, S. & McGarigal, K. (2008) Multi-scale features for identifying individuals in large biological databases: an application of pattern recognition technology to the marbled salamander *Ambystoma opacum*. *Journal of Applied Ecology*, **45**, 170–180.

Gilman, A., Hupman, K., Stockin, K.A. & Pawley, M.D.M. (2016) Computer-assisted recognition of dolphin individuals using dorsal fin pigmentations. In *2016 International Conference on Image and Vision Computing New Zealand (IVCNZ)*. pp. 1–6.

Gope, C., Kehtarnavaz, N., Hillman, G. & Würsig, B. (2005) An affine invariant curve matching method for photo-identification of marine mammals. *Pattern Recognition*, **38**, 125–132.

Hagler, J.R. & Jackson, C.G. (2001) Methods for marking insects: current techniques and future prospects. *Annual Review of Entomology*, **46**, 511–543.

Hall, C.L., Howard, D.R., Smith, R.J. & Mason, A.C. (2015) Marking by elytral clip changes stridulatory characteristics and reduces reproduction in the American burying beetle, *Nicrophorus americanus*. *Journal of Insect Conservation*, **19**, 155–162.

Jackson, R.M., Roe, J.D., Wangchuk, R. & Hunter, D.O. (2006) Estimating snow leopard population abundance using photography and capture-recapture techniques. *Wildlife Society Bulletin*, **34**, 772–781.

Jain, A.K. (2007) Biometeric recognition. *Nature*, **449**, 38–40.

Karanth, K.U. & Nichols, J.D. (1998) Estimation of tiger densities in India using photographic captures and recaptures. *Ecology*, **79**, 2852–2862.

Karanth, K.U., Nichols, J.D., Kumar, N.S. & Hines, J.E. (2006) Assessing tiger population dynamics using photographic capture-recapture sampling. *Ecology*, **87**, 2925–2937.

Kim, M.Y., Borzée, A., Kim, J.Y. & Jang, Y. (2017) Treefrog lateral line as a mean of individual identification through visual and software assisted methodologies. *Journal of Ecology and Environment*, **41**, 42.

Kozol, A.J., Scott, M.P. & Traniello, J.F.A. (1988) The American burying beetle, *Nicrophorus americanus*: studies on the natural history of a declining species. *Psyche: A Journal of Entomology*, **95**, 167–176.

Lomolino, M.V. & Creighton, J.C. (1996) Habitat selection, breeding success and conservation of the endangered American burying beetle *Nicrophorus americanus*. *Biological Conservation*, **77**, 235–241.

Lomolino, M.V., Creighton, J.C., Schnell, G.D. & Certain, D.L. (1995) Ecology and conservation of the endangered American burying beetle (*Nicrophorus americanus*). *Conservation Biology*, **9**, 605–614.

López-Pantoja, G., Nevado, L. & Sánchez-Osorio, I. (2008) Mark-recapture estimates of the survival and recapture rates of *Cerambyx welensii* Küster (Coleoptera cerambycidae) in a cork oak dehesa in Huelva (Spain). *Open Life Sciences*, **3**, 431–441.

Lowe, D.G. (2004) Distinctive image features from scale-invariant keypoints. *International Journal of Computer Vision*, **60**, 91–110.

Marshall, A.D. & Pierce, S.J. (2012) The use and abuse of photographic identification in sharks and rays. *Journal of Fish Biology*, **80**, 1361–1379.

McClintock, B.T. & White, G.C. (2009) A less field-intensive robust design for estimating demographic parameters with mark–resight data. *Ecology*, **90**, 313–320.

Mckenna-Foster, A., Perrotti, L., Blyth, J., LoPresti, E. & Kennedy, R.S. (2016) Measuring success of a reintroduced population of the American burying beetle (*Nicrophorus americanus* Olivier) to Nantucket Island, MA. *Journal of Insect Conservation*, **20**, 895–904.

McMahon, C., Hoff, J. van der & Burton, H. (2005) Handling intensity and the short- and long-term survival of elephant seals: addressing and quantifying research effects on wild animals. *Ambio*, **34**, 426–429.

Meek, P.D., Ballard, G., Claridge, A., Kays, R., Moseby, K., O'Brien, T., *et al.* (2014) Recommended guiding principles for reporting on camera trapping research. *Biodiversity and Conservation*, **23**, 2321–2343.

Meek, P.D. & Pittet, A. (2012) User-based design specifications for the ultimate camera trap for wildlife research. *Wildlife Research*, **39**, 649.

Morris, W.F. & Doak, D.F. (2002) *Quantitative conservation biology: theory and practice of population viability analysis*. Sinauer, Sunderland, Massachusetts.

Morrison, T.A., Keinath, D., Estes-Zumpf, W., Crall, J.P. & Stewart, C.V. (2016) Individual identification of the endangered Wyoming toad *Anaxyrus baxteri* and implications for monitoring species recovery. *Journal of Herpetology*, **50**, 44–49.

Morrison, T.A., Yoshizaki, J., Nichols, J.D. & Bolger, D.T. (2011) Estimating survival in photographic capture–recapture studies: overcoming misidentification error. *Methods in Ecology and Evolution*, **2**, 454–463.

Otis, D.L., Burnham, K.P., White, G.C. & Anderson, D.R. (1978) Statistical inference from capture data on closed animal populations. *Wildlife Monographs*, **62**, 3–135.

Perrotti, L. & Mckenna-Foster, A. (2019) A report on the 25 year effort to establish the American burying beetle (*Nicrophours americanus* Olivier) to Nantucket Island, Massachusetts. Presented at the Entomology, St. Louis, MO.

Pierce, B.L., Lopez, R.R. & Silvy, N.J. (2012) Estimating animal abundance. In *The Wildlife Techniques Manual: Research*. The Johns Hopkins University Press, Baltimore, pp. 84–310.

Raithel, C.J., Ginsberg, H.S. & Prospero, M.L. (2006) Population trends and flight behavior of the American burying beetle, *Nicrophours americanus* (Coleoptera: Silphidae), on Block Island, RI. *Journal of Insect Conservation*, **10**, 317–322.

Recio, P., Rodríguez-Ruiz, G., Ortega, J. & Martín, J. (2019) PIT-Tags as a technique for marking fossorial reptiles: insights from a long-term field study of the amphisbaenian *Trogonophis wiegmanni*. *Acta Herpetologica*, **14**, 101–107.

Rovero, F., Zimmermann, F., Bersi, D. & Meek, P. (2013) "Which camera trap type and how many do I need?" A review of camera features and study designs for a range of wildlife research applications. *Hystrix, the Italian Journal of Mammology*, **24**, 148–156.

Rugh, D.J., Braham, H.W. & Miller, G.W. (1992) Methods for photographic identification of bowhead whales, *Balaena mysticetus*. *Canadian Journal of Zoology*, **70**, 617–624.

Ruzzante, D.E., McCracken, G.R., Førland, B., MacMillan, J., Notte, D., Buhariwalla, C., *et al.* (2019) Validation of close-kin mark–recapture (CKMR) methods for estimating population abundance. *Methods in Ecology and Evolution*, **10**, 1445–1453.

Scherer, R.D., Muths, E., Noon, B.R. & Corn, P.S. (2005) An evaluation of weather and disease as causes of decline in two populations of boreal toads. *Ecological Applications*, **15**, 2150–2160.

Schnell, G.D., Hiott, A.E., Creighton, J.C., Smyth, V.L. & Komendat, A. (2008) Factors affecting overwinter survival of the American burying beetle, *Nicrophorus americanus* (Coleoptera: Silphidae). *Journal of Insect Conservation*, **12**, 483–492.

Seber, G.A.F. (1986) A review of estimating animal abundance. *Biometrics*, **42**, 267.

Sherley, R., Burghardt, T., Barham, P., Campbell, N. & Cuthill, I. (2010) Spotting the difference: towards fully-automated population monitoring of African penguins *Spheniscus demersus*. *Endangered Species Research*, **11**, 101–111.

Stevick, P.T., Palsbøll, P.J., Smith, T.D., Bravington, M.V. & Hammond, P.S. (2001) Errors in identification using natural markings: rates, sources, and effects on capture recapture estimates of abundance. *Canadian Journal of Fisheries and Aquatic Sciences*, **58**, 1861–1870.

Suriyamongkol, T. & Mali, I. (2018) Feasibility of using computer-assisted software for recognizing individual Rio Grande cooter (*Pseudemys gorzugi*). *Copeia*, **106**, 646–651.

Sutherland, W.J. (2006) *Ecological census techniques: a handbook*. Second Edition. Cambridge University Press.

Tikkamäki, T. & Komonen, A. (2011) Estimating population characteristics of two saproxylic beetles: a mark-recapture approach. *Journal of Insect Conservation*, **15**, 401–408.

Torres-Vila, L.M., Sanchez-González, Á., Ponce-Escudero, F., Martín-Vertedor, D. & Ferrero-García, J.J. (2012) Assessing mass trapping efficiency and population density of *Cerambyx welensii* Küster by mark-recapture in dehesa open woodlands. *European Journal of Forest Research*, **131**, 1103–1116.

Town, C., Marshall, A. & Sethasathien, N. (2013) Manta Matcher: automated photographic identification of manta rays using keypoint features. *Ecology and Evolution*, **3**, 1902–1914.

Treilibs, C.E., Pavey, C.R., Hutchinson, M.N. & Bull, C.M. (2016) Photographic identification of individuals of a free-ranging, small terrestrial vertebrate. *Ecology and Evolution*, **6**, 800–809.

Trolliet, F., Huynen, M.-C., Vermeulen, C. & Hambuckers, A. (2014) Use of camera traps for wildlife studies. a review. *Biotechnol. Agron. Soc. Environ.*, **18**, 446–445.

White, G.C., Anderson, D.R., Burnham, K.P. & Otis, D.L. (1982) *Capture-recapture and removal methods for sampling closed populations* (LA-8787-NERP). Los Alamos National Laboratory, Los Alamos, NM.

White, G.C. & Burnham, K.P. (1999) Program MARK: survival estimation from populations of marked animals. *Bird Study*, **46**, S120–S139.

Williams, B.K., Nichols, J.D. & Conroy, M.J. (2002) *Analysis and management of animal populations*. Academic Press, San Diego, California, USA.

Wilson, G.J. & Delahay, R.J. (2001) A review of methods to estimate the abundance of terrestrial carnivores using field signs and observation. *Wildlife Research*, **28**, 151–164.

Yoshizaki, J., Pollock, K.H., Brownie, C. & Webster, R.A. (2009) Modeling misidentification errors in capture–recapture studies using photographic identification of evolving marks. *Ecology*, **90**, 3–9.

