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1 **An inordinate fondness for beetles? Variation in seasonal dietary preferences of night**
2 **roosting big brown bats (*Eptesicus fuscus*)**

3 Elizabeth L. Clare¹, William O.C. Symondson², and M. Brock Fenton³

4

5 | ¹School of Biological and Chemical Sciences, Queen Mary; University of London, Mile End

6 Road, London E1 4NS, UK

7 | ²Cardiff School of Biosciences, The Sir Martin Evans Building, Museum Avenue, Cardiff, CF10

8 3AX, UK

9 | ³ Department of Biology, Western University, London Ontario, Canada, N6A 5B7

10

11 **Corresponding Author**

12 Elizabeth L. Clare

13 | School of Biological and Chemical Sciences, Queen Mary; University of London, Mile End

14 Road, London E1 4NS, UK, e-mail: elclare.evol.biology@gmail.com, Fax: +44 (0)20 7882 7732

15

16 **Key Words:** insectivores, species' interactions, molecular diet analysis

17 **Running Title:** Molecular analysis of big brown bat diet

18 **Abstract**

19 Generalist species with numerous food web interactions are thought to provide stability to
20 ecosystem dynamics however it is not always clear whether habitat generality translates into
21 dietary diversity. Big brown bats are common across North America and employ a flexible
22 foraging strategy ~~—flying~~ over water, dense forested areas, along forest edges and ~~in~~ rural and
23 urban settings ~~in search of prey~~. Despite this generalist use of habitat, they are paradoxically
24 characterized as beetle specialists. However, hard carapaces may preferentially survive digestion
25 leading to overrepresentation during morphological analysis of ~~faeces diet and~~ This
26 specialization has not been evaluated independently using molecular analysis and species level
27 identification of prey. We used next generation sequencing to assess the diet of big brown bats
28 ~~from fecal samples collected in 2008 and 2011~~. Beetles were consumed in the highest frequency
29 but Lepidoptera species richness was highest among identified prey. The consumption of species
30 showed strong seasonal and annual variation ~~(2008, $\chi^2=20.6$, $p=0.005$, 2011, $\chi^2=23.2$, $p=0.004$)~~
31 ~~but also varied between years (($\chi^2=19.7$, $p=0.04$)).~~ While Coleoptera consumption varied
32 ~~seasonally~~, Lepidoptera and Ephemeroptera were relatively constant dietary component ~~in all~~
33 ~~years and over the entire summer~~. Dietary diversity increased in late summer when insect
34 diversity decreases. Our results indicate that ~~the diet of~~ big brown bats is are dietary omnivorous
35 generalists and, while beetles are an important component of the diet, Lepidoptera are equally

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36 important, and Lepidoptera and Ephemeroptera are the only stable prey resource exploited. As
37 resources become limited big brown bats may respond by increasing the species richness of prey
38 and thus their connectedness in the ecosystem. This characterization of diet corresponds well
39 with a generalist approach to foraging and this ~~extreme generalist strategy~~ makes them an
40 ~~fundamentally~~ important species in encouraging and maintaining ecosystem stability.

41

42 **Introduction**

43 Understanding interactions among species is fundamental to assessing the way in which
44 ecosystems function and respond to variation. ~~Predator-prey~~Species' interactions, particularly
45 those involving generalists and omnivores, ~~predators~~ can be ~~particularly~~ important in promoting
46 ecosystem stability. The importance of behavioural flexibility and resource use has been widely
47 modelled in studies of food-web stability. Food webs appear most susceptible to the removal of
48 the most linked (generalist) species (e.g. Solé & Montoya 2001). In general, increased
49 behavioural flexibility of species in these networks allows a wider variety of species to interact
50 in response to local resource availability, and this functional redundancy may stabilize ecological
51 networks (Kondoh 2003) e.g. by directly stabilizing both predator and prey population sizes
52 (Singer & Bernays 2003) or via indirect control on lower level food web links (Rosenheim &
53 Corbett 2003) ~~_although they are among the most difficult to document.~~

54 In response to resource limitations, species may compete for resources or alter the prey
55 they choose. Over many generations, resource limitation may drive the evolution of
56 morphological or behavioural specialization and adaptive radiations and sympatric species are
57 thought to evolve and co-exist through partitioning available resources and niche ~~specialization~~
58 differentiation (Ricklefs 2007). Alternatively, competition for resources may result in increasing

59 niche flexibility (Grant & Grant 1987; Tebbich *et al.* 2004). ~~Behavioural flexibility is key to~~
60 ~~ecosystem functioning and is viewed as a stabilizing force in food webs which buffers the impact~~
61 ~~of species loss (Solé & Montoya 2001; Dunne *et al.* 2004).~~ Spatial-temporal variation in resource
62 use is an important form of behavioural flexibility which is particularly adaptive when resource
63 availability fluctuates.

