What they do in the shadows: stable isotope analysis reveal that spatial and temporal heterogeneity explain dietary niche variation in *Myotis lucifugus* in Newfoundland

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Abstract

Individuals must balance competitive and environmental pressures with obtaining the nutrients necessary to survive and reproduce. The goal of this project was to infer on individual dietary adaptations of adult female *Myotis lucifugus* from a maternity group. Therefore, I conducted stable isotope analysis on feces (*n* = 127), arthropods (*n* = 110), and hair (*n* = 120) collected from known individuals across two timescales (feces sampled May-August 2017; hair sampled 2012-2017). I used a Bayesian mixing model (MixSIAR) and an information-theoretic approach to determine models that best explained variation in isotopic niche. Isotopic niche variation across both timescales was strongly explained by spatial and temporal heterogeneity, with little explanatory power provided by inter-individual or reproductive group heterogeneity. Diets of individual bats were opportunistic, with strong dependence on the most abundant prey groups, although diets of most individuals contained a limited amount of all prey groups.

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Introduction

Variation among individuals of the same species in a population provides a mechanism by which multiple members of a species may survive and coexist. This may be accomplished through both limitation of intraspecific competition and the ability of a population to take maximum advantage of available resources (Bolnick et al. 2003; Svanbäck and Bolnick 2005; Sargeant 2007; Araújo et al. 2011). Additionally, genetic variation among individuals represents a tangible object upon which natural selection may act (Van Valen 1965; Bolnick et al. 2011). In ecology there are concepts used to define and understand properties of biological systems, such as relevance of foraging decisions (e.g., optimal foraging theory) or the interactions of an organism with it's environment (e.g., niche theory). I will use these concepts to provide a framework within which to quantify inter-individual dietary niche variation in an insectivorous bat species.

To begin, the ecological niche concept is defined as the genetically (or evolutionarily) determined range of tolerances and pattern of biological response of individuals, populations, or species to environmental conditions (Maguire, Jr. 1973). Environmental conditions may include all physical (e.g., temperature, wind, pH) and biological (e.g., competition, population density, predation, parasitism) conditions with which an individual, population, or species may interact (Maguire, Jr. 1973). Traditionally, the niche concept assumed that conspecifics were functionally equivalent in the environment (Elton 1946; Hutchinson 1957; Levins 1968; Colwell and Futuyma 1971). In terms of diet, this assumption held that conspecifics consumed various prey types in the same proportions. In many cases this assumption was disproven or has not been tested (Bolnick et al. 2003; Sargeant 2007; Araújo et al. 2011). Therefore, assuming functional equivalence is only valid if niche variation among individuals has little or no biological significance (Bolnick et al. 2003).

Niche theory predicts that if individuals of a species do not consume the same proportions of prey items at the same time, then individuals (or subsets of individuals) within a population partition resources (i.e., the realized niche of an individual is smaller than the fundamental niche (Hutchinson 1957)). The “width” of the foraging niche of an individual may depend on its phenotype and the diversity (or complexity) and abundance of local resources (Araújo et al. 2011). Therefore, morphological or behavioral variation among individuals may reduce intraspecific competition and increase resource exploitation and fitness (Hutchinson 1957; Schoener 1974; Finke and Snyder 2008). To represent the relationship between subgroups of individuals, the niche width concept apportions a populations’ total niche width into three components: the variation in resource use within individuals, among individuals, and among subgroups of individuals (e.g., social units (Roughgarden 1972; Bolnick et al. 2002)). If different individuals consume prey in different proportions, among-individual and among-subgroup components of variation will be greater than those explained by variation within individuals: resulting in among-individual partitioning of local resources (Roughgarden 1972; Bolnick et al. 2002; Newsome et al. 2012; Rosenblatt et al. 2015). From an evolutionary perspective, high levels of intraspecific competition may generate selection for increased population-level fundamental niche width by increasing the number of accessible resources (Araújo et al. 2011), while interspecific competition generates selection for decreased total fundamental niche width by limiting access to resources (MacArthur and Levins 1964; Slatkin 1980). In this way, existing variation among individuals or groups represents a heritable amount of variation in resource use, providing the genetic basis for evolution of a species' dietary niche (Araújo et al. 2011).

Similarly, the niche variation concept maintains that competitive release leads to expansion of the niche width of a population via increased among-individual variation (Van Valen 1965; Bolnick et al. 2007; Araújo et al. 2011). Under this concept, inter-individual variation within populations of ecological generalists is best explained by phenotypic or behavioral heterogeneity relative to ecological specialists (i.e., individuals within generalist populations are more variable in traits and behaviors, and therefore better suited to heterogeneous environments than specialists (Van Valen 1965; Levins 1968; Bolnick et al. 2007; Araújo et al. 2011)). The first assumption of this concept is that a subset of individuals of a population have higher relative fitness under certain, but not all environmental conditions. Second, variation in fitness among subsets must be heritable (e.g., genetic or learned). Third, selection of mates is not random. For example, individuals may preferentially occupy a given environment more than other environments, or select mates from individuals in a particular subset more frequently than from other subsets (Van Valen 1965).

Optimal foraging theory (MacArthur and Pianka 1966) can be used to explain inter-individual variation in foraging niches (Araújo et al. 2011). It predicts that an individual's deviation from an "optimal" diet is explained by: 1) phenotypic or learned differences in foraging behavior (i.e., ability to locate, track, handle, or consume alternative prey); 2) differences in physiological condition, energetic, or nutritional requirements (e.g., due to differential costs of reproduction); and/or 3) differences in ability to obtain an optimal diet (e.g., due to social status or rank (Morse 1974; Araújo et al. 2011)).

Central place foraging theory is a specific application of optimal foraging theory, where individuals forage from and return to a fixed location (Kacelnik 1984; Elliot 1988; Daniel et al. 2008; Olsson et al. 2008; Olsson and Bolin 2014). This predicts that individuals maximize long-term energy gain by expending more effort (or time) in patches at shorter distances from a fixed location (Schoener 1979; Daniel et al. 2008; Olsson et al. 2008; Rainho and Palmeirim 2011). Limitations in access to optimal prey items within an optimal distance may increase levels of intraspecific competition close to the central location (Kacelnik 1984; Elliot 1988). This increase in competition at shorter distances to the central location may in turn drive selection pressures acting on individual behavioral foraging plasticity or niche variation.

Stable isotope analysis (SIA) is a powerful tool that can be used to make inferences about diets of individual organisms. Many elements in nature have one or more stable isotopes that are heavier in mass and do not decay. Heterogeneity in the relative composition of these isotopes leads to naturally occurring spatial variance in these isotopes. Consumers assimilate and reflect this variance from the environments in which they feed. Two isotopes most commonly used to illustrate trophic interactions among individuals in a population are stable carbon and stable nitrogen. Stable carbon ($\delta^{13}C$) is an effective biological tracer of energy flow within food webs, because stable carbon values of organisms from a given environment fall within the ranges of stable carbon values of plants (or other primary producers) in a particular environment (DeNiro and Epstein 1978). This relationship is explained by differential carbon uptake by plants during photosynthesis. When coupled with knowledge of photosynthetic pathways used by plants in an environment (i.e., C_3, C_4, CAM), SIA of stable carbon reveals landscape origins of assimilated diet items (DeNiro and Epstein 1978; Kelly 2000; Fry 2006; Araújo et al. 2007; Crawford et al. 2008). Isotopes of stable nitrogen ($\delta^{15}N$) are preferentially incorporated into consumer tissues with an increase in concentration between two (2) and four (4) parts per thousand (‰) at each trophic level throughout known systems (Kelly 2000; Fry 2006). This phenomenon may illustrate trophic interactions among individual diets if potential food sources have different stable nitrogen values (DeNiro and Epstein 1981). In this way, stable nitrogen isotopes have been used to provide evidence of trophic resource partitioning within communities (e.g., higher and lower order consumers using similar resources) (Crawford et al. 2008).

Isotopic niche composition in a mixing space (δ-space) closely resembles the n-dimensional niche space conceptualized by niche theory (Hutchinson 1957; Newsome et al. 2007). Stable isotope analysis merges information on both resource (bionomic) and habitat (scenopoetic) use into a continuous, quantitative representation of the diet of individuals (Newsome et al. 2007; Crawford et al. 2008). Stable isotope analysis can provide information over different temporal and spatial scales, because sample materials (e.g., skin, organs, blood, hair, claws) have different turnover rates and isotopes can originate from different sources (Newsome et al. 2007; Crawford et al. 2008). For these reasons, SIA is particularly well-suited for investigations of dietary niche variation among individuals (Urton and Hobson 2005; Araújo et al. 2007; Newsome et al. 2007; Cryan et al. 2012; Tinker et al. 2012; Robertson et al. 2014; Rosenblatt et al. 2015).

Bats (Order Chiroptera) are a useful model for examining predictions of niche, optimal foraging, and central place theory. Most temperate bat species forage primarily on insects and other arthropods, which restricts the majority of foraging to times of the year when these organisms are active (Black 1974). Bats are unusually long-lived for a small mammal, reproduce slowly, and often roost together in large numbers (Altringham 1996). Many species of bats are capable of learning and teaching complex behaviors and patterns (Barclay 1982; Jones and Ransome 1993; Gillam 2007; Wright et al. 2011; Clarin et al. 2014), and because of their long lifespan, may be able to increase the efficiency of foraging behaviors over years or seasons. Due to these factors (e.g., restricted access to nutrients and energy, the energetic costs of flight and reproduction, and the behavioral and competitive pressures surrounding access), insectivorous bats provide a practical natural system to test predictions of empirical ecological theories over short and long timescales. Previous studies of bats suggest the presence of individual niche variation in species of insectivorous and frugivorous bats (reviewed by Bolnick et al. (2003) and Araújo et al. (2011)). However, few studies have specifically examined the question (Herrera et al. 2008; Cryan et al. 2012). Of those studies, only one (Herrera et al. 2008) incorporated source dietary isotopic values collected from fruits into their estimation of individual niche variation in Egyptian fruit bats (*Rousettus aegyptiacus*). The other study (Cryan et al. 2012) provided evidence for individual niche variation among adult female insectivorous big brown bats (*Eptesicus fuscus*) using a variance components analysis of four different stable isotope elements ($\delta^2H, \delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) but cited logistical difficulty and limitations in scope as the primary reasons to abandon characterization of isotopic composition of potential prey items (Cryan et al. 2012). This leaves estimation of individual niche variation in a way that incorporates isotopic signatures and relative dietary contributions of prey groups within insectivorous bats largely unstudied (e.g., Broders et al. 2014).