**Table 5.1.** HotSpotter Performance

False rejection rates (i.e., the frequency that two photos of the same individual were falsely identified) of laboratory-reared *Nicrophorus americanus* and *N. orbicollis* and wild-caught *N. orbicollis* using Hotspotter. Higher FRR and values imply poorer matching performance. We included multiple images of each individual including low-quality photos (ones with glare and lower resolution) and high-quality photos (ones with less glare and high resolution) in our dataset. Additionally, we randomly selected two images per individual and calculated a misidentification probability from this subset of images (Bendik et al. 2013; Morrison et al. 2016). We repeated this random process 1000 times and reported the mean misidentification probability across all iterations for the entire data set.

| Category | Sample size (Number of photos) | False rejection rate (FRR) | Mean misidentification probability |
|---|---|---|---|
| Laboratory-reared | | | |
| *N. americanus* | 50 | 0.160 | 0.075 |
| *N. orbicollis* | 500 | 0.174 | 0.235 |
| Photo quality | | | |
| Low-quality *N. americanus* | 15 | 0.533 | 0.419 |
| High-quality *N. americanus* | 35 | 0.030 | 0.049 |
| Low-quality *N. orbicollis* | 164 | 0.488 | 0.313 |
| High-quality *N. orbicollis* | 336 | 0.027 | 0.027 |
| Wild-caught | | | |
| *N. orbicollis* | 518 | 0.026 | 0.041 |
| *N. orbicollis* recaptures | 35 | 0.111 | 0.152 |
| Photo quality | | | |
| Low-quality wild-caught | 60 | 0.283 | 0.059 |
| High-quality wild-caught | 493 | 0.039 | 0.012 |

**Table 5.2.** Closed Model Selection Program MARK

Program MARK closed model selection results for *Nicrophorus orbicollis* collected in west-central Indiana, 2019. Akaike Information Criteria ($AIC_c$), the difference in $AIC_c$ values between the ith model and the model with the lowest $AIC_c$ value ($\Delta_i$), Akaike weights ($w_i$), number of parameters (K), and deviance are presented.

| Model | Description | $AIC_c$ | $\Delta_i$ | $w_i$ | K | Deviance |
|---|---|---|---|---|---|---|
| $M_o$ | Constant capture probability | -419.68 | 0.000 | 1.000 | 5 | 3.960 |
| $M_t$ | Time varying capture probability | -413.93 | 5.750 | 0.052 | 2 | 15.877 |
| $M_b$ | Behavioral response | -412.747 | 6.933 | 0.029 | 3 | 15.019 |
| $M_h$ | Heterogeneous capture probability | -381.10 | 38.58 | 0.000 | 2 | 48.706 |

**Table 5.3.** Model Parameter Estimates

Parameter estimates for *Nicrophorus orbicollis* inside the study area in west-central Indiana in July 2019. Parameter estimates are for the top-ranked $AIC_c$ model.

| Parameter [a] | Estimate | SE | CI | |
|---|---|---|---|---|
| | | | Lower | Upper |
| 1:*p* | 0.347 | 0.048 | 0.260 | 0.446 |
| 2:*p* | 0.672 | 0.059 | 0.549 | 0.775 |
| 3:*p* | 1.000 | 0.337E-5 | 0.999 | 1.000 |
| 4:*c* | 0.088 | 0.049 | 0.029 | 0.240 |
| 5:*c* | 0.104 | 0.035 | 0.053 | 0.194 |
| 6:f0 | 0.248E-8 | 0.161E-4 | 0.674E-12 | 0.914E-5 |

[a] *p* = probability of capture; *c* = probability of recapture.

**Table 5.4.** Population Size Estimates

Estimates from program MARK of the total population size of *Nicrophorus orbicollis* inside the study area in west-central Indiana in July 2019.

| Model | Description | $\widehat{N}$ | SE | 95% CI | |
|---|---|---|---|---|---|
| | | | | Lower | Upper |
| $M_o$ | Constant capture probability | 343 | 89 | 222 | 584 |
| $M_t$ | Time varying capture probability | 350 | 98 | 219 | 621 |
| $M_b$ | Behavioral response | 200 | 80 | 125 | 493 |
| $M_h$ | Heterogeneous capture probability | 184 | 13 | 162 | 214 |

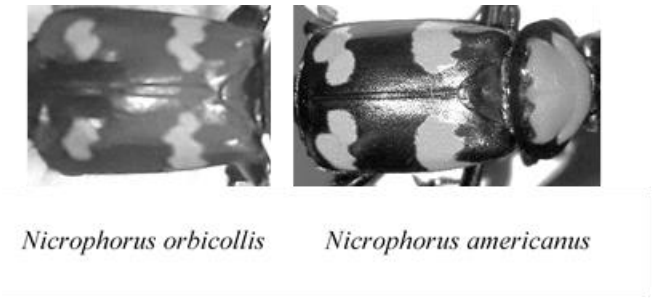


**Figure 5.1.** High-Quality and Low-Quality Photographs

Example of photos used for spot pattern recognition in *Nicrophorus orbicollis* and *Nicrophorus americanus* from laboratory populations in the summer of 2018 and 2019. We cropped pictures of *N. orbicollis* to include the dorsal region from the anterior of the elytra to the posterior of the elytra. We cropped pictures of *N. americanus* to include the pronotum because of the potential of it being unique to an individual. Additionally, the picture of *N. orbicollis* represents a lower quality photograph with less contrast, whereas the picture of *N. americanus* represents a higher quality photograph with sharper contrast

# CHAPTER 6. SUMMARY

## 6.1 Summary

Overall, my dissertation incorporated multiple techniques to assess niche relationships, energetics, and techniques for population abundance estimates. Collectively, my dissertation highlights how stable isotope techniques can be used in entomological studies to further advance our knowledge of insect biology and the stable isotope ecology of insects, it evaluates resource use and trophic niche overlap in burying beetles from the genus *Nicrophorus* (Coleoptera: Silphidae: Nicrophorinae), it investigates energetic trade-offs associated with sexual maturation as well as resource use in relation to resource quality and size, and tests the feasibility and the application of photographic mark recapture methods in estimating population abundances for *Nicrophorus* spp. Because of the endangered status of the American burying beetle (*Nicrophorus americanus*), an understanding of population abundance, the resources used for reproduction, and the life history trade-offs associated with resource use is beneficial to future conservation efforts.

Although American burying beetle reintroduction sites are selected based on similar habitat and resource availability, potential vertebrate carrion species compositions sometimes differ between locations. For example, within the extant population on Block Island, Rhode Island, ring-necked pheasant (*Phasianus colchicus*) is abundant, however it is absent from the reintroduced site on Nantucket Island, Massachusetts. My ability to sample all co-occurring burying beetle species allowed me to simultaneously assess variability in the isotopic niche space among and within species. I provide baseline estimates of resource use that indicate that both avian and mammalian carrion are main components of co-occurring burying beetles' reproductive carrion. On both Block Island and Nantucket Island, I document evidence of large niche overlap between all burying beetle species, suggesting that co-occurring burying beetles are competing for similar reproductive carrion resources to raise their young. However, the large observed isotopic niche overlap may also be influenced by a lack of functionally diverse potential reproductive carrion and its availability at our study sites. Therefore, future studies determining the size and abundance of potential reproductive carrion as well as resource selectivity, in the context of isotopic diet analysis, is needed to provide clarity for resource use

between populations over time. Based on my results, management of American burying beetles should consider long term provisioning of farm-raised quail that may supplement a potential lack of naturally occurring reproductive carrion resources at reintroduction sites. Furthermore, studies evaluating inter- and intraspecific competition for carrion resources will provide managers with vital information needed to conserve endangered populations.

Ideally the composition of potential vertebrate carrion at reintroduction sites would be within the preferred carcass size range for American burying beetles, and competition for these resources would be low. However, this is not always the case. Increased rates of habitat fragmentation throughout the historical range of American burying beetles has led to a decrease in appropriately sized small mammal and avian communities. Furthermore, the increase in edge habitat has resulted in increasing competition for reproductive carrion with co-occurring burying beetles and vertebrate scavengers. These factors set the stage for situations where American burying beetles may be forced to use a vertebrate carrion resource that is of low quality (i.e. suboptimal size or not fresh). My dissertation identified energetic trade-offs associated with variation in resource quality during prehatching parental care. My results indicate that the metabolic cost associated with prehatching parental care is magnified by the time it takes beetles to preserve a carcass, providing new insight into how parents accumulate metabolic cost during prehatching parental care. However, further study is needed to determine the relationship between metabolic rate, optimal carcass size, larval performance, and parental performance on longevity and the energetic cost associated with parental care in burying beetles. Insight into these questions will improve our understanding of burying beetle resource use and its influence on life history trade-offs.

Lastly, successful monitoring of wildlife populations requires reliable estimates of abundance, dispersal, and population demographics. However, it is often problematic to collect accurate data for wildlife populations, especially if they are cryptic, elusive, nocturnal, occur at low densities, or are species of conservation concern such as the American burying beetle. Traditionally estimating the abundance of American burying beetle within extant populations has constituted a management and conservation challenge. Using machine-learning software for photographic-identification in *Nicrophorus* spp. my dissertation demonstrates that elytron spot patterns are individually unique in burying beetles and provide a suitable bases for identification, while having a lower potential for animal stress or negative survival effects. I document

individual identification using machine-learning paired with photographic-identification in a mark-recapture study with a success rate greater than using traditional methods (i.e. bee-tags and elytron notching). There are many studies focused on the understanding of population dynamics and dispersal in burying beetles, however few have been conducted within reintroduced populations. Further study is needed to evaluate abundance, dispersal, and population demographics within extant and reintroduced populations of American burying beetles. Additionally, long-term population dynamics of burying beetles are poorly understood, and thus photographic-mark-recapture should be conducted over many years and at different field sites. This will further clarify how populations respond to environmental pressures and management strategies over time.