64 There are two main ~~hypotheses ways in~~ which ~~attempt to explain how~~ resource
65 distributions ~~are may be~~ related to the stability of food webs. First, increasing complexity within
66 food webs increases their stability and thus highly linked generalists promote ecosystem
67 functioning (Solé & Montoya 2001). Second, generalists that consume resources based on
68 frequency of encounters (Rosenheim & Corbett 2003) (rather than achieving generalism by
69 switching between highly specialized tactics) may respond to resource limitation by increasing
70 the abundance of a particular resource or increasing their flexibility and consuming a wider
71 variety of resources. In this context, behaviourally flexible highly linked omnivores ~~or~~
72 ~~generalists~~, that respond to limitations by increasing the variety of prey they consume, may be
73 particularly important components of ecosystem stability and documenting their ecosystem
74 function ~~is~~ vital to understanding ecosystem response to disturbance (Solé & Montoya 2001).

75 Behavioural flexibility in foraging by insectivorous bats has been well documented and
76 dietary diversity and choices may be influenced by habitat variation (Aldridge & Rautenbach

77 1987), temporal availability and abundance of prey (Rydell *et al.* 1996), gender (Belwood &
78 Fenton 1976) and or age (Adams 1997). In extreme cases, bats may switch between feeding
79 guilds and trophic levels to supplement diet (e.g. the ~~supposedly~~-nectivorous *Glossophaga*
80 *soricina* uses unique tactics incorporate insects in its diet (Clare *et al.* in ~~presseview~~)). This
81 degree of flexibility is unusual in a top predator and makes ~~them-bats~~ key ecosystem ~~species-taxa~~
82 and excellent models for the study of ecosystem functioning, though their cryptic behaviour
83 makes it extraordinarily difficult to directly document their behaviour in the wild.

84 A variety of molecular methods have been used to untangle ~~these-complex~~ ~~relationships~~
85 species' interactions (Symondson 2002; King *et al.* 2008), especially next generation sequencing
86 (NGS) methods (Pompanon *et al.* 2012) that can generate millions of ~~prey-sequences~~ ~~-at~~
87 relatively low cost. NGS methods can be applied to fragmentary, emulsified or mixed starting
88 materials such as stomach or faecal contents. This approach is based on sequencing in
89 extraordinary volume (so called "sequencing depth") to encompass the complete richness of
90 ~~targets~~ ~~species~~ within a system (Pompanon *et al.* 2012). This contrasts with cloning methods
91 (Zeale *et al.* 2011) that also begin with mixed starting materials but where the discovery of new
92 prey species is based on the inevitably limited number of sequenced clones rather than the NGS
93 mass screening approach. The NGS approach provides a possible solution to the problem of
94 understanding the complexity of interactions between generalist predators and their prey by

95 analyzing the prey exploited based on large samples. Using this method we can extend our
96 analyses beyond accurately documenting interactions (e.g., Deagle *et al.* 2009, 2010) to testing
97 specific predictions about how species' interactions vary in time and space (e.g., Razgour *et al.*
98 2011).

99 Several previous molecular analyses of bat diets have documented temporal variation in
100 resource use. In the first large scale molecular analysis of the diet of an insectivorous bat, Clare
101 *et al.* (2009) found little evidence that *Lasiurus borealis* (the eastern red bat) in Ontario, Canada
102 exhibited temporal variation in prey consumption. In contrast, molecular analyses of the feces of
103 another species, *Myotis lucifugus* in Ontario (Canada) (Clare *et al.* 2011, Clare *et al.* ~~in~~
104 ~~review~~[THIS ISSUE](#)) and *Plecotus* in the UK (Razgour *et al.* 2011) showed evidence of temporal
105 variation in diet. In the case of sister species of *Plecotus* this may lead to competition when
106 resources become limited (Razgour *et al.* 2011).

107 ~~The tendency for resident populations of bats to hunt locally and show strong temporal~~
108 ~~variation in resources use has significant implications for understanding ecosystem dynamics and~~
109 ~~their response to change.~~ Understanding ecosystem function and the services provided by
110 generalist predators is particularly important when population demography is unstable and this
111 “service” may fluctuate (Blehert *et al.* 2009). *Eptesicus fuscus*, the big brown bat, is common
112 across most of North America and ~~in~~ parts of Central and northern South American and the

113 Antilles (Simmons 2005). It is one of the bats best known to the ~~general~~ North American public
 114 because of its association with rabies (Nadin-Davis *et al.* 2010) and its propensity to roost in
 115 buildings (Kurta & Baker 1990). ~~E. fuscus~~ Individuals may frequently forage within 2 km of their
 116 roost (Kurta and Baker 1990), ~~or~~ but may also travel to sites up to 7 km away (Brigham 1991). In
 117 some areas, ~~E. fuscus~~ big brown bats use night roosts as places to temporarily stop and digest
 118 prey (Kurta & Baker 1990) so accumulations of droppings at night roosts provide an opportunity
 119 to determine which foods are consumed locally.