Little brown bats, *Myotis lucifugus* (Chiroptera: Vespertilionidae), are nearctic insectivorous bats that are thought to prey largely on midges and related flies (Diptera: Chironomidae and others), moths (Lepidoptera), mayflies (Ephemeroptera), caddisflies (Trichoptera), and small beetles (Coleoptera) (Belwood and Fenton 1976; Fenton and Morris 1976; Anthony and Kunz 1977; Whitaker, Jr. and Lawhead 1992; Whitaker, Jr. 2004; Clare et al. 2011; Clare et al. 2014). Previous research of isotopic niche widths of *M. lucifugus* in Atlantic Canada suggests a broad species-level trophic niche (Broders et al. 2014). *Myotis lucifugus* are thought to locate, capture, and ingest flying prey while in flight (Burles et al. 2008), although they have also been found to use other foraging techniques such as gleaning to capture stationary prey (e.g., spiders and beetles) (Ratcliffe and Dawson 2003). In this way, diet composition, niche width, and foraging strategy portray *M. lucifugus* as an opportunistic, generalist predator suited to consuming ephemeral patches of aquatic nocturnal insects.

Individual *M. lucifugus* at the edge of their habitable range experience more severe environmental constraints than conspecific individuals at the core of the range (Boyles et al. 2016). These constraints likely result in limitations on reproduction. Reproduction is energetically costly for females, and can have a substantive impact on the energy, nutritional, and time budgets of pregnant and lactating bats. In other Myotis bats, the timing of parturition has been linked to the availability of prey, further highlighting these energetic and nutritional costs (Arlettaz et al. 2001). Not surprisingly, energetic costs associated with reproduction equate to expansive differences in energy, nutritional, and time budgets throughout the period spanning emergence from hibernation. These expanses continue throughout gestation, parturition, and lactation when compared to juvenile (Belwood and Fenton 1976; Adams 1996), non-reproductive female, and male conspecifics (Anthony et al. 1981; Barclay 1989; Kurta et al. 1989; Barclay 1994; McLean and Speakman 1999). Therefore, energetic costs and budget differences among reproductive females and other reproductive classes, age classes, and sexes likely contribute to foraging niche variation. Interestingly, increased prey abundance leads to more selective feeding in pregnant and lactating females (Anthony and Kunz 1977; Clare et al. 2011), likely due to increased caloric and nutritional demands throughout gestation, parturition, and lactation (Barclay 1994). This observed increase in prey selectivity also likely reflects the increased prey abundance found throughout this season (Anthony and Kunz 1977).

Throughout gestation, parturition, and lactation, female *M. lucifugus* assemble in maternity groups numbering hundreds or thousands of individuals (Fenton and Barclay 1980). In these communal maternity groups, females display roost site fidelity and communally raise offspring (Dixon 2011). This affinity for such roost locations implicate female *M. lucifugus* as central-place foraging strategists, with the roost acting as the fixed point to which individuals return after each foraging bout (Olsson et al. 2008). Based on predictions of central place foraging theory and competition-based niche theory, this system may be costly from a nutrient/energy uptake perspective. Reproductive females in this system have high energetic and nutritional requirements, are limited late in pregnancy and early in lactation in both range of travel and maneuverability (Norberg and Rayner 1987; Kurta et al. 1989), and therefore may be more limited in access to resources than non-reproductive or male conspecifics. These limitations may equate to increased intraspecific competitive pressure acting on reproductive female individuals in the system. Why may a social system be advantageous in spite of increased intraspecific competitive pressures and resource limitations at the most energetically costly stage of the life cycle of an adult female individual? I suggest that this system may persist for the following reasons: 1) due to energetic or nutritional resources that are abundant beyond the point of limitation; 2) social behaviors associated with communal living (e.g., huddling, information sharing, altruism) may equate to profound energy savings, increasing the fitness of individuals belonging to the system (Wilkinson 1988; Gilbert et al. 2010; Clarin et al. 2014; Boratyński et al. 2015); 3) competitive pressures may be driving selection for niche width expansion via increased inter-individual variation (i.e., reproductive females in this system may select different proportions of prey items or forage in different areas).

The goal of this project was to investigate foraging niche variation among individuals and subgroups of adult female *M. lucifugus* belonging to a communal maternity colony using a stable isotope mixing model analysis. I was specifically interested in the potential fitness trade-offs associated with short and long timescale membership in a central-place foraging maternity system and the ecological mechanisms underlying the persistence of these systems. Therefore, I hypothesized that dietary niche variation among individuals or subgroups would explain more of the variation in population-level niche width than within-individual variation and would constitute the primary mechanism facilitating maternity system persistence. As such, I assumed that access to resources was limiting, and energy savings associated with communal living were not profound enough to perpetuate the system. To test this hypothesis, I created two suites of candidate models to explain dietary variation, incorporate hierarchical levels of population structure, and assess variation associated with short and long timescales (e.g., capture date for short timescale models and capture year for long timescale models). If variation among individuals or subgroups explained dietary variation on a given night, then I expected models containing individual and hierarchical subgroup effects to perform better than models without these effects. Finally, I expected the pattern to be more pronounced across years or seasons due to increased selection for inter-individual variation throughout the social system over time and long timescale stabilization of variance among assimilation of isotopes and stabilization of foraging patterns.

Materials and Methods

Study site: This research took place in the greater Salmonier Nature Park, approximately 20 km southwest of Holyrood, Newfoundland (UTM 22T 0327283m E 5237135m N or 47.26494° N, -53.2832° W), the site of a long-term research program to monitor various aspects of the ecology and basic life history of *M. lucifugus*. The approximately 2000 individuals in the study system use 11 bat roost boxes as roost structures. Distances among boxes ranged from 5 m to 1000 m. The dimensions and design of each of the roost boxes were the same (i.e., four chamber nursery house (Tuttle et al. 2013)), but heights, aspects, and solar exposure of each roost box differed (Besler 2018 unpublished data).

The study site is characterized by mean daily summer temperatures of approximately 14° C, 1200-1500 mm of cumulative annual precipitation, and mean relative humidity of approximately 80% (Banfield 1983). Average wind speed in the region is approximately 20 km/hour (Khan and Iqbal 2004). Land cover in this region is comprised of wet boreal forests, predominantly containing balsam fir (*Abies balsamea)* (Thompson et al. 2003), barrens dominated by heaths (Ericaceae), and peatlands dominated by peat mosses (Sphagnaceae) (Protected Areas Association of Newfoundland and Labrador 2008). In this province, reproductive female *M. lucifugus* are thought to be limited by access to suitable natural roosts and endure relatively short and harsh gestation and lactation periods (Park and Broders 2012). It is likely that this study site has lower benthic arthropod diversity, biomass, and productivity relative to locations at the core of the range (Larson and Colbo 1983; Clarke 1995; Clarke and Scruton 1997). Thus, obtaining sufficient caloric resources may be more challenging for individual bats in this location than in the core of the range. Impacts of both environmental and biological conditions (e.g., weather and prey abundance/competition) may be multiplicative, and can have profound effects on diet and reproductive success of *M. lucifugus* and other insectivorous bats (Burles et al. 2008; Burles et al. 2009).

Fecal and hair sample collection: I captured free-flying bats in mist nets (Avinet, Inc., PO Box 1103 Dryden, NY 13053 USA.) as they returned from nightly bouts of foraging a minimum of once per week from 18 May through 27 July 2017. I did not attempt to capture bats if the ambient temperature was lower than zero degrees (0°) Celsius, if precipitation exceeded more than one mm/hour, or if sustained wind speeds exceeded 25 km/hour. If a night could not be sampled due to weather or other unforeseen circumstances, the next consecutive satisfactory night was sampled. Once captured, individual bats were held in paper bags (#5; dimensions 13 x 8 x 27 cm) for 10-60 minutes inside either a building or vehicle to safely allow the individual to defecate in the bag. Each individual was assigned to age-class based on ossification of the epiphyseal growth plate of the metacarpals (Brunet-Rossini and Wilkinson 2009), sexed by examination of the genitals, assessed for reproductive condition by palpation of the abdomen (Racey 2009) and weighed. I used estimates of digestion time in active female *M. lucifugus* (35-50 minutes (Buchler 1975)), and time required for the materials in feces to acquire a digested isotopic signature (1-2 hours (Salvarina et al. 2013)) to restrict the sampling period. This period began at 00:00 and continued until sunrise to ensure samples collected on a night were representative of nightly consumption. A sampling night was considered valid if samples were collected from no fewer than eight individuals. Using this restriction, a total of six representative sample nights were selected (18 May, 12 June, 23 June, 06 July, 17 July, 27 July 2017). Hair samples were collected from between the scapulae of captured individuals using cuticle scissors (Tweezerman® International, 2 Tri Harbor Court, Port Washington, NY U.S.A.). Fecal and hair samples were placed individually in labelled 1.5 ml polypropylene micro-centrifuge tubes (Fisher Scientific Company, 112 Colonnade Road Ottawa, ON K2E 7L6 Canada), and frozen within 3 hours for long term storage at -20°C (field conditions) or -80°C (laboratory conditions (Hobson et al. 1997)). All analyses were restricted to adult female individual bats to control for niche variation due to age class or sex (Belwood and Fenton 1976; Anthony and Kunz 1977; Adams 1996).

Arthropod sample collection: I concurrently collected potential prey of little brown bats (i.e., nocturnal arthropods) throughout a feeding period using a combination of aquatic emergence traps (Modified model WEEK (LeSage and Harrison 1979)), Malaise traps (BugDorm/MegaView Science Co., Ltd. No. 656-2, Fuya Rd., Taichung 40762 Taiwan), UV black-light insect traps (BioQuip Products, 2321 Gladwick Street Rancho Dominguez, CA 90220 USA), and light-assisted pitfall traps (Luminoc, BIOCOM, 2120 Lavoisier, Sainte-Foy, Quebec, Canada G1N 4B1). This combination of arthropod traps was selected in an attempt to avoid the inherent biases associated with each method and to create a more complete snapshot of available prey in the area. I supplemented infrequent trap collections of spiders (order Araneae) with hand-collected spiders from aquatic, forested, and anthropogenic habitat types on the night of 15 August 2017. Once collected, samples were stored in high-grade ethanol (70-90%) and stored at -20ºC or -80ºC (Hobson et al. 1997). I identified insect specimens to order or higher resolution using a dissecting scope and an insect key (Borror et al. 1989).

Animal care: This research was conducted under permit from the Saint Mary's University Animal Care Committee, the Canadian Council on Animal Care, and the Department of Fisheries and Land Resources of Newfoundland and Labrador. Per the scientific permit agreement, no equipment used on this project was used on any other project at any location to prevent accidental spread of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome, an infectious fungal disease responsible for the deaths of millions of North American bats since 2006 (Blehert et al. 2009; Lorch et al. 2011; Vonhof et al. 2015).