# APPENDIX

### Table A.6.1. Appendix Table Stable Isotope Results

Stable isotope results from potential reproductive carrion and wild-caught burying beetles ($\delta^{13}C$ and $\delta^{15}N$). Means are shown. Stable isotopes are presented in delta notation (δ). All isotope values are presented in per mil (‰). We used the mathematical correction described in Post et al. (2007) to correct for effects of lipids on $\delta^{13}C$ values when C/N > 4.

| Species | Population | $\delta^{13}C$(‰) | C% | $\delta^{15}N$(‰) | N% | C/N |
|---|---|---|---|---|---|---|
| *Nicrophorus americanus* | Nantucket Island | -24.216 | 48.302 | 5.982 | 9.750 | 5.0 |
| *Nicrophorus americanus* | Nantucket Island | -23.654 | 48.589 | 6.225 | 9.936 | 4.9 |
| *Nicrophorus americanus* | Nantucket Island | -26.958 | 46.992 | 7.522 | 9.738 | 4.8 |
| *Nicrophorus americanus* | Nantucket Island | -23.356 | 48.906 | 6.293 | 10.027 | 4.9 |
| *Nicrophorus americanus* | Nantucket Island | -24.237 | 48.402 | 5.749 | 10.058 | 4.8 |
| *Nicrophorus americanus* | Nantucket Island | -23.877 | 46.996 | 5.490 | 8.906 | 5.3 |
| *Nicrophorus americanus* | Nantucket Island | -23.19 | 47.807 | 6.740 | 10.008 | 4.8 |
| *Nicrophorus americanus* | Nantucket Island | -22.532 | 47.920 | 6.416 | 10.301 | 4.7 |
| *Nicrophorus americanus* | Nantucket Island | -27.43 | 47.539 | 5.618 | 9.950 | 4.8 |
| *Nicrophorus americanus* | Nantucket Island | -25.641 | 46.439 | 7.222 | 10.944 | 4.2 |
| *Nicrophorus americanus* | Nantucket Island | -24.789 | 48.337 | 5.013 | 10.125 | 4.8 |
| *Nicrophorus americanus* | Nantucket Island | -23.536 | 48.053 | 5.564 | 10.239 | 4.7 |
| *Nicrophorus americanus* | Nantucket Island | -24.029 | 48.490 | 4.830 | 10.266 | 4.7 |
| *Nicrophorus americanus* | Nantucket Island | -27.03 | 47.172 | 5.279 | 10.218 | 4.6 |
| *Nicrophorus americanus* | Nantucket Island | -20.455 | 45.353 | 7.978 | 9.678 | 4.7 |
| *Nicrophorus americanus* | Nantucket Island | -20.662 | 45.173 | 7.644 | 9.513 | 4.7 |
| *Nicrophorus americanus* | Block Island | -23.11 | 48.549 | 8.908 | 10.069 | 4.8 |
| *Nicrophorus americanus* | Block Island | -26.461 | 41.510 | 5.534 | 8.438 | 4.9 |
| *Nicrophorus americanus* | Block Island | -24.144 | 48.444 | 8.009 | 9.788 | 4.9 |
| *Nicrophorus americanus* | Block Island | -25.103 | 48.365 | 6.699 | 9.697 | 5.0 |
| *Nicrophorus americanus* | Block Island | -25.953 | 48.802 | 8.305 | 9.899 | 4.9 |
| *Nicrophorus americanus* | Block Island | -21.521 | 48.789 | 9.775 | 9.859 | 4.9 |
| *Nicrophorus americanus* | Block Island | -22.592 | 48.980 | 6.835 | 10.214 | 4.8 |
| *Nicrophorus americanus* | Block Island | -26.672 | 48.363 | 7.822 | 10.007 | 4.8 |
| *Nicrophorus americanus* | Block Island | -25.473 | 48.094 | 5.902 | 9.786 | 4.9 |
| *Nicrophorus americanus* | Block Island | -25.478 | 49.656 | 6.797 | 10.184 | 4.9 |
| *Nicrophorus americanus* | Block Island | -26.794 | 48.466 | 8.268 | 10.026 | 4.8 |
| *Nicrophorus americanus* | Block Island | -24.352 | 47.185 | 7.105 | 9.878 | 4.8 |
| *Nicrophorus americanus* | Block Island | -26.356 | 48.730 | 9.613 | 9.934 | 4.9 |
| *Nicrophorus americanus* | Block Island | -24.756 | 48.038 | 6.967 | 9.846 | 4.9 |

Table A.3.1 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| *Nicrophorus americanus* | Block Island | -24.225 | 48.031 | 7.059 | 9.761 | 4.9 |
| *Nicrophorus americanus* | Block Island | -25.561 | 48.111 | 8.407 | 10.085 | 4.8 |
| *Nicrophorus americanus* | Block Island | -25.088 | 49.352 | 7.609 | 9.867 | 5.0 |
| *Nicrophorus americanus* | Block Island | -24.62 | 48.534 | 5.284 | 9.865 | 4.9 |
| *Nicrophorus americanus* | Block Island | -21.993 | 48.468 | 9.790 | 9.845 | 4.9 |
| *Nicrophorus americanus* | Block Island | -25.749 | 48.985 | 7.931 | 10.129 | 4.8 |
| *Nicrophorus americanus* | Block Island | -25.812 | 49.950 | 5.949 | 9.987 | 5.0 |
| *Nicrophorus americanus* | Block Island | -25.903 | 49.069 | 6.630 | 9.979 | 4.9 |
| *Nicrophorus americanus* | Block Island | -24.905 | 49.541 | 7.077 | 9.900 | 5.0 |
| *Nicrophorus americanus* | Block Island | -25.685 | 48.680 | 7.974 | 9.986 | 4.9 |
| *Nicrophorus americanus* | Block Island | -27.123 | 48.397 | 7.447 | 9.934 | 4.9 |
| *Nicrophorus americanus* | Block Island | -23.939 | 49.137 | 8.883 | 9.939 | 4.9 |
| *Nicrophorus americanus* | Block Island | -24.923 | 49.051 | 7.407 | 10.067 | 4.9 |
| *Nicrophorus americanus* | Block Island | -23.166 | 48.226 | 5.962 | 9.849 | 4.9 |
| *Nicrophorus americanus* | Block Island | -23.048 | 48.257 | 5.666 | 9.828 | 4.9 |
| *Nicrophorus americanus* | Block Island | -26.725 | 48.337 | 6.822 | 9.935 | 4.9 |
| *Nicrophorus americanus* | Block Island | -25.906 | 49.869 | 6.586 | 10.100 | 4.9 |
| *Nicrophorus americanus* | Block Island | -25.901 | 47.941 | 6.237 | 9.744 | 4.9 |
| *Nicrophorus americanus* | Block Island | -21.684 | 49.261 | 4.203 | 9.989 | 4.9 |
| *Nicrophorus americanus* | Block Island | -23.787 | 47.561 | 9.243 | 9.867 | 4.8 |
| *Nicrophorus americanus* | Block Island | -28.177 | 47.820 | 5.751 | 9.798 | 4.9 |
| *Nicrophorus americanus* | Block Island | -27.337 | 48.721 | 6.509 | 9.896 | 4.9 |
| *Nicrophorus americanus* | Block Island | -25.552 | 43.735 | 6.632 | 8.873 | 4.9 |
| *Nicrophorus americanus* | Block Island | -26.327 | 49.115 | 7.540 | 9.941 | 4.9 |
| *Nicrophorus americanus* | Block Island | -22.491 | 47.112 | 7.546 | 9.630 | 4.9 |
| *Nicrophorus tomentosus* | Nantucket Island | -23.705 | 48.813 | 7.627 | 9.858 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -26.037 | 47.321 | 6.843 | 9.066 | 5.2 |
| *Nicrophorus marginatus* | Nantucket Island | -26.852 | 48.265 | 4.658 | 9.331 | 5.2 |
| *Nicrophorus marginatus* | Nantucket Island | -26.197 | 48.250 | 4.829 | 9.542 | 5.1 |
| *Nicrophorus marginatus* | Nantucket Island | -25.833 | 47.500 | 4.544 | 9.521 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -27.2 | 48.168 | 5.737 | 9.473 | 5.1 |
| *Nicrophorus marginatus* | Nantucket Island | -26.179 | 49.217 | 3.985 | 9.860 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -24.665 | 45.731 | 6.745 | 9.425 | 4.9 |
| *Nicrophorus marginatus* | Nantucket Island | -24.326 | 50.553 | 5.398 | 9.401 | 5.4 |
| *Nicrophorus marginatus* | Nantucket Island | -27.208 | 50.428 | 4.197 | 9.179 | 5.5 |
| *Nicrophorus marginatus* | Nantucket Island | -24.985 | 51.396 | 5.116 | 9.283 | 5.5 |
| *Nicrophorus marginatus* | Nantucket Island | -27.527 | 50.565 | 4.215 | 9.624 | 5.3 |
| *Nicrophorus marginatus* | Nantucket Island | -27.046 | 49.454 | 5.241 | 9.642 | 5.1 |
| *Nicrophorus marginatus* | Nantucket Island | -26.963 | 47.360 | 4.128 | 9.632 | 4.9 |

Table A.3.1 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| *Nicrophorus marginatus* | Nantucket Island | -27.327 | 49.108 | 4.532 | 9.756 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -25.811 | 48.592 | 5.396 | 9.744 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -26.968 | 48.202 | 3.873 | 9.611 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -27.417 | 47.916 | 3.744 | 9.416 | 5.1 |
| *Nicrophorus marginatus* | Nantucket Island | -26.312 | 48.055 | 5.224 | 9.691 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -26.07 | 48.599 | 7.788 | 9.759 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -26.368 | 49.753 | 3.902 | 9.768 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.68 | 51.269 | 4.662 | 9.367 | 5.5 |
| *Nicrophorus orbicollis* | Nantucket Island | -24.454 | 50.836 | 5.107 | 9.605 | 5.3 |
| *Nicrophorus orbicollis* | Nantucket Island | -27.921 | 50.072 | 4.922 | 9.828 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -27.539 | 51.912 | 7.155 | 9.317 | 5.6 |
| *Nicrophorus orbicollis* | Nantucket Island | -24.442 | 54.121 | 4.535 | 8.837 | 6.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -24.661 | 54.698 | 6.049 | 8.778 | 6.2 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.318 | 54.389 | 6.313 | 8.603 | 6.3 |
| *Nicrophorus orbicollis* | Nantucket Island | -23.477 | 51.631 | 5.376 | 9.546 | 5.4 |
| *Nicrophorus orbicollis* | Nantucket Island | -24.753 | 49.223 | 5.069 | 10.003 | 4.9 |
| *Nicrophorus orbicollis* | Nantucket Island | -24.58 | 53.554 | 5.311 | 9.217 | 5.8 |
| *Nicrophorus orbicollis* | Nantucket Island | -24.873 | 49.906 | 7.391 | 9.877 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.956 | 51.491 | 6.540 | 9.504 | 5.4 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.435 | 49.321 | 6.993 | 9.845 | 5.0 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.041 | 49.536 | 5.531 | 10.041 | 4.9 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.682 | 52.192 | 6.028 | 9.686 | 5.4 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.27 | 49.847 | 4.957 | 10.008 | 5.0 |
| *Nicrophorus orbicollis* | Nantucket Island | -24.9 | 49.429 | 4.969 | 9.690 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.31 | 48.393 | 5.446 | 10.112 | 4.8 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.235 | 48.985 | 6.033 | 9.856 | 5.0 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.268 | 49.401 | 4.769 | 9.706 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.791 | 49.685 | 7.585 | 9.568 | 5.2 |
| *Nicrophorus orbicollis* | Nantucket Island | -28.238 | 47.100 | 5.129 | 9.388 | 5.0 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.207 | 47.787 | 6.549 | 9.626 | 5.0 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.21 | 48.726 | 6.340 | 9.572 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.218 | 48.867 | 6.800 | 9.621 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.594 | 49.675 | 5.755 | 9.942 | 5.0 |
| *Nicrophorus orbicollis* | Nantucket Island | -27.056 | 49.919 | 3.632 | 9.698 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -22.84 | 50.079 | 8.179 | 9.446 | 5.3 |
| *Nicrophorus orbicollis* | Nantucket Island | -24.788 | 49.551 | 6.584 | 9.597 | 5.2 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.231 | 50.043 | 4.493 | 9.456 | 5.3 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.115 | 50.276 | 5.979 | 9.456 | 5.3 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.18 | 49.543 | 5.457 | 9.570 | 5.2 |