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120 While many bats used mixed foraging habitats (and sympatric species may overlap in
 121 their foraging (Furlonger *et al.* 1987)) some general trends in habitat use are apparent for the
 122 common species in the study site (Ontario Canada). For example, hoary bats and Eastern-eastern
 123 red batss often forage in cover or along edges (Furlonger *et al.* 1987) and frequently in
 124 concentrations of insects at street lights (Hickey & Fenton 1996; Acharya & Fenton 1999), while
 125 the three small *Myotis* bats little brown bats mainly forage are much more habitat restricted and
 126 forage over riparian systems water (Furlonger *et al.* 1987;-) very close to their roosts (Clare *et al.*
 127 2011) more commonly than the othe Clare *et al.* 2011) species.- In contrast, Big-big brown bats
 128 appear to employ one of the mosta very general-flexible forage strategies strategy with no
 129 significant habitat associations (Furlonger *et al.* 1987)s. Acoustic monitoring has measured big
 130 brown bat activity in all rural settings. They have been reported to fly over-including over water,

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131 forested areas, along edges ~~and at street lights and in both rural and urban settings~~ (Geggie &
132 Fenton 1985; Furlonger *et al.* 1987) ~~and they are the only species to make significant use of~~
133 ~~urban areas~~ (Furlonger *et al.* 1987). Although they employ a generalist strategy across these
134 landscapes, big brown bats have been ~~paradoxically~~ called beetle specialists (Coleoptera; e.g.,
135 Freeman 1981; Ober and Hayes 2008; Agosta *et al.* 2003). However, the ~~alleged-observed~~
136 preference for beetles (as high as 96% of diet by mass, Agosta *et al.* 2003) has not been
137 challenged by accurate species-level identification of their prey. The hard carapace of beetles
138 may preferentially survive digestion leading to an overrepresentation of these taxa during
139 morphological analysis of prey remains in faeces. Molecular analysis has been demonstrated to
140 accurately identify small, soft bodied prey and rare taxa (Clare *et al.* 2009) and ~~it~~ presents an
141 excellent diagnostic tool ~~to assess the complete~~ ~~providing species-level analysis of the~~ diet of big
142 brown bats. ~~These data will and~~ answer the question of whether their generalist foraging strategy
143 translates into a far more general diet than previously documented.²

144 We assessed the diet of a group of night-roosting big brown bats during ~~their colony~~
145 ~~establishment periods in~~ ~~the summer of~~ both 2008 and ~~in~~ 2011 (total of 25 weeks). The roost was
146 located in a forested patch along the Grand River in ~~Cambridge~~ Ontario. The bats roosted in an
147 overhanging section of soffit and fascia. Continuous observation and inspection of the home
148 showed no evidence that the bats roosted in the home during the day ~~however they -E.fuscus~~

149 foraged in the area in the apparent absence of other bat species and fresh guano (faeces)
150 accumulated each night. Given this, We-we concluded that we were sampling faeces left by
151 night-roosting bats rather than a permanent colony. If the bats whose diets we documented fed
152 locally, we predicted that the diet would vary reflecting local availability of prey.

153 We tested three hypotheses about the diet of this species. First, we tested the hypothesis
154 that resident big brown bats exhibit seasonal variation in their diet reflecting a preference for
155 local feeding, tracking generally established fluctuations in prey abundance. Second, we tested
156 the hypothesis that variation in diet between years is minimal so that overall dietary diversity and
157 seasonal variation in diet are stable across years. Finally, we tested the prediction that E. fuscus
158 isbig brow bats are beetle specialists by estimating the relative importance of prey groups in the
159 diet of this species over two years of monitoring. We also compared ordinal level analysis of
160 prey (as traditionally conducted during morphological dietary analysis) with analysis at the
161 species level made possible using molecular methods.

162

163 **Materials and Methods**

164 *Sample collection, DNA Extraction, Amplification and Sequencing*

165 We placed collection sheets under the colony for weeklong periods between May and
166 September in 2008 and again in 2011. We collected the accumulated faeces weekly and stored it

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167 at -20°C prior to analysis for a total of 25 weeks of monitoring (15 weeks of continuous
168 monitoring in 2008 and 10 weeks in 2011 reflecting differential colony establishment times in
169 the two years, we observed no variation in sequencing success due to length of storage).