Stable Isotope Analysis (SIA): A stable isotope sample consisted of a targeted mass of 0.35 ± 0.02 mg of ground, oven-dried (at 60°C for 24 hours) arthropods (n = 110), feces (n = 127), or lipid-extracted hair samples hair (n = 120, N = 359, mean mass = 0.346, SD = 0.026). Each sample was weighed into 3.5 x 5 mm tin capsules using a microbalance (UMT2, Mettler-Toledo, LLC, 1900 Polaris Parkway, Columbus, OH, 43240 USA). Lipids present in the tissue or pelage of organisms can skew SIA results, leading to misinterpretations of consumer $\delta^{13}C$ values. To address this, I extracted lipids from hair samples using the procedures of Folch et al. (1956) and Blight and Dyer (1959) for lipid extraction from organismal tissue. Each hair sample was washed three times in a 2:1 chloroform:methanol solution for a period of 15 minutes each with a final air dry beneath a fume hood for 12 hours (Folch et al. 1957; Bligh and Dyer 1959; Cryan et al. 2012). Each sample was analyzed for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) through combustion conversion of sample material to gas through a 4010 elemental analyzer (Costech International, Via Firenze 30A, 20063, Cernusco S/Nav, Milan, Italy) coupled to a Delta Plus XL (Thermo Fisher Scientific, 168 3rd Avenue, Waltham, MA 02451 USA) continuous flow isotope ratio mass spectrometer at the Environmental Isotope Lab at the University of Waterloo (Waterloo, Ontario, Canada). The stable carbon ($\delta^{13}C$) reported is the corrected delta value in per mil (‰) units against the primary reference scale of Vienna Pee Dee Belemnite. Likewise, stable nitrogen ($\delta^{15}N$) reported is also the corrected delta value in per mil (‰) units against the primary reference scale of atmospheric air. Quality control was monitored and certified using international reference materials. Of the total sample number in an analytical run, no less than 20% of samples were standardized or reference materials. Standardized and reference materials were used to infer data normalization, to quantify daily mass spectroscopy precision, and to assess linearity issues or spectroscopy drift throughout the duration of an analytical sample run.

Statistical analysis: All data were analyzed in R Version 3.4.2 (R Core Team 2017). I used a parallelized version of the hierarchical Bayesian mixing model framework MixSIAR (Semmens and Stock 2016). The goal of a Bayesian approach is to estimate the probability of a model parameter being a certain value given the data and prior knowledge about the system before the data are collected. In this way, our understanding about the system (and its associated parameters) is updated, in a statistically robust way, by the collection of data, in which the process reassigns probabilities across possibilities (hypotheses) for each parameter. The prior probabilities represent the range of potential values each model parameter can take, and the associated likelihoods (Gelman et al. 2004; Kruschke 2014). Markov Chain Monte Carlo (MCMC) methods are used to estimate these probabilities (Gelman et al. 2004; Kruschke 2014). The results of Bayesian analysis (i.e., posterior estimates) yield the probability of a given model based on collected data and explicitly-specified prior beliefs (Gelman et al. 2004). I chose the MixSIAR framework because it was specifically developed for studies like this, with the aim of estimating posterior probability distributions for proportional contribution of each source (prey item) to the mixture (diet) of interest (Moore and Semmens 2008), while simultaneously estimating variance in diet composition (σ) across multiple hierarchical levels of a consumer's population structure (Semmens et al. 2009).

Each model was checked for convergence using the Gelman-Rubin diagnostic test, Geweke diagnostic test, and a visual confirmation of the Markov chain traceplots. The Gelman-Rubin diagnostic test represents the potential scale reduction factor, and was calculated based on the weighted within-chain and among-chain variance (Gelman and Rubin 1992; Brooks and Gelman 1998). Approximate convergence of the MCMC process was diagnosed when this ratio equaled one (i.e., the between-chain variance is equal to the within chain variance (Gelman and Rubin 1992; Brooks and Gelman 1998; Gelman et al. 2004)). The Geweke diagnostic is a two-sided z-test that compares the means of two non-overlapping parts of a Markov chain. If the value of the Geweke diagnostic was greater than 5% of the total number of variables, then the Markov chains had not converged.

I used this framework to calculate the Convex Hull Area (CHA) value for the model input data resulting from the stable isotope analysis of feces and hair. The CHA constitutes the total area encompassed by the isotopic $\delta^{13}C$ and $\delta^{15}N$ signatures of all individuals of the sample population in $\delta^{13}C$-$\delta^{15}N$ bi-plot space (Layman et al. 2007). I used the CHA to compare the relative amount of inter-individual variation among the input data into the model suite for each timescale. If the CHA value was larger, the area required to encompass the isotopic signatures of individuals in the study population was higher, equating to more variation among individuals within that population at that timescale.

I also used the MixSIAR framework to calculate the Deviance Information Criterion (DIC) value for each model. The DIC was created as a Bayesian analogue to the Akaike Information Criterion (AIC) capable of comparing complex hierarchical models, in which the effective number of parameters in each model is not clearly defined (Spiegelhalter et al. 2002). The DIC calculates the number of effective parameters and adds this with the estimate of mean posterior deviance (used as a Bayesian measure of model fit or adequacy), allowing comparisons to be made across hierarchical models as in an information-theoretic approach (Spiegelhalter et al. 2002; Spiegelhalter et al. 2014). I used DIC values to conduct a multi-model, information-theoretic approach following Burnham and Anderson (2002).

Additionally, I reported median (distribution center) and modal (most frequently occurring) values for posterior estimates of variance in diet composition for each variable and calculated 95% highest density intervals (i.e., the span of values that are most credible and cover 95% of the distribution) to illustrate the precision of each estimate

(Kruschke 2014). Finally, I reported posterior estimates of proportional contribution for each source to diet for the variable from the best-performing model determined from the multi-model inference. I created the resulting figures using the R graphics and ggplot2 packages (Wickham 2009).

To test the prediction that dietary niches of individuals or subgroups of individuals within the study population vary on a given night, I used SIA ratios of $\delta^{13}C$ and $\delta^{15}N$ from arthropods (source) and feces (mixture) collected from individual bats across six nights (18 May, 12 June, 23 June, 06 July, 17 July, 27 July 2017) throughout the gestation, parturition, and lactation periods. Arthropod prey orders were combined into five prey groups based on relative abundance, estimated contribution to diet (Clare et al. 2011; Clare et al. 2014), and measured isotopic values and variances (Phillips et al. 2005). The “rare” group constituted prey orders which cumulatively represented < 2% of overall abundance over the six dates in 2017. I did this to ensure that the solution of the mixing models was tenable and made the most biological sense.

I constructed a short timescale suite of models following Semmens et al. (2009). The global model included three potential predictor variables: individual, reproductive class (e.g., pregnant, lactating, post-lactating, non-reproductive, and not-obviously-pregnant) and capture/collection date. I nested the individual variable within reproductive class to represent the existing structure of the study population. Due to current limitations on the capabilities of MixSIAR, each model for this objective contained only one or two of the potential categorical variables from the global model.

Each model was analyzed using both generalist and opportunist prior distributions. A generalist prior held that each prey order or group had an equally likely probability of being consumed by the predator (Figure 1: grey). This prior assumed that consumers were pure ecological generalists, consuming all potential prey groups with no discernment. I created an opportunist prior (Figure 1: red) using weighted prey abundance data summarized from arthropod capture data across six survey nights May-July 2017. This prior assumed that consumers were ecological opportunists, with an increased likelihood of consumption of a prey group if that group was more abundant in their environment. Prey abundance was represented as the number of individual arthropods from an order/group captured in proportion to the total number of individuals captured from all orders across six (6) nights surveyed. I defined abundance in this way to link likelihood of encounter to consumption by individual bats.

To test the prediction that inter-individual and subgroup variation best explained dietary niche variation across years, I used SIA ratios of $\delta^{13}C$ and $\delta^{15}N$ from hair samples collected from individual bats that were sampled a minimum of two years over a six-year period (2012-2017). I attempted to control for variation across seasons by selecting samples collected from a given individual on the same month across years. I used the source data from SIA ratios of $\delta^{13}C$ and $\delta^{15}N$ from the binned arthropod groups collected over the survey season in 2017. Due to differential assimilation of $\delta^{13}C$ and $\delta^{15}N$ based on fractionation of stable isotopes in the tissues of consumers, one must adjust the mixture values to the source values using a laboratory-calculated and species-specific trophic discrimination factor (TDF) (DeNiro and Epstein 1978; DeNiro and Epstein 1981; Fry 2006). Because TDF for $\delta^{13}C$ and $\delta^{15}N$ in the tissues of *M. lucifugus* has not been calculated, I was forced to use TDF originally calculated for *M. nattererii*, an ecologically-similar European congener (Siemers et al. 2011). To adjust the mixture values to source values, I used TDF of 3.2 ± 0.34‰ for $\Delta^{13}C$ and 3.2 ± 0.27‰ for $\Delta^{15}N$.

I also constructed a model suite for this timescale following Semmens et al. (2009). The global model included three potential predictor variables: individual, reproductive class (e.g., pregnant, lactating, post-lactating, non-reproductive, and not-obviously-pregnant) and capture/collection year. As per the short timescale model suite, each MixSIAR model contained only one or two potential predictor variables for analysis. I used the same generalist and opportunist prior distributions as in the short timescale model suite.

Results

Arthropod abundance: The most abundant prey group across six nights May-June 2017 at Salmonier Nature Park, Newfoundland were Diptera, with an overall abundance of 62% over the 2017 survey period (Table 1 & Figure 2). Next were Trichoptera, with an abundance of 18%, followed by Lepidoptera and Ephemeroptera, with an abundance of approximately 9% each. Finally, the "rare" prey group contained all other prey orders and cumulatively represented 1.7% of overall abundance over the 2017 survey period. The peak of prey abundance appeared to be 17 July 2017, although abundance varied greatly on each of the sample nights.

The arthropod capture data reflected a heavy bias associated with the use of phototactic attractant traps (Table 2), despite attempts to prevent this. However, use of 'passive' trap types such as Malaise and aquatic emergent traps did make up nearly one quarter of arthropods captured at Salmonier Nature Park, Newfoundland on six survey nights in 2017.

Raw isotopic data: The CHA values for individual adult female *M. lucifugus* in both short and long timescales indicated a similar value for $\delta^{13}C$ and $\delta^{15}N$ SIA ratios (short timescale (feces) CHA = 1.327, long timescale (hair) CHA = 1.307), suggesting similar diet niche widths across short and long timescales. Prior to performing the mixing model analysis, visual estimation of 95% confidence ellipses encapsulating subcategories within the predictor variables of short timescale fecal isotope ratios illustrated less overlap (or higher variance) among capture dates than among reproductive classes (Figure 3). Furthermore, continued visual estimation of 95% confidence ellipses encapsulating subcategories of long timescale isotope ratios indicated less overlap among capture years (Figure 4). Despite these observed differences, there was a high amount of overlap assessed using visual estimation of input data and CHA values, suggesting *a priori* that the hypotheses and predictions about inter-individual and subgroup variation were not supported by the data.