Table A.3.1 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| *Nicrophorus orbicollis* | Nantucket Island | -26.333 | 51.669 | 5.818 | 9.283 | 5.6 |
| *Nicrophorus orbicollis* | Nantucket Island | -24.515 | 48.458 | 5.509 | 9.549 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.783 | 48.984 | 6.907 | 9.619 | 5.1 |
| *Nicrophours marginatus* | Block Island | -25.538 | 50.121 | 9.081 | 9.748 | 5.1 |
| *Nicrophorus orbicollis* | Block Island | -26.228 | 44.596 | 5.186 | 8.803 | 5.1 |
| *Nicrophorus orbicollis* | Block Island | -27.074 | 48.474 | 7.145 | 9.775 | 5.0 |
| *Nicrophours marginatus* | Block Island | -27.752 | 47.873 | 9.460 | 9.661 | 5.0 |
| *Nicrophours marginatus* | Block Island | -26.055 | 49.277 | 4.203 | 9.319 | 5.3 |
| *Nicrophours marginatus* | Block Island | -27.418 | 48.482 | 5.042 | 9.242 | 5.2 |
| *Nicrophours marginatus* | Block Island | -26.84 | 48.203 | 4.306 | 9.379 | 5.1 |
| *Nicrophorus orbicollis* | Block Island | -25.951 | 47.982 | 7.470 | 9.658 | 5.0 |
| *Nicrophours marginatus* | Block Island | -27.761 | 49.605 | 4.986 | 9.304 | 5.3 |
| *Nicrophours marginatus* | Block Island | -27.537 | 47.754 | 6.120 | 9.399 | 5.1 |
| *Nicrophorus orbicollis* | Block Island | -25.897 | 46.935 | 6.465 | 9.603 | 4.9 |
| *Nicrophours marginatus* | Block Island | -26.994 | 47.235 | 7.198 | 9.560 | 4.9 |
| *Nicrophours marginatus* | Block Island | -25.583 | 45.940 | 4.131 | 8.934 | 5.1 |
| *Nicrophours marginatus* | Block Island | -26.763 | 47.343 | 5.053 | 9.486 | 5.0 |
| *Nicrophours marginatus* | Block Island | -27.338 | 50.890 | 4.991 | 10.100 | 5.0 |
| *Nicrophours marginatus* | Block Island | -26.939 | 48.093 | 4.515 | 9.438 | 5.1 |
| *Nicrophours marginatus* | Block Island | -26.865 | 46.822 | 5.941 | 9.510 | 4.9 |
| *Nicrophours marginatus* | Block Island | -28.571 | 49.279 | 4.547 | 9.221 | 5.3 |
| *Nicrophours marginatus* | Block Island | -28.308 | 48.694 | 4.696 | 9.570 | 5.1 |
| *Agelaius phoeniceus* | Nantucket Island | -19.426 | 46.100 | 6.115 | 13.746 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -24.814 | 47.873 | 5.281 | 13.947 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -24.888 | 49.119 | 5.996 | 14.195 | 3.5 |
| *Agelaius phoeniceus* | Nantucket Island | -21.59 | 45.665 | 5.732 | 13.753 | 3.3 |
| *Cardinalis cardinalis* | Nantucket Island | -24.201 | 44.523 | 5.488 | 13.437 | 3.3 |
| *Dumetella carolinensis* | Nantucket Island | -25.389 | 45.937 | 6.884 | 13.780 | 3.3 |
| *Dumetella carolinensis* | Nantucket Island | -24.637 | 46.330 | 3.789 | 14.077 | 3.3 |
| *Dumetella carolinensis* | Nantucket Island | -26.089 | 46.676 | 8.151 | 14.206 | 3.3 |
| *Dumetella carolinensis* | Nantucket Island | -25.616 | 33.958 | 7.050 | 9.951 | 3.4 |
| *Dumetella carolinensis* | Nantucket Island | -24.947 | 45.188 | 6.182 | 13.766 | 3.3 |
| *Dumetella carolinensis* | Nantucket Island | -24.989 | 43.989 | 7.703 | 13.104 | 3.4 |
| *Dumetella carolinensis* | Nantucket Island | -25.092 | 42.655 | 5.599 | 12.875 | 3.3 |
| *Cardinalis cardinalis* | Nantucket Island | -24.551 | 48.093 | 5.567 | 13.049 | 3.7 |
| *Turdus migratorius* | Nantucket Island | -25.563 | 42.904 | 7.126 | 13.150 | 3.3 |
| *Bombycilla cedrorum* | Nantucket Island | -25.125 | 43.296 | 4.274 | 12.852 | 3.4 |
| *Sciurus carolinensis* | Nantucket Island | -25.395 | 54.493 | 2.457 | 10.706 | 5.1 |
| *Sciurus carolinensis* | Nantucket Island | -25.568 | 52.040 | 0.175 | 10.309 | 5.0 |

Table A.3.1 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| *Sciurus carolinensis* | Nantucket Island | -24.104 | 50.397 | 6.994 | 11.929 | 4.2 |
| *Sciurus carolinensis* | Nantucket Island | -23.551 | 46.478 | 4.783 | 13.157 | 3.5 |
| *Sciurus carolinensis* | Nantucket Island | -24.337 | 45.416 | 7.020 | 12.689 | 3.6 |
| *Sciurus carolinensis* | Nantucket Island | -25.001 | 50.778 | 2.123 | 11.191 | 4.5 |
| *Sciurus carolinensis* | Nantucket Island | -24.455 | 45.846 | 5.154 | 13.731 | 3.3 |
| *Sciurus carolinensis* | Nantucket Island | -24.306 | 47.951 | 6.017 | 11.709 | 4.1 |
| *Sciurus carolinensis* | Nantucket Island | -24.032 | 44.084 | 1.717 | 13.338 | 3.3 |
| *Rattus norvegicus* | Nantucket Island | -23.738 | 54.261 | 7.960 | 11.378 | 4.8 |
| *Rattus norvegicus* | Nantucket Island | -24.583 | 53.829 | 5.238 | 11.777 | 4.6 |
| *Rattus norvegicus* | Nantucket Island | -23.381 | 59.050 | 5.706 | 8.609 | 6.9 |
| *Rattus norvegicus* | Nantucket Island | -26.89 | 53.742 | 8.653 | 9.905 | 5.4 |
| *Rattus norvegicus* | Nantucket Island | -25.795 | 53.893 | 5.495 | 9.544 | 5.6 |
| *Rattus norvegicus* | Nantucket Island | -24.601 | 51.919 | 5.469 | 11.093 | 4.7 |
| *Sciurus carolinensis* | Nantucket Island | -23.457 | 48.262 | 0.367 | 12.977 | 3.7 |
| *Sciurus carolinensis* | Nantucket Island | -23.99 | 46.661 | 2.565 | 13.731 | 3.4 |
| *Sciurus carolinensis* | Nantucket Island | -23.946 | 44.886 | 1.812 | 12.537 | 3.6 |
| *Turdus migratorius* | Nantucket Island | -25.759 | 44.886 | 6.066 | 12.537 | 3.6 |
| *Turdus migratorius* | Nantucket Island | -25.297 | 45.061 | 7.553 | 13.250 | 3.4 |
| *Turdus migratorius* | Nantucket Island | -25.46 | 44.750 | 6.081 | 13.938 | 3.2 |
| *Turdus migratorius* | Nantucket Island | -25.253 | 43.499 | 4.910 | 13.533 | 3.2 |
| *Dumetella carolinensis* | Nantucket Island | -23.718 | 42.197 | 7.458 | 12.400 | 3.4 |
| *Colaptes auratus* | Nantucket Island | -26.153 | 45.711 | 6.787 | 13.971 | 3.3 |
| *Cyanocitta cristata* | Nantucket Island | -23.321 | 45.918 | 4.156 | 13.481 | 3.4 |
| *Scolopaz minor* | Nantucket Island | -24.309 | 48.930 | 4.481 | 12.260 | 4.0 |
| *Scolopaz minor* | Nantucket Island | -25.247 | 46.747 | 6.606 | 12.475 | 3.7 |
| *Anas platyrhynchos* | Nantucket Island | -23.43 | 43.696 | 7.936 | 13.370 | 3.3 |
| *Larus argentatus* | Nantucket Island | -17.719 | 44.900 | 13.948 | 13.622 | 3.3 |
| *Corvus brachyrhynchos* | Nantucket Island | -21.133 | 45.841 | 6.952 | 13.083 | 3.5 |
| *Cartharus guttatus* | Nantucket Island | -26.198 | 45.443 | 7.135 | 12.954 | 3.5 |
| *Aix sponsa* | Nantucket Island | -28.69 | 50.111 | 6.623 | 12.690 | 3.9 |
| *Fulica americana* | Nantucket Island | -23.483 | 52.816 | 8.786 | 12.004 | 4.4 |
| *Melanitta nigra* | Nantucket Island | -18.189 | 41.472 | 11.199 | 12.033 | 3.4 |
| *Clangula hyemalis* | Nantucket Island | -17.241 | 45.062 | 13.163 | 13.391 | 3.4 |
| *Somateria mollissima* | Nantucket Island | -18.074 | 45.451 | 11.983 | 13.347 | 3.4 |
| *Colinus virginianus* | Nantucket Island | -20.267 | 47.360 | 2.857 | 13.945 | 3.4 |
| *Colinus virginianus* | Nantucket Island | -20.236 | 47.552 | 3.164 | 13.987 | 3.4 |
| *Colinus virginianus* | Nantucket Island | -20.074 | 47.067 | 3.119 | 13.980 | 3.4 |
| *Colinus virginianus* | Nantucket Island | -20.064 | 47.614 | 2.905 | 14.067 | 3.4 |
| *Sylvilagus floridanus* | Nantucket Island | -23.917 | 48.240 | 0.938 | 14.090 | 3.4 |

Table A.3.1 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| *Sciurus carolinensis* | Nantucket Island | -23.835 | 49.060 | 1.157 | 13.372 | 3.7 |
| *Sciurus carolinensis* | Nantucket Island | -24.118 | 48.259 | 2.006 | 13.331 | 3.6 |
| *Sciurus carolinensis* | Nantucket Island | -23.244 | 47.681 | 1.276 | 13.898 | 3.4 |
| *Sciurus carolinensis* | Nantucket Island | -25.097 | 48.900 | 1.413 | 11.352 | 4.3 |
| *Rattus norvegicus* | Nantucket Island | -26.199 | 51.756 | 7.232 | 11.962 | 4.3 |
| *Sciurus carolinensis* | Nantucket Island | -23.415 | 47.368 | 2.266 | 14.351 | 3.3 |
| *Sciurus carolinensis* | Nantucket Island | -25.582 | 55.359 | 2.687 | 10.275 | 5.4 |
| *Sciurus carolinensis* | Nantucket Island | -25.043 | 52.419 | 0.174 | 11.363 | 4.6 |
| *Sciurus carolinensis* | Nantucket Island | -24.252 | 49.443 | 0.134 | 13.821 | 3.6 |
| *Sciurus carolinensis* | Nantucket Island | -23.779 | 47.935 | 2.514 | 14.025 | 3.4 |
| *Sciurus carolinensis* | Nantucket Island | -26.98 | 56.043 | 2.061 | 8.982 | 6.2 |
| *Sylvilagus floridanus* | Nantucket Island | -26.652 | 43.344 | 3.522 | 13.148 | 3.3 |
| *Sylvilagus floridanus* | Nantucket Island | -28.538 | 46.099 | 3.009 | 13.030 | 3.5 |
| *Sylvilagus floridanus* | Nantucket Island | -27.731 | 44.240 | 4.004 | 13.000 | 3.4 |
| *Sylvilagus floridanus* | Nantucket Island | -27.537 | 30.381 | 3.291 | 9.447 | 3.2 |
| *Sylvilagus floridanus* | Nantucket Island | -27.035 | 43.828 | 1.877 | 13.039 | 3.4 |
| *Dumetella carolinensis* | Nantucket Island | -24.73 | 46.597 | 6.698 | 12.326 | 3.8 |
| *Dumetella carolinensis* | Nantucket Island | -24.849 | 43.578 | 9.407 | 10.832 | 4.0 |
| *Passer domesticus* | Nantucket Island | -25.928 | 46.094 | 5.660 | 11.443 | 4.0 |
| *Dumetella carolinensis* | Block Island | -24.777 | 45.925 | 7.049 | 12.331 | 3.7 |
| *Rattus norvegicus* | Block Island | -26.672 | 45.347 | 5.864 | 13.449 | 3.4 |
| *Rattus norvegicus* | Block Island | -23.955 | 43.909 | 7.279 | 12.837 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -24.01 | 49.138 | 1.609 | 9.722 | 5.1 |
| *Peromyscus leucopus* | Nantucket Island | -24.463 | 47.535 | 2.079 | 13.834 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -23.452 | 46.084 | 1.504 | 13.505 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -23.047 | 41.351 | 2.979 | 12.327 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -25.755 | 47.137 | 2.089 | 13.984 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -23.958 | 46.084 | 0.816 | 13.505 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -23.931 | 48.593 | 2.243 | 14.238 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -24.496 | 47.011 | 1.695 | 13.940 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -23.831 | 48.063 | 0.644 | 13.916 | 3.5 |
| *Microtus pennsylvanicus* | Nantucket Island | -23.411 | 20.906 | 0.469 | 6.299 | 3.3 |
| *Microtus pennsylvanicus* | Nantucket Island | -25.221 | 21.738 | 1.443 | 6.504 | 3.3 |
| *Microtus pennsylvanicus* | Nantucket Island | -24.413 | 47.451 | 1.038 | 14.181 | 3.3 |
| *Microtus pennsylvanicus* | Nantucket Island | -24.372 | 32.344 | 2.936 | 9.549 | 3.4 |
| *Microtus pennsylvanicus* | Nantucket Island | -24.438 | 47.657 | 2.933 | 12.788 | 3.7 |
| *Microtus pennsylvanicus* | Nantucket Island | -25.952 | 48.168 | 0.820 | 14.388 | 3.3 |
| *Microtus pennsylvanicus* | Nantucket Island | -25.856 | 31.118 | 1.146 | 9.298 | 3.3 |
| *Microtus pennsylvanicus* | Nantucket Island | -23.813 | 45.629 | 0.253 | 13.777 | 3.3 |

Table A.3.1 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| *Microtus pennsylvanicus* | Nantucket Island | -24.497 | 45.098 | 0.829 | 13.559 | 3.3 |
| *Microtus pennsylvanicus* | Nantucket Island | -25.387 | 39.966 | 1.167 | 12.050 | 3.3 |
| *Microtus pennsylvanicus* | Nantucket Island | -25.605 | 15.028 | 0.647 | 4.576 | 3.3 |
| *Microtus pennsylvanicus* | Nantucket Island | -25.118 | 46.484 | 1.251 | 14.227 | 3.3 |
| *Microtus pennsylvanicus* | Nantucket Island | -25.382 | 44.969 | 0.424 | 13.800 | 3.3 |
| *Microtus pennsylvanicus* | Nantucket Island | -24.485 | 18.832 | 1.134 | 5.890 | 3.2 |
| *Phasianus colchicus* | Block Island | -23.636 | 43.957 | 4.415 | 13.893 | 3.2 |
| *Phasianus colchicus* | Block Island | -24.458 | 47.745 | 3.789 | 14.625 | 3.3 |
| *Phasianus colchicus* | Block Island | -24.059 | 47.508 | 6.210 | 15.109 | 3.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -27.798 | 41.803 | 5.591 | 7.965 | 5.2 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.453 | 50.594 | 4.188 | 9.351 | 5.4 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.299 | 50.209 | 4.737 | 9.770 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.758 | 49.761 | 7.775 | 9.383 | 5.3 |
| *Nicrophorus orbicollis* | Nantucket Island | -24.791 | 49.993 | 5.778 | 9.774 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -22.121 | 49.577 | 4.993 | 9.900 | 5.0 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.49 | 50.741 | 4.847 | 9.718 | 5.2 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.607 | 46.869 | 8.710 | 9.323 | 5.0 |
| *Nicrophorus orbicollis* | Nantucket Island | -27.194 | 48.695 | 6.436 | 9.866 | 4.9 |
| *Nicrophorus orbicollis* | Nantucket Island | -24.677 | 48.318 | 5.282 | 9.633 | 5.0 |
| *Nicrophorus orbicollis* | Nantucket Island | -27.394 | 51.459 | 4.566 | 9.908 | 5.2 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.993 | 49.854 | 5.253 | 9.523 | 5.2 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.794 | 49.820 | 4.573 | 9.859 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -28.766 | 49.549 | 7.494 | 9.768 | 5.1 |
| *Nicrophorus tomentosus* | Nantucket Island | -27.382 | 46.905 | 5.939 | 10.246 | 4.6 |
| *Nicrophorus tomentosus* | Nantucket Island | -23.928 | 46.988 | 7.521 | 10.188 | 4.6 |
| *Nicrophorus marginatus* | Nantucket Island | -27.334 | 51.070 | 5.274 | 9.853 | 5.2 |
| *Nicrophorus marginatus* | Nantucket Island | -27.96 | 49.045 | 4.609 | 9.498 | 5.2 |
| *Nicrophorus marginatus* | Nantucket Island | -25.2 | 50.131 | 6.486 | 10.052 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -28.006 | 49.761 | 5.606 | 9.966 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -28.145 | 50.430 | 3.770 | 9.283 | 5.4 |
| *Nicrophorus orbicollis* | Nantucket Island | -24.17 | 50.413 | 6.495 | 9.858 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -27.084 | 50.514 | 4.208 | 9.985 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -22.931 | 50.028 | 4.737 | 9.910 | 5.0 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.235 | 51.016 | 6.042 | 9.612 | 5.3 |
| *Nicrophorus orbicollis* | Nantucket Island | -27.865 | 50.693 | 4.009 | 9.700 | 5.2 |
| *Nicrophorus orbicollis* | Nantucket Island | -24.643 | 46.722 | 6.895 | 9.352 | 5.0 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.431 | 50.560 | 3.570 | 9.718 | 5.2 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.37 | 50.443 | 5.314 | 9.646 | 5.2 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.09 | 49.680 | 5.330 | 9.780 | 5.1 |

Table A.3.1 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| *Nicrophorus marginatus* | Nantucket Island | -26.926 | 50.858 | 7.625 | 10.034 | 5.1 |
| *Nicrophorus marginatus* | Nantucket Island | -25.972 | 49.795 | 4.894 | 9.947 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -27.815 | 51.438 | 4.247 | 9.769 | 5.3 |
| *Nicrophorus marginatus* | Nantucket Island | -27.38 | 50.908 | 5.028 | 9.858 | 5.2 |
| *Nicrophorus marginatus* | Nantucket Island | -26.864 | 50.658 | 3.903 | 9.866 | 5.1 |
| *Nicrophorus marginatus* | Nantucket Island | -27.343 | 50.570 | 6.349 | 9.742 | 5.2 |
| *Nicrophorus marginatus* | Nantucket Island | -26.535 | 50.693 | 5.185 | 10.278 | 4.9 |
| *Nicrophorus marginatus* | Nantucket Island | -27.486 | 50.260 | 5.309 | 10.062 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -28.235 | 50.611 | 5.631 | 10.224 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -26.004 | 50.163 | 4.539 | 10.114 | 5.0 |
| *Nicrophorus marginatus* | Nantucket Island | -24.963 | 50.052 | 5.841 | 10.809 | 4.6 |
| *Nicrophorus orbicollis* | Nantucket Island | -26.389 | 51.667 | 4.313 | 10.012 | 5.2 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.331 | 50.097 | 5.853 | 10.078 | 5.0 |
| *Nicrophorus orbicollis* | Nantucket Island | -27.845 | 50.747 | 6.815 | 9.872 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.275 | 50.659 | 6.497 | 9.912 | 5.1 |
| *Nicrophorus orbicollis* | Nantucket Island | -28.118 | 51.221 | 6.977 | 9.572 | 5.4 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.937 | 50.435 | 5.895 | 10.366 | 4.9 |
| *Nicrophorus orbicollis* | Nantucket Island | -25.784 | 51.651 | 4.782 | 9.855 | 5.2 |
| *Nicrophorus tomentosus* | Block Island | -27.31 | 46.386 | 7.729 | 10.901 | 4.3 |
| *Nicrophorus tomentosus* | Block Island | -25.654 | 47.091 | 9.992 | 11.065 | 4.3 |
| *Nicrophorus tomentosus* | Block Island | -25.032 | 47.012 | 7.070 | 10.881 | 4.3 |
| *Nicrophorus tomentosus* | Block Island | -26.388 | 47.488 | 7.755 | 10.996 | 4.3 |
| *Nicrophorus tomentosus* | Block Island | -28.527 | 47.538 | 6.682 | 11.000 | 4.3 |
| *Nicrophorus tomentosus* | Block Island | -25.335 | 48.574 | 7.597 | 10.862 | 4.5 |
| *Nicrophorus tomentosus* | Block Island | -26.124 | 47.509 | 6.232 | 10.525 | 4.5 |
| *Nicrophorus tomentosus* | Block Island | -27.802 | 48.688 | 4.610 | 10.446 | 4.7 |
| *Nicrophorus tomentosus* | Block Island | -24.978 | 44.204 | 7.162 | 11.043 | 4.0 |
| *Nicrophorus tomentosus* | Block Island | -27.388 | 46.963 | 6.926 | 10.724 | 4.4 |
| *Nicrophorus tomentosus* | Block Island | -25.104 | 45.339 | 6.552 | 10.964 | 4.1 |
| *Nicrophorus tomentosus* | Block Island | -24.888 | 47.515 | 7.841 | 10.027 | 4.7 |
| *Nicrophorus tomentosus* | Block Island | -25.229 | 47.202 | 8.600 | 11.245 | 4.2 |
| *Nicrophorus tomentosus* | Block Island | -26.492 | 43.390 | 9.326 | 10.945 | 4.0 |
| *Nicrophorus tomentosus* | Block Island | -27.2 | 46.625 | 8.421 | 11.143 | 4.2 |
| *Nicrophorus tomentosus* | Block Island | -24.413 | 47.073 | 9.958 | 10.937 | 4.3 |
| *Nicrophorus tomentosus* | Block Island | -26.133 | 46.169 | 8.454 | 11.168 | 4.1 |
| *Nicrophorus tomentosus* | Block Island | -26.643 | 47.857 | 4.600 | 9.838 | 4.9 |
| *Nicrophorus tomentosus* | Block Island | -24.668 | 47.125 | 7.736 | 11.232 | 4.2 |
| *Nicrophorus marginatus* | Block Island | -27.193 | 49.687 | 4.487 | 9.761 | 5.1 |
| *Nicrophorus marginatus* | Block Island | -27.164 | 49.332 | 5.346 | 9.786 | 5.0 |