Multi-model inference and Bayesian posterior estimates: DIC scores calculated from the short timescale isotope ratios indicated that the best performing models included capture date and reproductive class variables (Tables 3 & 4). The best performing model in both generalist and opportunist model suites was a univariate model containing only the capture date variable. The other models making up the 95% confidence set of generalist prior and opportunist prior model sets included the capture date variable, making this the most important relative variable (Generalist $Nw_i = 1$, Opportunist $Nw_i = 1$). Reproductive class variables had low relative importance weights (Generalist $Nw_i = 0.173$, Opportunist $Nw_i = 0.066$). Models containing individual effects performed comparatively poorly, and were excluded from further interpretation.

Posterior estimates of variance (σ) from the best performing model from both generalist prior and opportunist prior model suites indicated that short timescale dietary variation was primarily due to temporal heterogeneity, followed by reproductive class heterogeneity, suggesting that the main driver of dietary variance was temporal and spatial heterogeneity (Figures 5 & 6). Diptera and Trichoptera were highly important diet components across all capture dates in models informed by both prior assumptions (Figures 7 & 8). Due to the differences of assumptions between the generalist and opportunist prior distributions, proportional estimates of prey groups to dietary composition across survey dates differed among model suites. The generalist model illustrated an increased dependence on Ephemeroptera on 06 July 2017 and the "rare" group on 27 July 2017. The opportunist model indicated an increase in dependence on Ephemeroptera and Lepidoptera on 06 July 2017, followed by an increased dependence on Lepidoptera on 27 July 2017. Changes in diet among survey dates appeared to be closely correlated with prey abundance across the survey period in both generalist prior and opportunist prior models.

DIC scores calculated from the long timescale isotope ratios indicated the best performing model from both generalist prior and opportunist prior model suites was a univariate model containing the capture year variable (Generalist $\boldsymbol{w_i}$ = 1, Opportunist $\boldsymbol{w_i}$ = 1 (Tables 5 & 6)). Due to the lack of any other models in the 95% confidence set, no posterior estimates are presented for these model suites. Models in the opportunist prior suite containing individual effects performed very poorly and did not converge, so no interpretations were drawn from these variables and they were excluded from the multi-model analysis.

Similar to the short-term models, Diptera and Trichoptera were highly important diet components across all capture years in both model types (Figures 9 & 10). Interestingly, the heavily ephemeral prey group Ephemeroptera comprised a substantial diet component of individuals in the system on each of the survey years. Posterior distributions of contribution to diet for both generalist and opportunist model types indicated increased dietary reliance on Ephemeroptera in 2017. The hair samples collected in 2017 represent isotopic assimilation from the last molt period (e.g., 2016-2017), suggesting either that these individuals in the molt period had been assimilating a diet heavier in mayflies than prior years, or the assimilation of other prey items had not yet been reflected in the hair samples.

Discussion

Contrary to the predictions of this study, variation in diet across the survey timescales was not best explained by variation among individuals or subgroups, but instead was explained by temporal heterogeneity. I suggest that temporal heterogeneity was likely due to the influence of limitations on prey abundance (e.g., ephemerality and patchiness of available prey resources throughout both space and time). The arthropod collection data indicated that dietary complexity mirrors prey diversity as it fluctuates across a foraging season. The disparities among exact proportions of arthropod abundance data and the estimated diet of individuals was likely a reflection of individual bats foraging in different areas than those that I sampled for arthropods on a given night. The total area sampled for arthropods was likely much smaller than the area required for an individual bat to forage, which in this study system were found to travel up to 35 linear km on a single night (Besler, Broders & Fouts 2017, unpublished data). Despite the lack of fine scale dietary resolution, the mixing model analysis was sensitive enough to detect pulses of highly ephemeral resources, and dietary shifts. Temporal dietary shifts have often been observed in this species (Belwood and Fenton 1976; Anthony and Kunz 1977; Clare et al. 2011; Clare et al. 2014), and are thought to be linked to differential nutrient requirements of gestating and lactating individuals (Belwood and Fenton 1976; Barclay 1994). These patterns may in turn be due to the relative importance of essential fatty acids to the diet of gestating and lactating individuals (Schalk and Brigham 1995). Despite differences in dietary requirements, the effect of reproductive class was of inconsequential relative importance in tested model suites and had lower modal posterior estimates of variance from the best-performing short-timescale models. Consistent with dietary composition throughout other areas of the species' range (Anthony and Kunz 1977; Burles et al. 2008; Ober and Hayes 2008), data from Newfoundland suggest that Trichoptera and Diptera constitute a crucial component of diets of individuals across a single season and multiple years. However, most of the prey groups form important components of diets of individual bats at different periods. Interestingly, the timing and composition of dietary shifts detected in Newfoundland were similar to those in previous studies (Belwood and Fenton 1976; Ober and Hayes 2008; Clare et al. 2011). This suggests either the existence of some mechanism (e.g., genetic or learned) responsible for timing these shifts in bats across the entirety of the species continental range, or that the patterns of abundance in nocturnal arthropod prey across North America are spatially and temporally consistent.

The isotopic niche width (convex hull area (CHA)) of *M. lucifugus* in Newfoundland was smaller than those found in this species across other sites throughout Atlantic Canada (Broders et al. 2014). It is thought that individuals expand their niche widths (e.g., approach their fundamental niche width) during times of resource scarcity (Araújo et al. 2011), suggesting that prey limitations in Newfoundland may not be as profound as previously suggested. The lack of variation in isotopic niche among individuals in the system demonstrate limited spatial heterogeneity in isotopic composition of $\delta^{13}C$-$\delta^{15}N$ on the landscape of eastern Newfoundland. This is likely explained by the less diverse origin of primary production and arthropod community in both terrestrial and freshwater systems in the region than other areas across North America (Larson and Colbo 1983; Clarke 1995; Clarke and Scruton 1997). There was less apparent inter-individual and subgroup dietary variation across years than observed in the short-term samples, supporting the prediction that that short-term dietary trends are masked in the long-term assimilation of stable carbon and nitrogen.

In shorter timescales (across a single foraging season) foraging bats favor a more opportunistic approach (suggested by Anthony and Kunz 1977, Burles et al. 2008). These trends appear to continue in this system throughout years. I speculate that the seasonality and ephemerality of available resources linked with a highly heterogeneous landscape and prey base lead to selection for an opportunist dietary strategy to best optimize the diets of individual bats. Contrary to the predictions of the niche variation concept, which attribute generalism and opportunism with increased inter-individual and subgroup variation(Van Valen 1965; Bolnick et al. 2007), seasonality and heterogeneity of resources alone may be explanatory enough to facilitate this strategy.

Optimality of the diets of free-ranging individual bats may be restricted by limitations on prey abundance or intraspecific competitive pressures, as previously suspected (Barclay and Brigham 1994; Altringham 1996). However, optimality in diet

Conclusions: This work supports the conclusions of Roswag et al. (2015) regarding the importance of the consideration of multiple spatial and temporal scales in determining dietary variation or partitioning within a system. My study used trophic discrimination factors originally calculated for *M. natterii* due to a knowledge gap for the study species, *M. lucifugus*. This knowledge gap could be filled with a similar lab-based study (Siemers et al. 2011; Roswag et al. 2014; Roswag et al. 2015a) Additionally, this study made the assumption that individual encounter rates weighed heavily in the decisions of foraging bats, with tenuous support in the literature (Barclay and Brigham 1994; Schalk and Brigham 1995; Clare et al. 2011; Clare et al. 2014). I suggest that future work should focus on linking the diversity of available prey with the dietary richness of individual bats. It would be interesting to relate dietary variation with fitness (e.g., fecundity) or predation risk of populations from different areas throughout the range (as in Darimont et al. 2007). This work also illustrated the importance of prey composition and abundance as a potential recovery tool in the management of post-exposure white nose syndrome. Given the lack of a "silver bullet" treatment for white-nose syndrome in North American bats, I suggest that recovery efforts for maternity colonies instead focus on managing for healthy levels of nocturnal arthropods. Some areas have already observed a precipitous decline in flying insects (Conrad et al. 2006; Hallmann et al. 2017), and linked declines in arthropods with changes in diet of aerial insectivores (English et al. 2018). *Myotis lucifugus*, would benefit most from management practice which ensure the health of both water bodies and forested landscapes in areas where these individuals roost and forage (Belwood and Fenton 1976; Anthony and Kunz 1977; Whitaker, Jr. and Lawhead 1992; Clare et al. 2011; Clare et al. 2014). This is especially important for areas already on the edge of the species' range, which likely are more heavily limited by restrictions on prey abundance (Boyles et al. 2016; Kaupas and Barclay 2018).

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Table 1: Percent abundance of arthropod prey orders collected using a combination of Malaise, ultraviolet black-light, aquatic-emergent, and light assisted pitfall traps on six survey nights in 2017 at Salmonier Nature Park, Newfoundland. Prey orders with a cumulative abundance of > 2% were combined into a "rare" prey group for analysis.

Order	**Percent abundance**
Diptera	62.12%
Trichoptera	17.97%
Ephemeroptera	9.18%
Lepidoptera	9.00%
Collembola	0.60%
Araneae	0.40%
Coleoptera	0.33%
Hymenoptera	0.18%
Neuroptera	0.14%
Hemiptera	0.03%
Isopoda	0.02%
Geophilomorpha	0.01%
Homoptera	0.01%
Psocoptera	0.01%

Table 2: Percent of total arthropod capture by trap type from Salmonier Nature Park, Newfoundland on six survey nights in 2017.

Trap type	Percent of total capture
UV black light	77.64%
Malaise	21.11%
Aquatic emergence	1.17%
Light-assisted pitfall	0.09%

Table 3: Multi-model inference results for generalist prior models explaining variation in diet across six sample nights in 2017 at Salmonier Nature Park, Newfoundland. Each model contained one or two variables representing variation at the individual, reproductive class, and capture date level, and included residual * process error structure. The models in bold make up the 95% confidence set. Models with a star (*) have nested subgroup variables.

Generalist Short Timescale Models

Model	**DIC**	Δ_i	w_i	$\sum w_i$
Capture Date	**1910.360**	**0**	**0.827**	**0.827**
Capture Date + Reproductive Class	**1913.493**	**3.133**	**0.173**	**1**
Reproductive Class	1941.534	31.174	0	0
Individual	3853.379	1943.019	0	0
Capture Date + Individual	4159.622	2249.262	0	0
Reproductive Class + Individual *	4481.890	2571.530	0	0

Table 4: Multi-model inference results for opportunist prior models explaining variation in diet across six sample nights in 2017 at Salmonier Nature Park, Newfoundland. Each model contained one or two variables representing variation at individual, reproductive class, and capture date levels, and included residual * process error structure. The models in bold make up the 95% confidence set. The star (*) represents models with nested subgroup variables.