Table A.3.1 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| *Nicrophorus marginatus* | Block Island | -28.12 | 49.584 | 5.555 | 9.820 | 5.0 |
| *Nicrophorus marginatus* | Block Island | -26.84 | 48.903 | 5.486 | 9.786 | 5.0 |
| *Nicrophorus marginatus* | Block Island | -27.788 | 50.367 | 5.880 | 10.167 | 5.0 |
| *Nicrophorus marginatus* | Block Island | -28.014 | 49.471 | 5.007 | 9.802 | 5.0 |
| *Nicrophorus marginatus* | Block Island | -27.514 | 47.848 | 7.039 | 9.868 | 4.8 |
| *Nicrophorus marginatus* | Block Island | -26.029 | 49.751 | 6.348 | 9.951 | 5.0 |
| *Nicrophorus marginatus* | Block Island | -28.048 | 50.748 | 5.596 | 10.014 | 5.1 |
| *Nicrophorus marginatus* | Block Island | -27.922 | 49.832 | 5.365 | 10.065 | 5.0 |
| *Nicrophorus marginatus* | Block Island | -27.998 | 49.378 | 6.515 | 10.069 | 4.9 |
| *Nicrophorus marginatus* | Block Island | -26.544 | 50.163 | 7.303 | 10.502 | 4.8 |
| *Nicrophorus marginatus* | Block Island | -26.669 | 49.599 | 6.014 | 10.036 | 4.9 |
| *Nicrophorus marginatus* | Block Island | -28.279 | 49.270 | 6.208 | 10.214 | 4.8 |
| *Nicrophorus marginatus* | Block Island | -26.644 | 48.733 | 7.724 | 10.361 | 4.7 |
| *Nicrophorus orbicollis* | Block Island | -26.124 | 47.725 | 8.741 | 9.336 | 5.1 |
| *Nicrophorus orbicollis* | Block Island | -25.736 | 49.628 | 7.523 | 10.142 | 4.9 |
| *Nicrophorus orbicollis* | Block Island | -25.809 | 49.710 | 8.549 | 10.686 | 4.7 |
| *Nicrophorus orbicollis* | Block Island | -26.537 | 49.763 | 4.452 | 10.083 | 4.9 |
| *Nicrophorus orbicollis* | Block Island | -25.889 | 49.172 | 6.005 | 10.103 | 4.9 |
| *Nicrophorus orbicollis* | Block Island | -27.942 | 50.005 | 6.745 | 10.515 | 4.8 |
| *Nicrophorus orbicollis* | Block Island | -26.164 | 49.156 | 10.986 | 10.752 | 4.6 |
| *Nicrophorus orbicollis* | Block Island | -26.71 | 49.969 | 6.821 | 10.178 | 4.9 |
| *Nicrophorus orbicollis* | Block Island | -27.03 | 48.786 | 6.483 | 10.550 | 4.6 |
| *Nicrophorus orbicollis* | Block Island | -24.544 | 50.364 | 16.650 | 10.826 | 4.7 |
| *Nicrophorus orbicollis* | Block Island | -26.584 | 50.470 | 7.145 | 9.848 | 5.1 |
| *Nicrophorus orbicollis* | Block Island | -24.739 | 49.097 | 7.687 | 10.391 | 4.7 |
| *Nicrophorus orbicollis* | Block Island | -25.789 | 50.612 | 8.037 | 9.941 | 5.1 |
| *Nicrophorus orbicollis* | Block Island | -23.669 | 49.783 | 8.588 | 9.880 | 5.0 |
| *Nicrophorus orbicollis* | Block Island | -25.542 | 50.846 | 7.045 | 10.087 | 5.0 |
| *Nicrophorus orbicollis* | Block Island | -26.095 | 49.192 | 7.093 | 10.162 | 4.8 |
| *Nicrophorus orbicollis* | Block Island | -25.948 | 50.014 | 6.504 | 10.176 | 4.9 |
| *Colinus virginianus* | Nantucket Island | -20.933 | 46.198 | 3.825 | 9.058 | 5.1 |
| *Colinus virginianus* | Nantucket Island | -20.058 | 35.257 | 4.471 | 10.366 | 3.4 |
| *Colinus virginianus* | Nantucket Island | -20.157 | 43.750 | 3.720 | 12.646 | 3.5 |
| *Colinus virginianus* | Nantucket Island | -20.047 | 32.045 | 3.472 | 9.883 | 3.2 |
| *Colinus virginianus* | Nantucket Island | -19.663 | 36.889 | 4.369 | 11.390 | 3.2 |
| *Colinus virginianus* | Nantucket Island | -19.898 | 34.818 | 4.056 | 10.083 | 3.5 |
| *Colinus virginianus* | Nantucket Island | -19.966 | 44.135 | 3.905 | 13.481 | 3.3 |
| *Colinus virginianus* | Nantucket Island | -20.152 | 46.070 | 3.146 | 14.461 | 3.2 |
| *Peromyscus leucopus* | Block Island | -25.627 | 40.597 | 4.805 | 9.365 | 4.3 |

Table A.3.1 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| *Peromyscus leucopus* | Nantucket Island | -24.343 | 43.866 | 5.649 | 12.934 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -24.269 | 44.279 | 5.361 | 13.000 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -24.839 | 48.756 | 6.534 | 14.179 | 3.4 |
| *Turdus migratorius* | Nantucket Island | -25.564 | 30.256 | 12.508 | 9.084 | 3.3 |
| *Dumetella carolinensis* | Nantucket Island | -23.939 | 47.174 | 5.977 | 14.276 | 3.3 |
| *Dumetella carolinensis* | Nantucket Island | -26.585 | 46.526 | 7.611 | 13.915 | 3.3 |
| *Dumetella carolinensis* | Nantucket Island | -25.247 | 45.885 | 5.144 | 13.553 | 3.4 |
| *Cardinalis cardinalis* | Nantucket Island | -19.487 | 48.190 | 7.400 | 14.138 | 3.4 |
| *Cardinalis cardinalis* | Nantucket Island | -24.247 | 45.246 | 4.046 | 13.859 | 3.3 |
| *Peromyscus leucopus* | Block Island | -25.47 | 46.766 | 5.158 | 13.391 | 3.5 |
| *Peromyscus leucopus* | Block Island | -25.362 | 47.913 | 5.127 | 14.097 | 3.4 |
| *Peromyscus leucopus* | Block Island | -24.048 | 46.217 | 4.705 | 13.692 | 3.4 |
| *Peromyscus leucopus* | Block Island | -25.348 | 48.563 | 5.400 | 14.152 | 3.4 |
| *Peromyscus leucopus* | Block Island | -24.737 | 31.115 | 5.245 | 9.092 | 3.4 |
| *Peromyscus leucopus* | Block Island | -25.119 | 47.971 | 6.184 | 13.977 | 3.4 |
| *Peromyscus leucopus* | Block Island | -24.199 | 48.096 | 4.263 | 14.123 | 3.4 |
| *Peromyscus leucopus* | Block Island | -24.67 | 49.118 | 5.015 | 14.299 | 3.4 |
| *Peromyscus leucopus* | Block Island | -25.584 | 45.452 | 4.969 | 13.451 | 3.4 |
| *Peromyscus leucopus* | Block Island | -24.551 | 47.591 | 4.829 | 14.414 | 3.3 |
| *Peromyscus leucopus* | Block Island | -25.16 | 45.932 | 5.017 | 13.463 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -24.487 | 48.537 | 4.982 | 14.490 | 3.3 |
| *Peromyscus leucopus* | Nantucket Island | -23.314 | 35.232 | 3.316 | 10.346 | 3.4 |
| *Microtus pennsylvanicus* | Nantucket Island | -22.967 | 42.153 | 5.385 | 12.500 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -26.18 | 47.170 | 2.318 | 13.692 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -23.509 | 43.148 | 3.724 | 12.674 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -23.566 | 49.010 | 3.309 | 14.298 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -23.334 | 44.847 | 1.993 | 12.843 | 3.5 |
| *Peromyscus leucopus* | Nantucket Island | -23.408 | 46.989 | 4.065 | 13.784 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -23.411 | 49.317 | 5.585 | 14.374 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -23.013 | 26.862 | 4.228 | 7.739 | 3.5 |
| *Peromyscus leucopus* | Nantucket Island | -23.832 | 42.346 | 3.171 | 11.937 | 3.5 |
| *Peromyscus leucopus* | Nantucket Island | -23.849 | 48.030 | 3.677 | 13.789 | 3.5 |
| *Peromyscus leucopus* | Nantucket Island | -25.166 | 44.273 | 5.360 | 13.110 | 3.4 |
| *Peromyscus leucopus* | Nantucket Island | -23.794 | 46.636 | 3.069 | 13.696 | 3.4 |
| *Microtus pennsylvanicus* | Nantucket Island | -23.493 | 43.768 | 2.767 | 12.550 | 3.5 |
| *Microtus pennsylvanicus* | Nantucket Island | -26.384 | 48.605 | 2.367 | 14.672 | 3.3 |
| *Microtus pennsylvanicus* | Nantucket Island | -26.429 | 42.824 | 0.823 | 13.160 | 3.3 |
| *Microtus pennsylvanicus* | Nantucket Island | -26.697 | 45.405 | 0.988 | 13.209 | 3.4 |
| *Microtus pennsylvanicus* | Nantucket Island | -26.494 | 43.498 | 2.091 | 13.265 | 3.3 |