Opportunist Short Timescale Models

Model	**DIC**	Δ_i	w_i	$\sum w_i$
Capture Date	**1908.131**	**0**	**0.934**	**0.934**
Capture Date + Reproductive Class	**1913.435**	**5.304**	**0.066**	**1**
Reproductive Class	1942.096	33.965	0	
Individual	3811.587	1903.456	0	
Reproductive Class + Individual *	4279.738	2371.607	0	
Capture Date + Individual	5240.654	3332.523	0	

Table 5: Multi-model inference results for generalist prior models explaining diet variation across years 2012-2017 at Salmonier Nature Park, Newfoundland. Each model contained one or two variables representing variation across the individual, reproductive class, and capture year levels. Models were analyzed using residual * process error structure. Models in bold make up the 95% confidence set.

Generalist Long Timescale Models

Model	**DIC**	Δ_i	w_i	$\sum w_i$
Capture Year	**1628.969**	**0**	**1**	**1**
Reproductive Class	1655.778	26.809	0	
Reproductive Class + Capture Year	1665.75	36.781	0	
Individual	1707.576	78.607	0	
Individual + Reproductive Class *	1724.233	95.264	0	
Individual + Capture Year	1763.701	134.732	0	

Table 6: Multi-model inference results for opportunist prior models explaining diet variation across years 2012-2017 at Salmonier Nature Park, Newfoundland. Each model contained one or two variables representing variation across the individual, reproductive class, and capture year levels. Models were analyzed using residual * process error structure. Models in bold make up the 95% confidence set.

Opportunist Long Timescale Models

Model	**DIC**	Δ_i	w_i	$\sum w_i$
Capture Year	**1624.286**	**0**	**1**	**1**
Reproductive Class	1650.321	26.035	0	
Reproductive Class + Capture Year	1669.637	45.351	0	
Individual + Reproductive Class *	Did not converge			
Individual + Capture Year	Did not converge			
Individual	Did not converge			

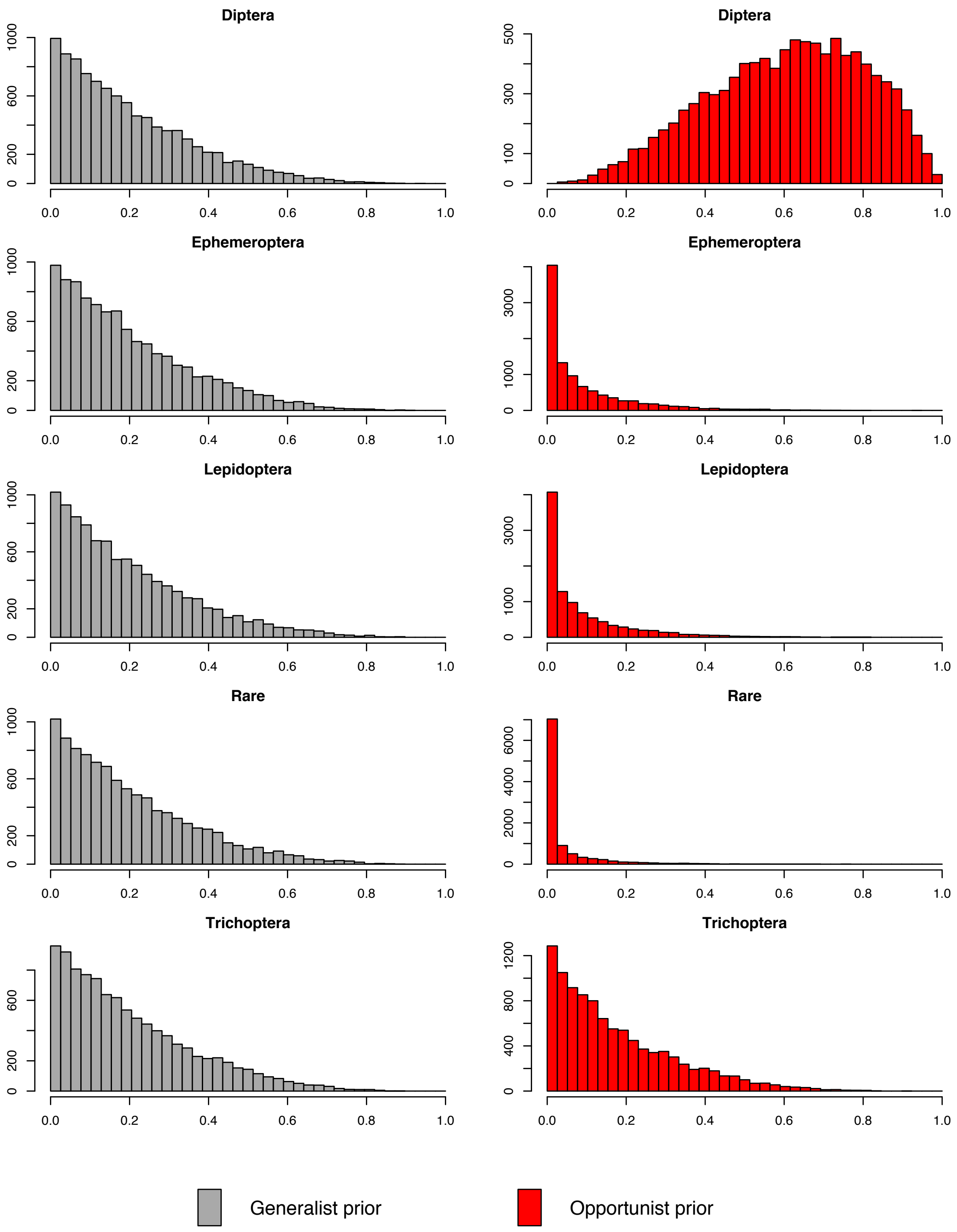


Figure 1: Generalist (grey) and opportunist (red) prior distributions used in both short and long timescale model suites. An opportunist prior distribution was calculated from overall arthropod abundance collected on six survey nights in 2017 at Salmonier Nature Park, Newfoundland. A generalist prior distribution assumed equal likelihood of consumption across all prey groups. The "rare" prey group constituted prey orders with a relative abundance of < 2% across the survey nights.

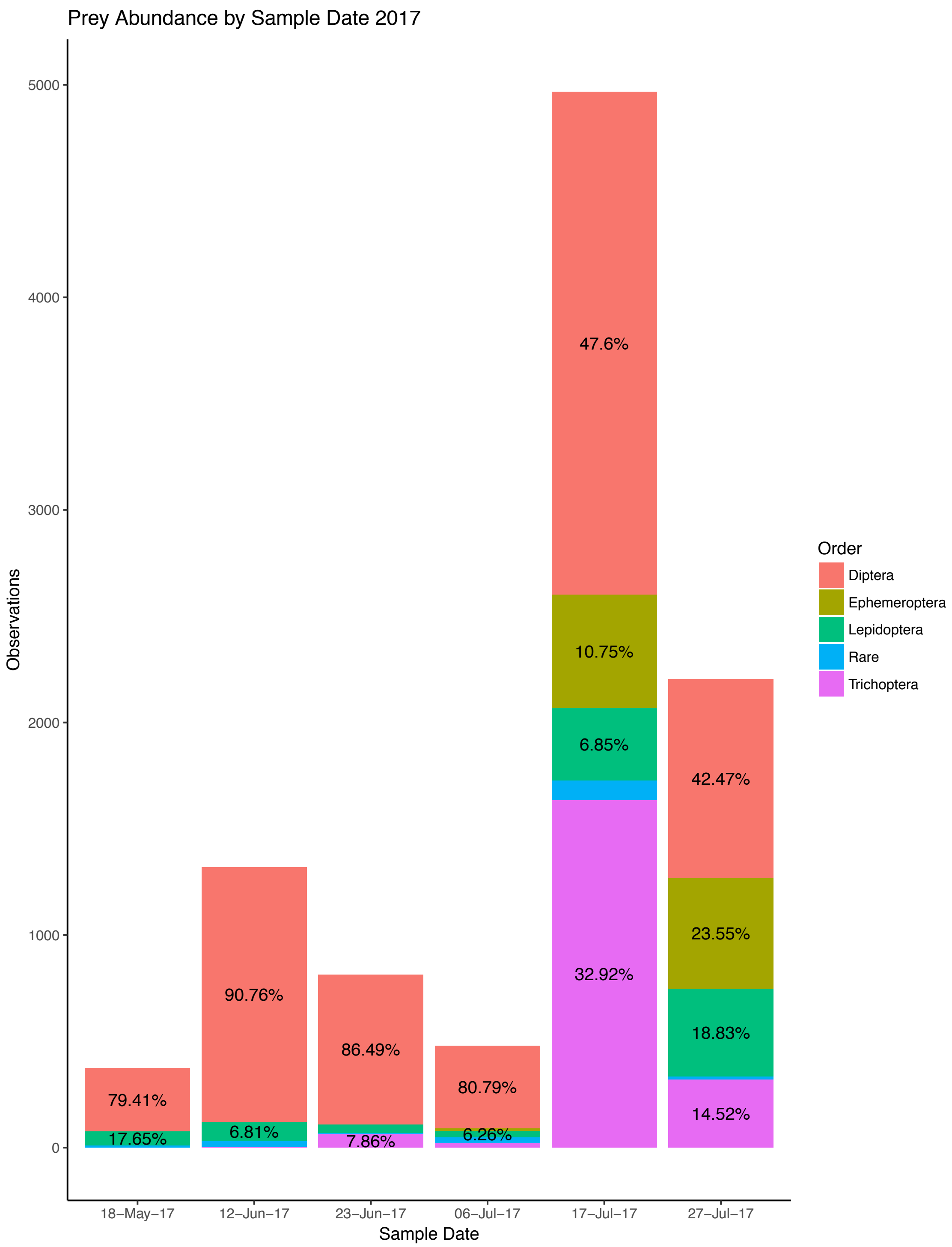


Figure 2: Nocturnal arthropod abundance at Salmonier Nature Park, Newfoundland over six survey nights in 2017, collected using a combination of Malaise, ultraviolet black-light, aquatic-emergent, and light assisted pitfall traps. Abundance is the proportion of individual prey items belonging to a prey group divided by the total number of individuals collected on that night. Prey proportions for each night on figure do not add to 100%, as percentages of prey groups that constituted > 6% abundance per night were not listed on the figure.