Table A.3.1 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| *Microtus pennsylvanicus* | Nantucket Island | -26.052 | 47.414 | 0.941 | 13.583 | 3.5 |
| *Microtus pennsylvanicus* | Nantucket Island | -26.091 | 48.628 | 1.676 | 14.654 | 3.3 |
| *Microtus pennsylvanicus* | Nantucket Island | -26.642 | 46.317 | 2.131 | 13.637 | 3.4 |
| *Microtus pennsylvanicus* | Nantucket Island | -26 | 32.085 | 1.627 | 9.559 | 3.4 |
| *Microtus pennsylvanicus* | Nantucket Island | -27.003 | 49.371 | 1.862 | 14.700 | 3.4 |
| *Microtus pennsylvanicus* | Nantucket Island | -25.79 | 46.075 | 0.691 | 13.759 | 3.3 |
| *Colinus virginianus* | Nantucket Island | -20.314 | 49.631 | 3.463 | 12.479 | 4.0 |
| *Colinus virginianus* | Nantucket Island | -20.155 | 45.095 | 3.372 | 12.697 | 3.6 |
| *Colinus virginianus* | Nantucket Island | -20.382 | 43.654 | 3.859 | 12.158 | 3.6 |
| *Colinus virginianus* | Nantucket Island | -21.163 | 50.718 | 3.648 | 10.467 | 4.8 |
| *Colinus virginianus* | Nantucket Island | -20.309 | 46.150 | 4.092 | 12.123 | 3.8 |
| *Colinus virginianus* | Nantucket Island | -20.864 | 49.822 | 3.633 | 10.919 | 4.6 |
| *Agelaius phoeniceus* | Nantucket Island | -23.321 | 47.026 | 13.915 | 14.512 | 3.2 |
| *Pipilo erythrophthalmus* | Nantucket Island | -22.314 | 48.008 | 6.645 | 14.867 | 3.2 |
| *Pipilo erythrophthalmus* | Nantucket Island | -24.217 | 45.178 | 8.061 | 13.909 | 3.2 |
| *Agelaius phoeniceus* | Nantucket Island | -18.639 | 46.636 | 8.519 | 14.603 | 3.2 |
| *Passer domesticus* | Nantucket Island | -24.729 | 44.365 | 10.137 | 13.682 | 3.2 |
| *Passer domesticus* | Nantucket Island | -23.189 | 47.645 | 5.015 | 14.159 | 3.4 |
| *Pipilo erythrophthalmus* | Nantucket Island | -24.815 | 47.512 | 6.612 | 14.538 | 3.3 |
| *Agelaius phoeniceus* | Nantucket Island | -23.38 | 47.196 | 9.197 | 14.180 | 3.3 |
| *Turdus migratorius* | Nantucket Island | -24.178 | 47.781 | 6.365 | 14.810 | 3.2 |
| *Pipilo erythrophthalmus* | Nantucket Island | -22.45 | 48.032 | 6.724 | 14.525 | 3.3 |
| *Nicrophorus americanus* | Block Island | -20.483 | 47.339 | 8.710 | 9.862 | 4.8 |
| *Nicrophorus americanus* | Block Island | -25.941 | 47.522 | 7.909 | 9.921 | 4.8 |
| *Nicrophorus americanus* | Block Island | -25.906 | 41.330 | 7.760 | 8.716 | 4.7 |
| *Nicrophorus americanus* | Block Island | -27.279 | 47.799 | 5.895 | 9.717 | 4.9 |
| *Nicrophorus americanus* | Block Island | -24.052 | 46.754 | 9.355 | 9.819 | 4.8 |
| *Nicrophorus americanus* | Block Island | -26.822 | 48.411 | 11.459 | 10.403 | 4.7 |
| *Nicrophorus americanus* | Block Island | -26.201 | 46.657 | 5.526 | 9.559 | 4.9 |
| *Nicrophorus americanus* | Block Island | -25.389 | 47.421 | 7.684 | 9.783 | 4.8 |
| *Nicrophorus americanus* | Block Island | -25.58 | 45.745 | 7.926 | 9.734 | 4.7 |
| *Nicrophorus americanus* | Block Island | -24.512 | 47.528 | 8.738 | 10.609 | 4.5 |
| *Nicrophorus americanus* | Block Island | -24.241 | 47.459 | 13.955 | 9.741 | 4.9 |
| *Nicrophorus americanus* | Block Island | -22.63 | 46.318 | 10.112 | 9.709 | 4.8 |
| *Nicrophorus americanus* | Block Island | -24.38 | 46.896 | 8.259 | 9.841 | 4.8 |
| *Nicrophorus americanus* | Block Island | -25.079 | 47.195 | 7.226 | 9.796 | 4.8 |
| *Nicrophorus americanus* | Block Island | -22.871 | 48.470 | 8.392 | 10.333 | 4.7 |
| *Nicrophorus americanus* | Block Island | -23.889 | 47.894 | 9.924 | 10.140 | 4.7 |
| *Nicrophorus americanus* | Block Island | -23.812 | 46.287 | 7.209 | 9.379 | 4.9 |

Table A.3.1 continued

| | | | | | | |
|---|---|---|---|---|---|---|
| *Nicrophorus americanus* | Block Island | -25.789 | 47.304 | 7.975 | 9.593 | 4.9 |
| *Nicrophorus americanus* | Block Island | -25.473 | 46.951 | 7.323 | 9.626 | 4.9 |
| *Nicrophorus americanus* | Block Island | -24.769 | 46.810 | 7.722 | 9.727 | 4.8 |
| *Nicrophorus americanus* | Block Island | -24.093 | 46.600 | 8.106 | 9.386 | 5.0 |
| *Nicrophorus americanus* | Block Island | -22.969 | 46.498 | 9.097 | 9.389 | 5.0 |
| *Nicrophorus americanus* | Block Island | -25.164 | 47.781 | 9.434 | 9.649 | 5.0 |
| *Nicrophorus americanus* | Block Island | -22.676 | 47.878 | 6.960 | 9.869 | 4.9 |
| *Nicrophorus americanus* | Block Island | -25.557 | 46.902 | 8.476 | 9.558 | 4.9 |
| *Nicrophorus americanus* | Block Island | -22.339 | 47.554 | 7.317 | 9.744 | 4.9 |
| *Nicrophorus americanus* | Nantucket Island | -22.491 | 46.991 | 6.438 | 9.436 | 5.0 |
| *Nicrophorus americanus* | Nantucket Island | -24.599 | 49.868 | 9.316 | 9.569 | 5.2 |
| *Nicrophorus americanus* | Nantucket Island | -22.076 | 48.789 | 9.619 | 10.126 | 4.8 |
| *Nicrophorus americanus* | Nantucket Island | -24.225 | 47.945 | 9.659 | 9.841 | 4.9 |
| *Nicrophorus americanus* | Nantucket Island | -23.162 | 49.881 | 9.596 | 10.729 | 4.6 |

**Table A.6.2.** Mammal and Avian Carrion Groups

Mammalian and avian species used to determine diet item groups, including sample size, sample type, and islands where we sourced carcasses. We collected all muscle samples from frozen carcasses donated to the Maria Mitchell Association of Nantucket. Group letters represent the final reproductive diet category and a significant ($\alpha = 0.05$) difference in $\delta^{13}C$ and $\delta^{15}N$ values calculated from a multivariate analysis of variance (MANOVA) with a post hoc Tukey's multiple comparison test (Zar 2014) and a k-nearest neighbor analysis (Rosing et al. 1998; Table 2).

| Species | Source Location | *n* | Sample type | Group |
|---|---|---|---|---|
| *Peromyscus leucopus* | Block Island | 13 | Blood | E |
| *Peromyscus leucopus* | Nantucket Island | 30 | Blood | K |
| *Sciurus carolinensis* | Nantucket Island | 22 | Muscle | K |
| *Rattus norvegicus* | Nantucket Island | 7 | Muscle | J |
| *Rattus norvegicus* | Block Island | 2 | Muscle | D |
| *Sylvilagus floridanus* | Nantucket Island | 7 | Muscle | K |
| *Microtus pennsylvanicus* | Nantucket Island | 31 | Blood | K |
| *Agelaius phoeniceus* | Nantucket Island | 6 | Blood | G |
| *Cardinalis cardinalis* | Nantucket Island | 3 | Blood | G |
| *Dumetella carolinensis* | Block Island | 14 | Feather/Muscle | A |
| *Dumetella carolinensis* | Nantucket Island | 3 | Blood | F |
| *Turdus migratorius* | Block Island | 1 | Blood | A |
| *Turdus migratorius* | Nantucket Island | 2 | Blood | F |
| *Bombycilla cedrorum* | Nantucket Island | 1 | Blood | F |
| *Colaptes auratus* | Nantucket Island | 1 | Muscle | H |
| *Cyanocitta cristata* | Nantucket Island | 1 | Muscle | G |
| *Scolopaz minor* | Nantucket Island | 2 | Muscle | H |
| *Anas platyrhynchos* | Nantucket Island | 1 | Muscle | F |
| *Sturnus vulgaris* | Block Island | 4 | Feather | A |
| *Sturnus vulgaris* | Nantucket Island | 20 | Blood/Muscle | F |
| *Larus argentatus* | Nantucket Island | 1 | Muscle | I |
| *Corvus brachyrhynchos* | Nantucket Island | 1 | Muscle | F |
| *Cartharus guttatus* | Nantucket Island | 1 | Muscle | H |
| *Aix sponsa* | Nantucket Island | 1 | Muscle | I |
| *Fulica americana* | Nantucket Island | 1 | Muscle | I |
| *Melanitta nigra* | Nantucket Island | 1 | Muscle | I |
| *Clangula hyemalis* | Nantucket Island | 1 | Muscle | I |
| *Somateria mollissima* | Nantucket Island | 1 | Muscle | I |
| *Colinus virginianus* | Nantucket Island | 18 | Muscle | C |
| *Pipilo erythrophthalmus* | Nantucket Island | 4 | Blood | F |
| *Passer domesticus* | Nantucket Island | 3 | Blood | G |
| *Phasianus colchicus* | Block Island | 12 | Feather | B |
| *Setophaga ruticilla* | Block Island | 2 | Feather | A |
| *Setophaga palmarum* | Block Island | 5 | Feather | A |

# VITA

**BRANDON QUINBY**

Graduate Research Assistant

Department of Forestry and Natural Resources

Purdue University

## EDUCATION

Purdue University, Wildlife Science, Ph.D., 2020

Purdue University, Calumet, Biology, M.S., 2016

Purdue University, North Central, Biology, B.S., 2013

## APPOINTMENTS

2016-2020 Graduate Research Assistant, Department of Forestry and Natural Resources, Purdue University

2016-2020 Graduate Teaching Assistant, Department of Forestry and Natural Resources, Purdue University

2013-2016 Graduate Teaching Assistant, Department of Biology, Purdue University Calumet

2013-2016 Graduate Research Assistant, Department of Biology, Purdue University Calumet

2011-2012 Undergraduate Teaching Assistant, Department of Biology, Purdue University North Central

## PUBLICATIONS

**Quinby, B.M.,** Creighton, J.C., and M.C. Belk. *Accepted* Behavioral constraints on local adaptation and counter-gradient variation: Implications for climate change. Ecology and Evolution.