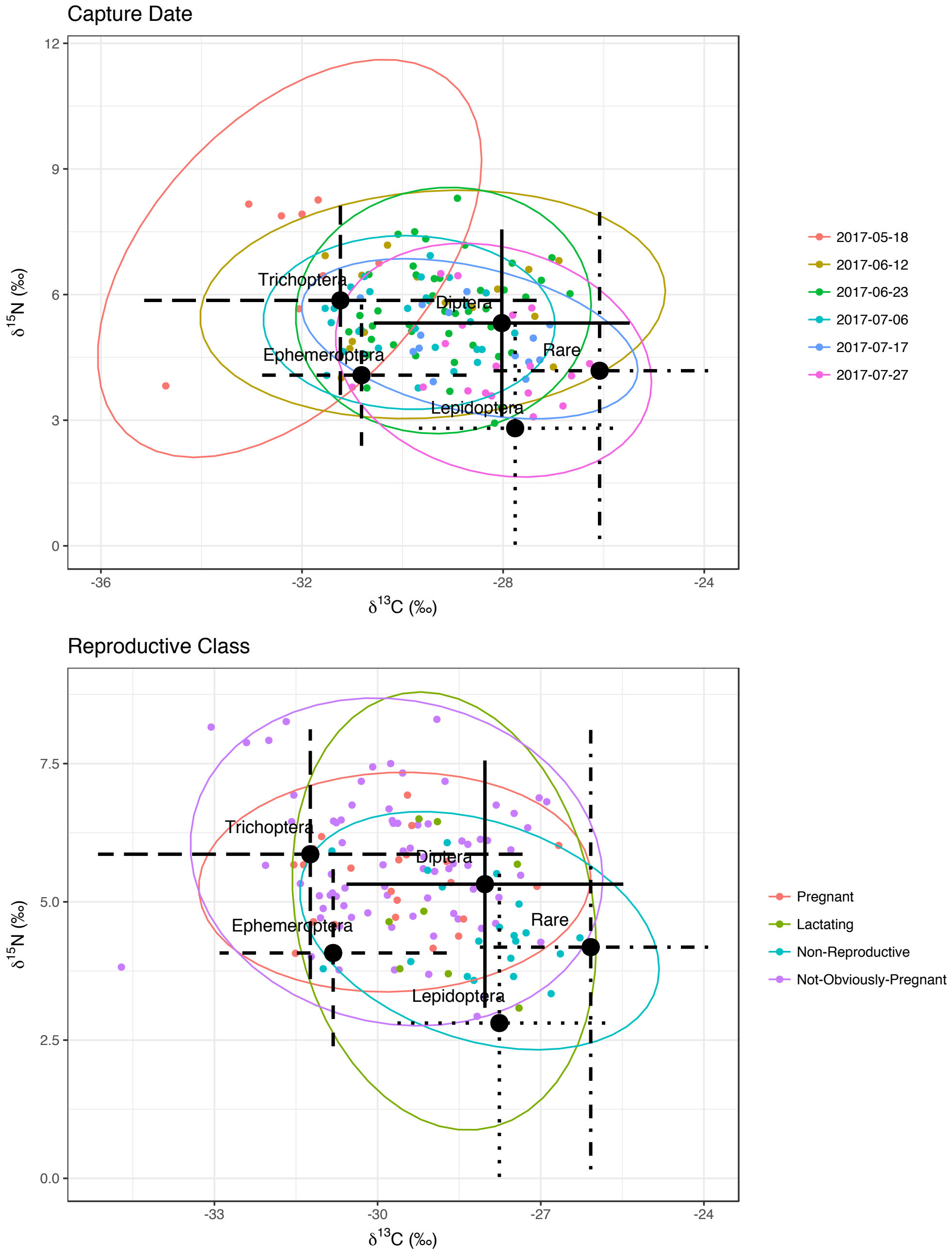


Figure 3: Stable isotope ratios of fecal input data for the short timescale model suite investigating isotopic niche variation on six sample nights in 2017 at Salmonier Nature Park, Newfoundland. The axes represent a $\delta^{13}C - \delta^{15}N$ bi-plot with stable carbon concentration in parts per thousand (‰) on the x-axis and stable nitrogen concentration in parts per thousand (‰) on the y-axis. Mixture data points (individual bats) are categorized by the date of capture and reproductive class. 95% confidence ellipses encompass a subcategory within either capture date or reproductive class. Mean prey groups are represented by large dots with cross bars illustrating ± 1 standard deviation. The “rare” prey group was the additive group created from all prey groups comprising > 2% of overall abundance over the survey period.

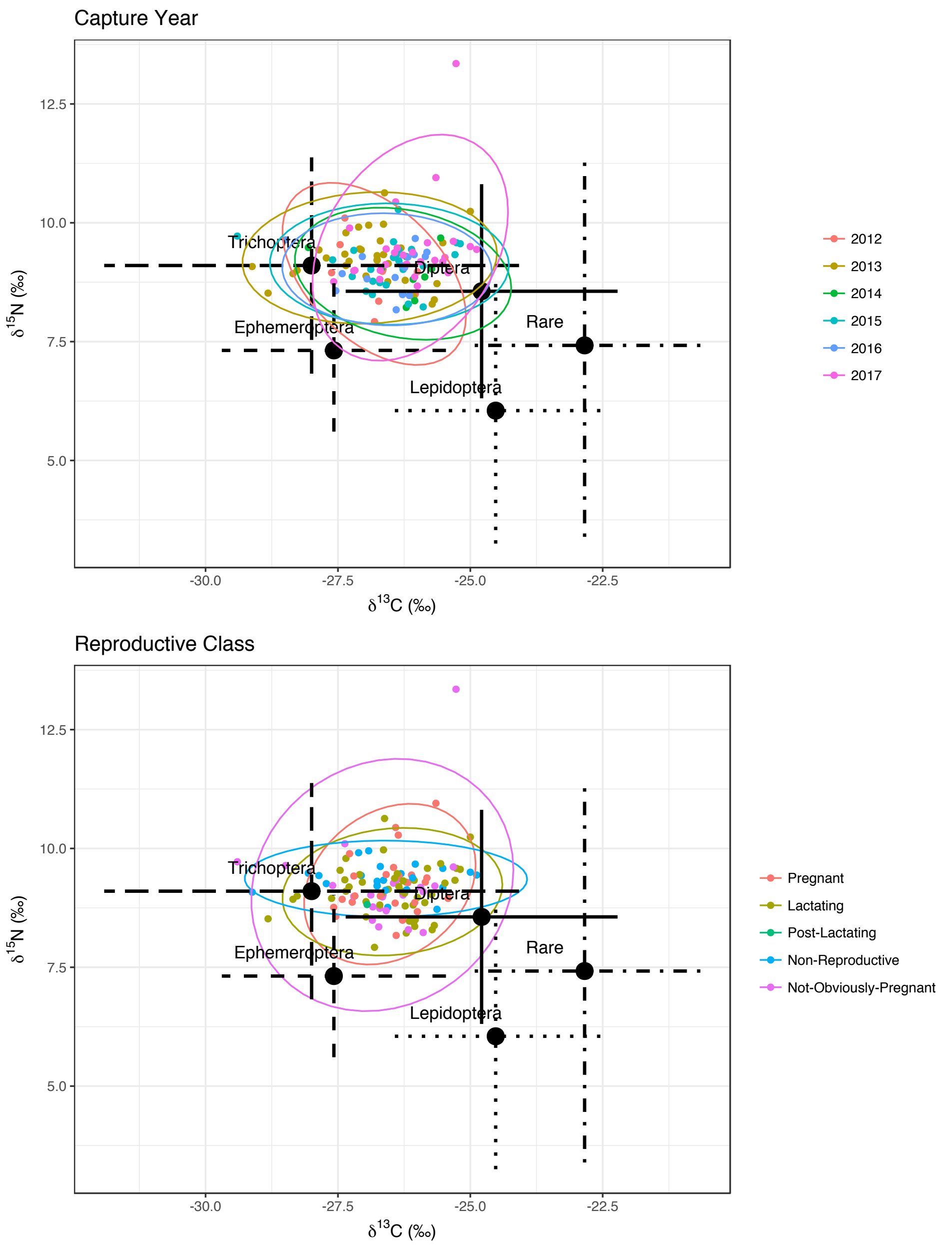


Figure 4: Stable isotope ratios of hair input data for the long timescale model suite investigating inter-individual and subgroup variation in isotopic niche across the sample years 2012 – 2017 at Salmonier Nature Park, Newfoundland. The axes represent a $\delta^{13}\mathrm{C} - \delta^{15}\mathrm{N}$ bi-plot with stable carbon concentration in parts per thousand (‰) on the x-axis and stable nitrogen concentration in parts per thousand (‰) on the y-axis. Mixture data points (individual bats) are categorized by capture year and reproductive class. 95% confidence ellipses encompass a subcategory within the respective variable. There were too few post-lactating individual data points to calculate an ellipse. Mean source (prey) groups are represented by large black dots with cross bars illustrating ± 1 of the adjusted source + discrimination standard deviation. The “rare” prey group was the additive group created from all prey groups comprising > 2% of overall abundance over the survey period.

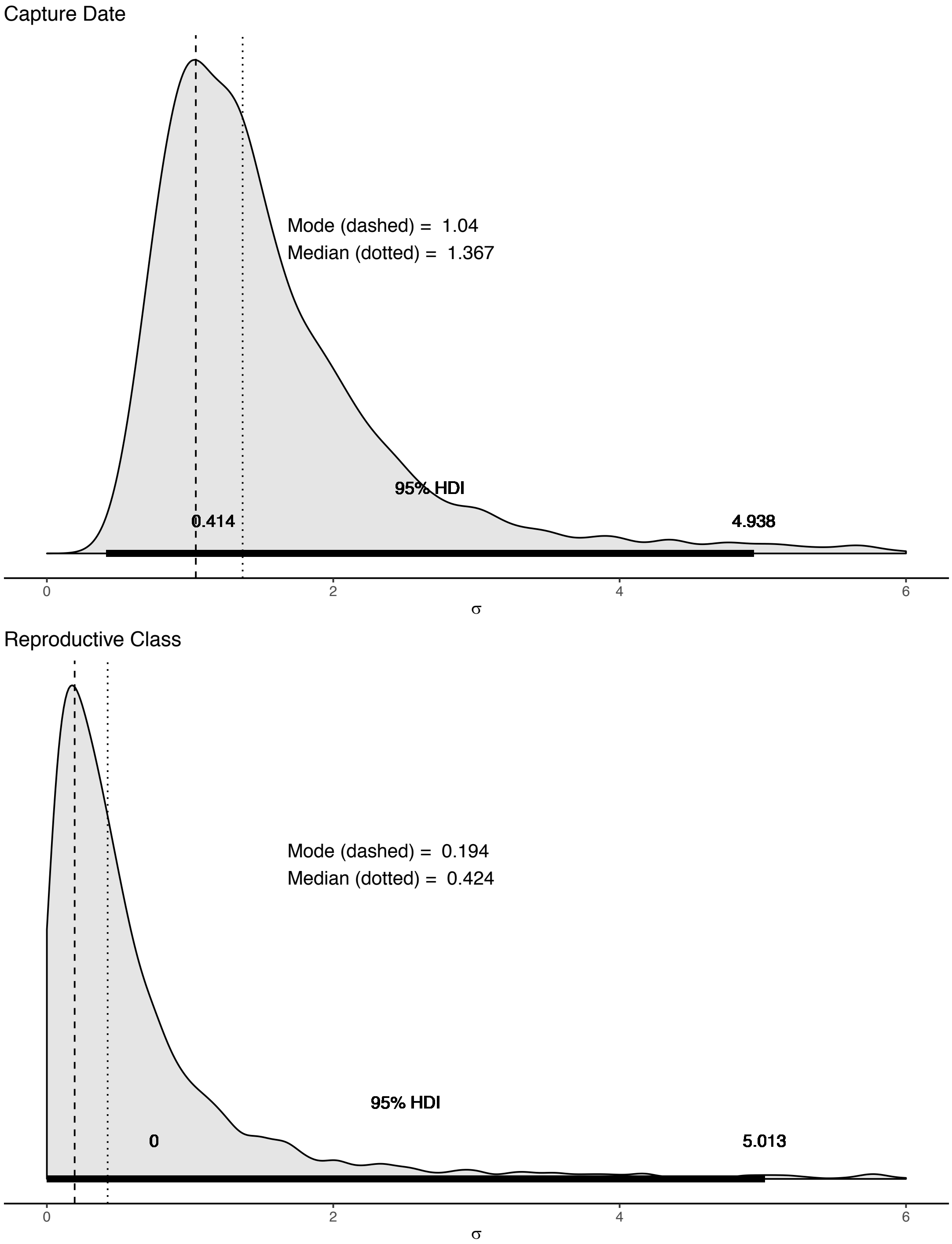


Figure 5: Posterior density estimates of variance (σ) in diet composition among capture date and reproductive class variables in the study system. Estimates are from the best-performing, generalist, short-timescale model containing that variable in the 95% confidence set. Median estimates are indicated by dotted vertical lines, modal estimates are indicated by dashed vertical lines, and bold horizontal bars with values are 95% highest density intervals (HDI), indicating 95% certainty that the true estimate falls within the given range.

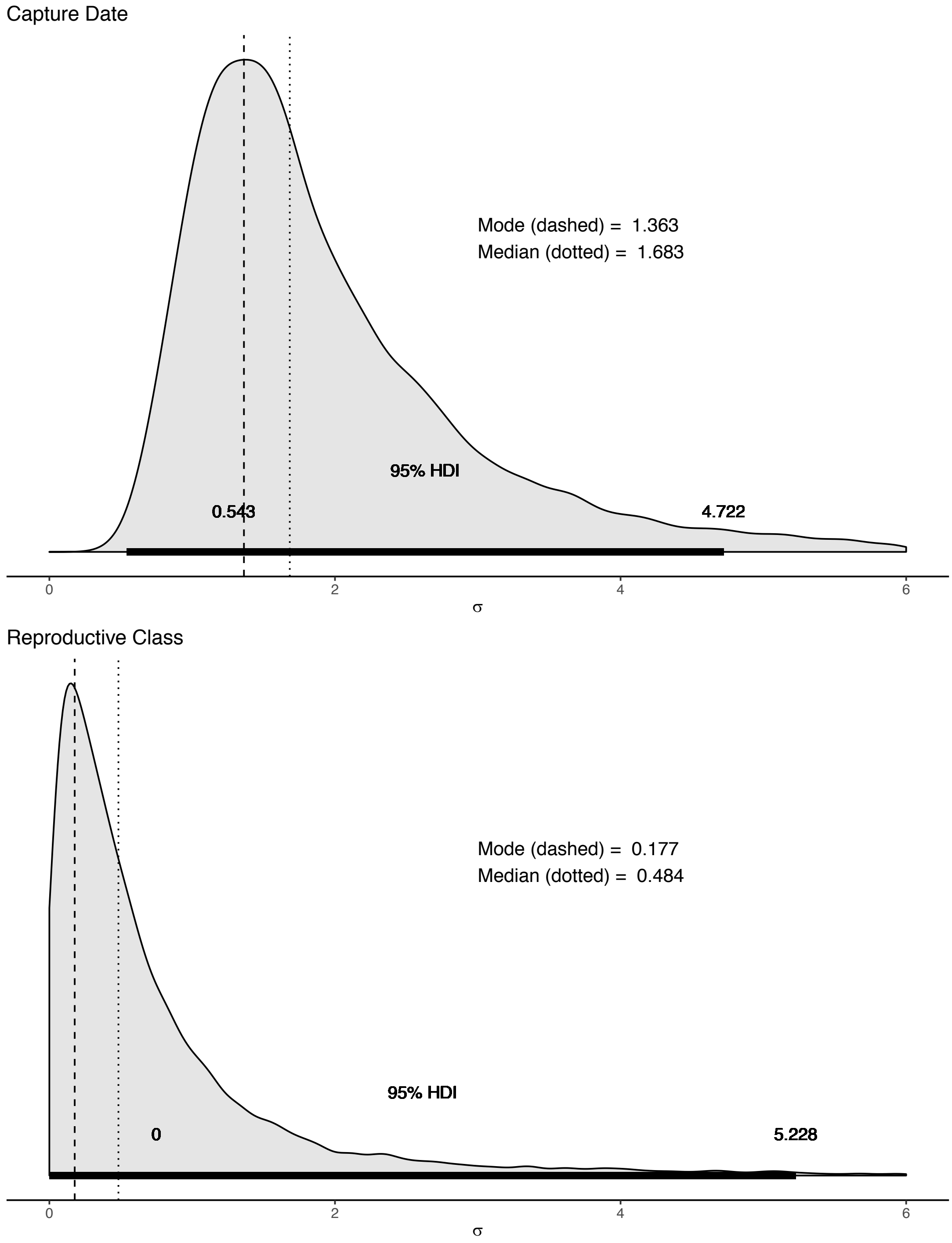
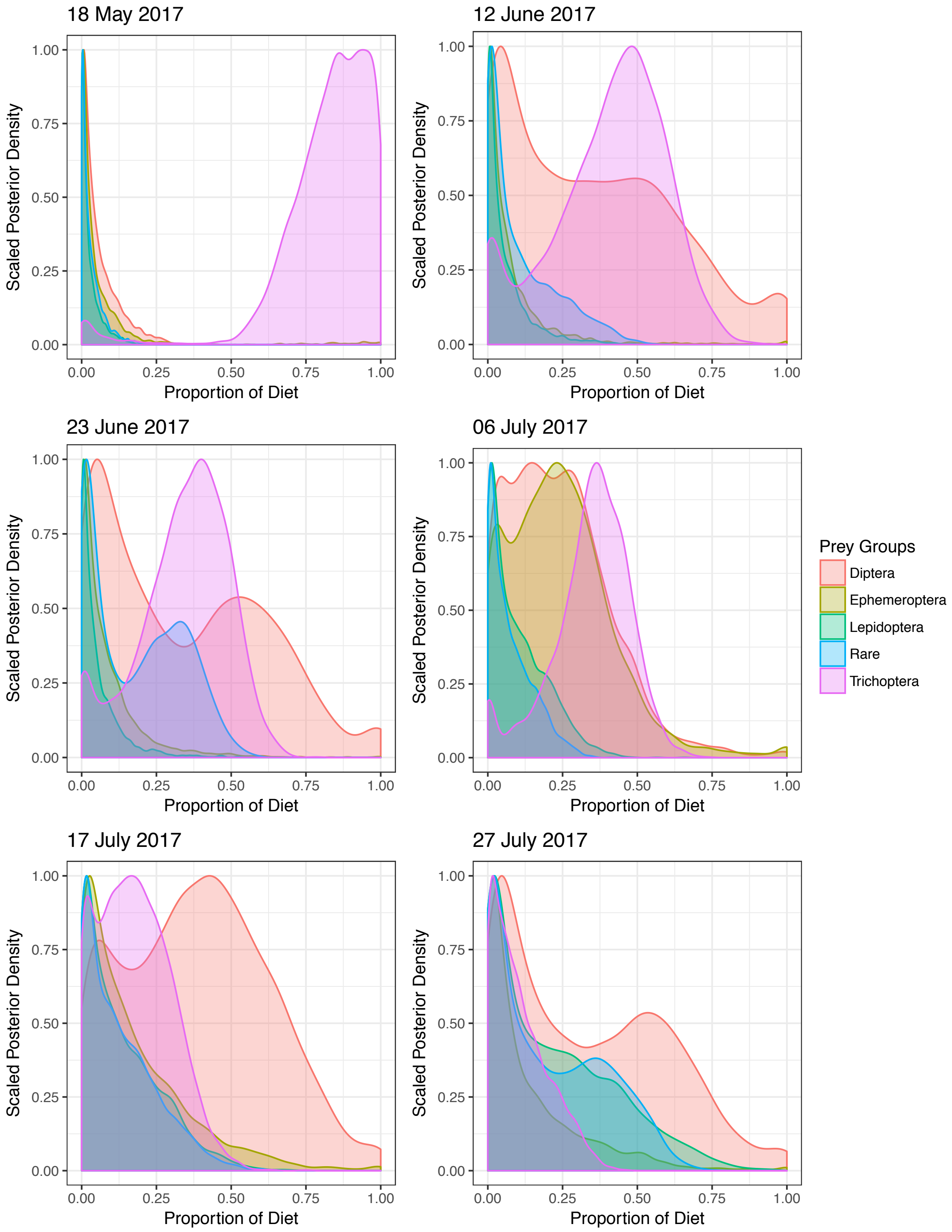


Figure 6: Posterior density estimates of variance (σ) in diet composition among the capture date and reproductive class variables in the study system. Estimates are from the best-performing, opportunist, short-timescale model containing that variable in the 95% confidence set. Median estimates are indicated by dotted vertical lines, modal estimates are indicated by dashed vertical lines, and bold horizontal bars with values are 95% highest density intervals (HDI), indicating 95% certainty that the true estimate falls within the given range.


18 May 2017

12 June 2017

23 June 2017

06 July 2017

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27 July 2017

Figure 7: Generalist posterior source contribution-to-diet distributions across six sample nights in 2017 at Salmonier Nature Park, Newfoundland. The generalist prior distribution assumed equal likelihood of contribution to diet across source categories.