**Quinby, B.M.**, Feldman*, N.S., Flaherty, E.A., Belk, M.C., Smith, A.D.F., and J.C. Creighton. (2020) Isotopic discrimination between carrion and elytra clippings of lab-reared American burying beetles (*Nicrophorus americanus*): Implications for conservation and evaluating feeding relationships in the wild. Rapid Communications in Mass Spectrometry, 34, (12), p.e8785

**Quinby, B.M.,** Creighton, J.C., and E.A. Flaherty. *Under Review June 2020*. Stable isotope ecology in insect studies: a review. Ecological Entomology.

**Quinby, B.M.,** Creighton, J.C., and E.A. Flaherty. *Under Review June 2020*. Photo-identification capture-mark-recapture using HotSpotter™ to estimate populations of burying beetles. Environmental Entomology.

**TEACHING EXPERIENCE**

Purdue University

FNR 348: Wildlife Investigational Techniques (2016-2018)

Laboratory Instructor

FNR 341: Wildlife Habitat Management (2019)

Laboratory Instructor

FNR 498: Optimizing TWS Meeting Attendance (conference preparation course; 2018)

Laboratory Instructor

SA 21268: Environment and Culture in the Galapagos (2019)

Purdue University Calumet

BIOL 102: Introductory Biology II (2013-2016)

Laboratory Instructor

BIOL 348: Evolution (2013-2016)

Laboratory Instructor

BIOL 333: Ecology (2013-2016)

Laboratory Instructor

BIOL 213: Anatomy and Physiology I (2015)

Laboratory Preparation and Grading

BIOL 214: Anatomy and Physiology II (2015)

Laboratory Preparation and Grading

**PRESENTATIONS (* = mentored student)**

**Quinby, B.M.**, Flaherty, E.A., and J.C. Creighton. Evaluation of the Vertebrate Carrion Resources used by the American Burying Beetle (*Nicrophorus americanus*). Contributed Talk -NBI 2019, 8th Biennial Research Conference of the Nantucket Biodiversity Initiative, Nantucket MA, November 2019.

**Quinby, B.M.**, Flaherty, E.A., and J.C. Creighton. Evaluation of the Prey Base and Feeding Relationships of the American Burying Beetle (*Nicrophorus americanus*). Contributed Talk- IECC 2019, 30th Annual Meeting of Invertebrates in Education and Conservation, Tucson U.S.A., July 2019.

**Quinby, B.M.**, Flaherty, E.A., Creighton, J.C. Evaluation of the Prey Base and Feeding Relationships of the American Burying Beetle (*Nicrophorus americanus*) Using Stable Isotope Analysis. Contributed Talk- Evolution 2019, Annual Meeting of the Society for the Study of Evolution (SSE), the Society of Systematic Biologist (SSB), and the American Society of Naturalists (ASN), Providence U.S.A., June 2019.

**Quinby, B.M.**, Flaherty, E.A., and J.C. Creighton. Evaluation of the Prey Base and Feeding Relationships of the American Burying Beetle (*Nicrophorus americanus*) on Nantucket Island. Contributed Poster- TWS 2018, 25th Meeting of The Wildlife Society, Cleveland U.S.A., October 2018.

**Quinby, B.M.**, Flaherty, E.A., and J.C. Creighton. Evaluation of the Prey Base and Feeding Relationships of the American Burying Beetle (*Nicrophorus americanus*) on Nantucket Island.. Contributed Poster- ISBE 2018, 17th Congress of the International Society of Behavioral Ecology, University of Minnesota, Minneapolis U.S.A, August 2018.

**Quinby, B.M.**, Flaherty, E.A., Creighton, J.C., Feldman, N., Smith, A.D.F., and M.C. Belk. Evaluation of the Prey Base and Feeding Relationships of the American Burying Beetle (*Nicrophorus americanus*) Using Stable Isotope Analysis. Contributed Talk-NBI 2017, 7th Biennial Research Conference of the Nantucket Biodiversity Initiative, Nantucket MA, November 2017.

**Quinby, B.M.**, Creighton, C. and M.C. Belk. Latitudinal Variation in Life History Strategies in the Burying Beetle, *Nicrophorus orbicollis.* Contributed Talk- ISBE 2016, 16th Congress of the International Society of Behavioral Ecology, University of Exeter, Exeter U.K., June 2016.

**Quinby, B.M.** and J.C. Creighton. Latitudinal Variation in Life History Strategies in the Burying Beetle, *Nicrophorus orbicollis.* Contributed Poster- ABS 2015, Meeting of the Animal Behavior Society, University of Alaska Anchorage, June 2015.

**Quinby, B.M.**, Palmer, M., Anspach, C., Rivera, O., Haro, J., and V. Quinn. Behavioral Consequences of Eavesdropping in Crayfish. Contributed Poster-Butler Undergraduate Research Conference, Butler University, April 2012.

Anspach, C., Palmer, M., **Quinby, B.M.**, Rivera, O., Haro, J., and V. Quinn. Crayfish Social Interaction and its Effects on Serotonin. Contributed Poster-Butler Undergraduate Research Conference, Butler University, April 2012.

**Quinby, B.M.**, Palmer, M., Anspach, C., Rivera, O., Haro, J., and V. Quinn. Behavioral Consequences of Eavesdropping in Crayfish. Contributed Poster-Indiana Academy of Sciences 2012, Purdue University West Lafayette, February 2012.

Anspach, C., Palmer, M., **Quinby, B.M.**, Rivera, O., Haro, J., and V. Quinn Crayfish Social Interaction and its Effects on Serotonin.. Contributed Poster-Indiana Academy of Sciences 2012, Purdue University West Lafayette, February 2012.

**Quinby, B.M.**, Flaherty, E.A., and J.C. Creighton. Individual Identification of Burying Beetles Using Hotspotter™. 2019. Contributed Poster-Forestry and Natural Resources Research Symposium, West Lafayette, IN.

Tauber, E.K*. **Quinby, B.M.**, Swihart, R.K., and E.A. Flaherty. Estimating Decay Rates of Deer Pellets in Central Indiana. Contributed Poster-2019 Forestry and Natural Resources Research Symposium, West Lafayette, IN.

**Quinby, B.M.**, Flaherty, E.A., and J.C. Creighton. Evaluation of the Prey Base and Feeding Relationships of the American Burying Beetle (*Nicrophorus americanus*) on Nantucket Island. Contributed Poster- 2018 Forestry and Natural Resources Research Symposium, Purdue University, West Lafayette IN, April 2018.

**Quinby, B.M.** and J.C. Creighton. Latitudinal Variation in Life History Strategies in the Burying Beetle, *Nicrophorus orbicollis*. Contributed Talk-Purdue University, Calumet Graduate Research Day, Purdue University, Calumet, March 2016.

**Quinby, B.M.**, Fieldman, N.S.*, and J.C. Creighton. Variation in Breeding Behavior, Development, and Immune Response Along a Latitudinal Gradient in the Burying Beetle, *Nicrophorus orbicollis*. Contributed Poster- Purdue University, Calumet Graduate Research Day, Purdue University, Calumet, March 2015.

**Quinby, B.M.**, Prieto, V.*, and J.C. Creighton. The Effects of Environmental Temperature on Immune Responses and Reproductive Behavior in the Burying Beetle, *Nicrophorus orbicollis.* Contributed Poster- Purdue University, Calumet Graduate Research Day, Purdue University, Calumet, March 2014.

## INVITED SEMINARS AND GUEST LECTURE

**2020** *Stable isotope ecology and ecophysiology of burying beetles.* Purdue University Northwest Seminar Series, Hammond, IN.

**2019** *Research Design and Caribou Case Study Lectures.* Wildlife Habitat Management. Purdue University, West Lafayette, IN.

**2018** *Live History Evolution Lecture.* Principals of Evolution. Purdue University Northwest, Hammond, IN.

**2018** *Sexual Selection Lecture.* Animal Behavior. Purdue University Northwest, Hammond, IN.

## SCHOLARSHIPS/GRANTS

**2019** Purdue University Graduate Student Council Travel Grant ($250)

**2019** Terrestrial Invertebrate Taxon Advisory Group ($500)

**2016-2018** Purdue University Graduate School, Ross Fellowship ($22,000)

**2018** Nantucket Biodiversity Initiative ($1,000)

**2017** Sophie Danforth Conservation Biology Fund ($1,000)

**2017** Theodore Roosevelt Memorial Fund ($1,000)

**2017** Nantucket Biodiversity Initiative ($1,044)

**2015** Purdue University Graduate Student Research Grant ($750)

**2015** Purdue University Graduate School, Conference Travel Grant ($1000)

**2011-2012** LSAMP Undergraduate Research Award ($500)

## HONORS AND AWARDS

**2020** Purdue Teaching Academy Graduate Teaching Award, Purdue University

**2020** Conservation Leaders for Tomorrow Professional Development Scholarship

**2020** Exemplary Graduate Student Service Award Purdue University

**2013-2016** Chancellor's List Purdue Calumet

**2013-2016** Semester Honors Purdue Calumet

**2009-2013** Chancellor's List Purdue North Central

**2009-2013** Semester Honors Purdue North Central

**2009-2013** Louis Stokes Alliance for Minority Participation Undergraduate Research

## SERVICE AND MENTORING

**2019-2020** Graduate student mentor for the Purdue University Chapter of The Wildlife Society

**2019** Graduate student mentor for Diversity in Faces, Spaces and Places Research and Extension Experiential Learning for Undergraduates (REEU)

**2018-2019** Graduate student mentor for undergraduate research project evaluating the use of deer pellets and their decay rate as an indirect measurement of deer density.

**2018** Graduate student mentor for Purdue Summer Stay Undergraduate Research Project evaluating the use morphometric and geometrics to identify individual sea turtles and burying beetles.

**2017-2020** Graduate student Ombudsman for the Forestry and Natural Resources Departmen.

**2017-2018** President of the Forestry and Natural Resources Graduate Student Council.

**2016-2017** Treasurer of the Forestry and Natural Resources Graduate Student Council.

**2014-2015** Science Olympiad Volunteer, Judge, and Exam writer. Developed and administered Entomology test

**2013-2019** Volunteer at department sponsored events. Made myself available for any department/club organization, new student orientation, recruitment events, and community activities.

**2012-2013** Student Administrator for campus wide Supplemental Instruction program Purdue North Central.

**2012-2013** Field Research Assistant to Dr. Vanessa Quinn Purdue University North Central. Tracked butterfly flight patterns in response to anthropogenic disturbance.

**2012-2013** Student Administrator for campus wide Supplemental Instruction program Purdue North Central.

**2012** Tri Beta Biological Honor Society Treasurer Purdue North Central

**2012** Inductee Beta Beta Beta National Biological Honor Society

**2011** Inductee Alpha Sigma Lambda National Honor Society for non-traditional students

**2011** President PNC Pre-Vet Club

**2010** President/Founding member PNC Gay-Straight Alliance

**2010** Treasurer PNC Pre-Vet Club