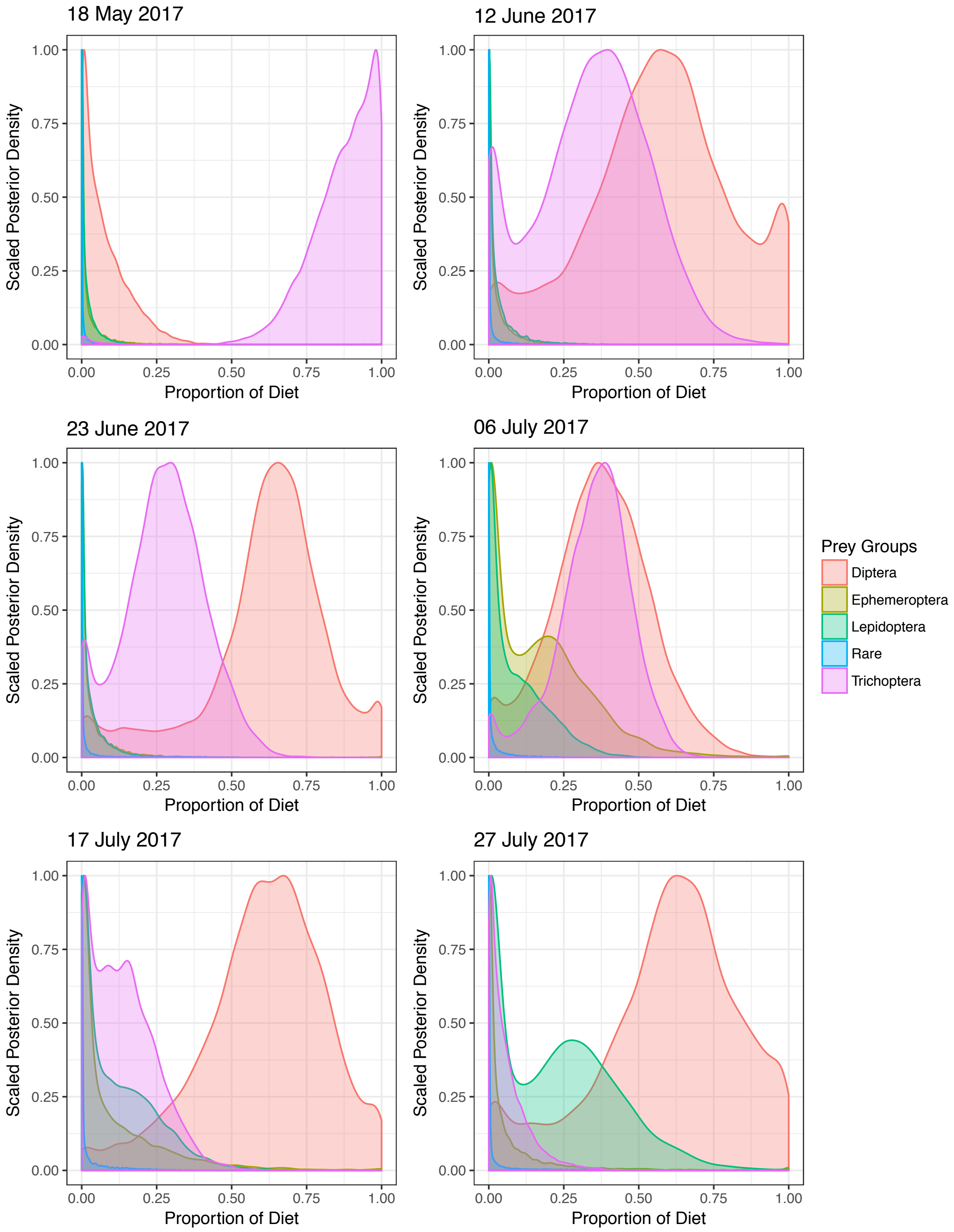

Figure 8: Opportunist posterior source contribution-to-diet distributions across six sample nights in 2017 at Salmonier Nature Park, Newfoundland. The opportunist prior distribution was calculated from arthropod abundance across the six survey nights in 2017.

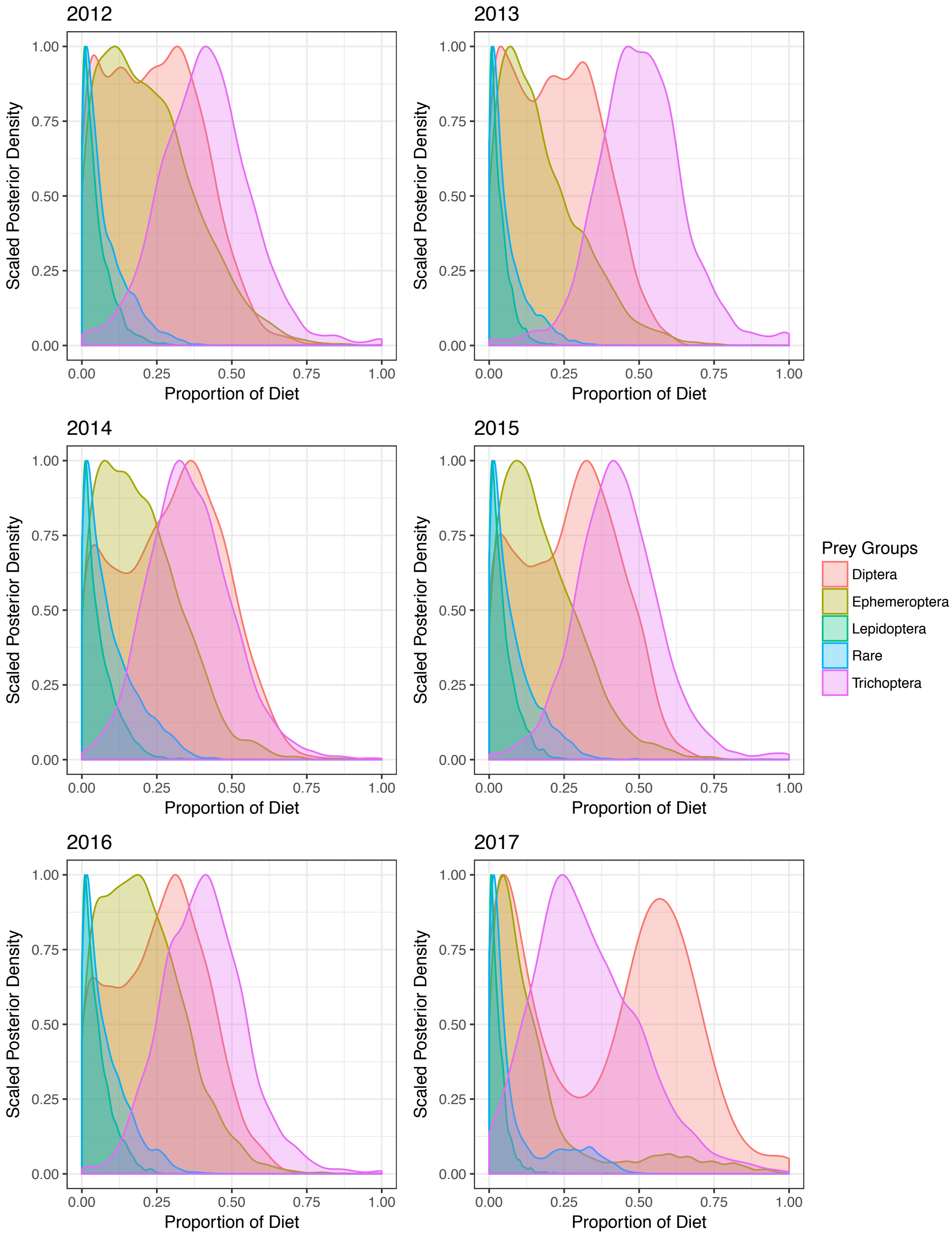

Figure 9: Generalist posterior source contribution-to-diet distributions across six years 2012-2017 at Salmonier Nature Park, Newfoundland. The generalist prior distribution assumed equal probability of contribution to diet across source categories.

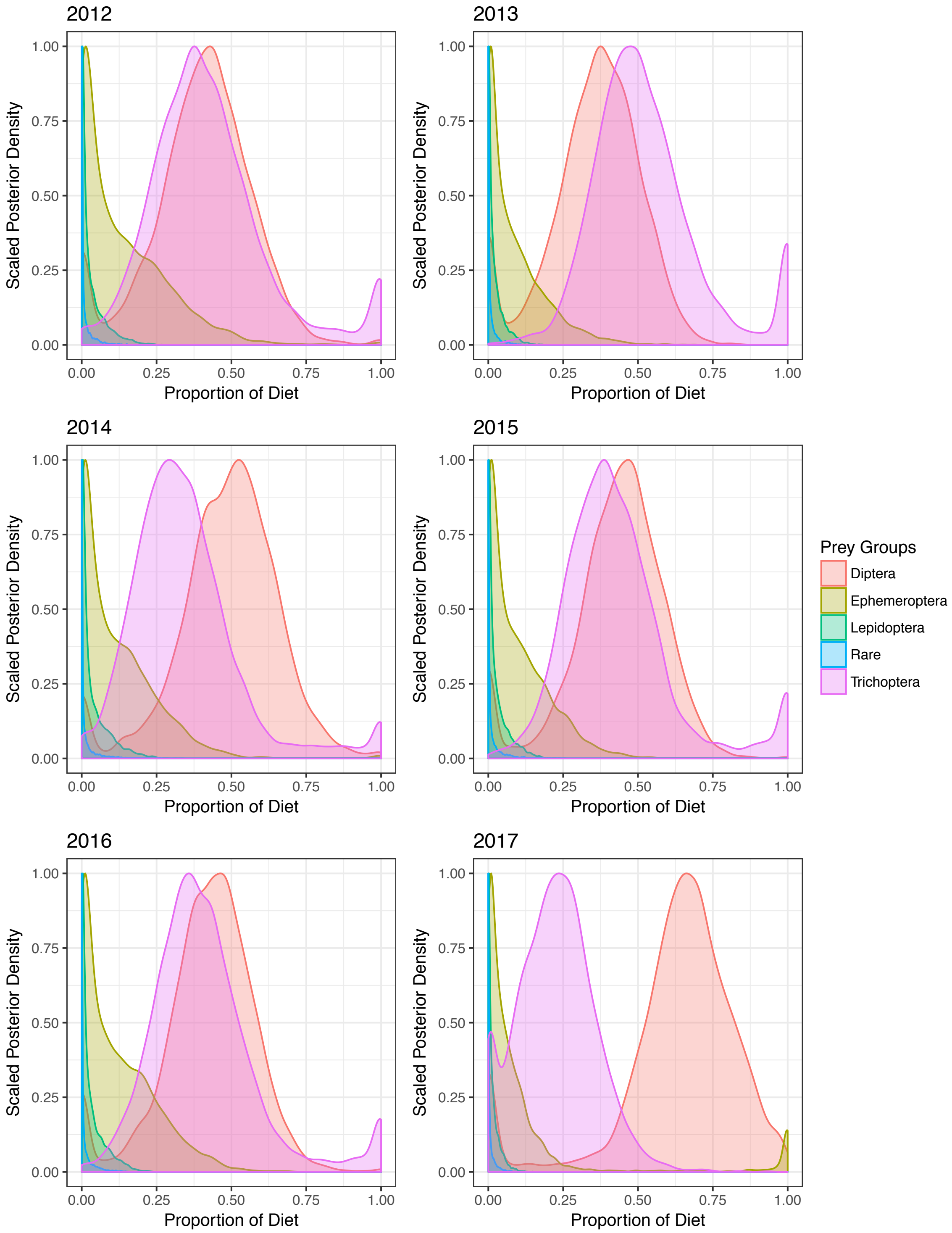

Figure 10: Opportunist posterior source contribution-to-diet distributions across six years 2012-2017 at Salmonier Nature Park, Newfoundland. The opportunist prior distribution was calculated from arthropod abundance across the six survey nights in 2017.