170 For each weekly sample we selected a minimum of 30 faecal pellets and homogenized
171 them as a single unit to ensure they were well mixed – hereafter we refer to these homogenized
172 pellets as a “sample”. From each sample we extracted DNA using the QIAmp DNA Stool Mini
173 Kit (Qiagen, ~~Valencia, CA~~UK) according to manufacturers instructions with the modifications
174 suggested by Zeale *et al.* (2011). In addition we made the following protocol additions; 1) to
175 encompass community diet in each sample, rather than the diet of a single individual bat, we
176 used many (at least 30) faecal pellets rather than just one giving a volume of 1-1.5ml of starting
177 material and 2) we extended the first centrifuge step (Zeale step 4) to 3 minutes to aid in
178 pelleting the particulate material produced by this large volume. We stored the extracted DNA at
179 -20°C prior to PCR amplification.

180 We tested all DNA extractions using unmodified primers ZBJ-ARTF1c and ZBJ-ArtR2c
181 from Zeale *et al.* (2011) to confirm extraction success. We then amplified each sample using
182 fusion primers (Figure 1) adapted for the Ion Torrent platform (Ion Torrent, Life Technologies).
183 These primers consisted of adaptor sequences (forward adaptor and trP regions) a unique DNA
184 sequence (MID) used to bioinformatically separate sequences for analysis and the original primer

185 sequence ZBJ-ARTF1c and ZBJ-ArtR2c from Zeale *et al.* (2011) as required for the sequencing
186 platform. In our design we [follow Brown et al. \(THIS ISSUE\) and](#) used unique combinations of
187 10 bp MID sequences on both the forward and reverse primer for each pooled sample. This
188 design allows fewer primers to be used to resolve the same number of samples (called sequence
189 libraries) (e.g., rather than 100 unique forward MID tagged primers for 100 samples, 10 unique
190 forward and 10 unique reverse~~s~~ can yield the same resolution power). This reduced the cost of
191 primers. We [sequenced only in the forward direction](#) ~~did not use bi-directional sequencing but~~
192 ~~sequenced only in the forward direction~~ further reducing the number of required primers.

193 We carried out PCR~~s reactions~~ following the amplification reaction described by
194 ~~(~~Bohmann *et al.* (2011) in a 20 ul reaction containing 1ul of template DNA using Qiagen
195 multiplex PCR kits (Qiagen, UK) as described with the following modifications: we did not use
196 either Q solution (from Qiagen) or BSA (as suggested by Bohmann *et al.* 2011). We visualized
197 all PCR products on a 1.5% agarose gel. We quantified the PCR products by measuring the
198 relative luminescence of 1ul of PCR product on a 1.5% agarose gel stained with ethidium
199 bromide (validated using Qubit low sensitivity dsDNA BR Assay Kit on a Qubit Flurometer,
200 Invitrogen Life Technologies~~)~~). We pooled equal molar quantities of each PCR product and then
201 size selected and purified these using a QIAquick Gel Extraction kit (Qiagen, UK). We
202 quantified the final mixed PCR product using a Qubit dsDNA BR Assay Kit (low sensitivity)

203 with a Qubit Fluorometer (Invitrogen Life Technologies). Sequencing of the product was
204 conducted at the University of Bristol Genomics facility (School of Biological Sciences, Bristol
205 UK) on an Ion Torrent personal gene machine (PGM) (Ion Torrent, Life Technologies) using a
206 316 chip and 300 bp chemistry. PCR products were quantified for sequencing using a
207 Bioanalyzer (high sensitivity kit, Agilent Technologies).

208

209 *Sequence Analysis*

210 We separated forward and reverse MIDNs, clipped primer and adapter sequence and
211 filtered sequences using the Galaxy platform (<https://main.g2.bx.psu.edu/root>, (Giardine *et al.*
212 2005; Blankenberg *et al.* 2010; Goecks *et al.* 2010) and Bioedit (T. Hall, [http://www.](http://www.Mbio.ncsu.edu/bioedit/bioedit.html)
213 [Mbio.ncsu.edu/bioedit/bioedit.html](http://www.Mbio.ncsu.edu/bioedit/bioedit.html)). We filtered all recovered sequences for rare haplotypes
214 (represented by <2 copies) and sequences much longer (>175 bp) or shorter (<100 bp) than the
215 expected 157 bp amplicon.

216 We clustered the sequences into molecular operational taxonomic units in the program
217 jMOTU (Jones *et al.* 2011) and tested thresholds from 1-10 bp. A graph (not shown) of
218 recovered MOTU vs. threshold suggests that a 4 bp cut-off was most appropriate for this data set
219 (see Razgour *et al.* 2011). We extracted representative sequences for each MOTU ~~and edited~~

220 ~~and aligned them manually to remove indels and to match~~ to compare with reference sequences
221 from known insect ~~sequence~~ taxa.

222 We compared these representatives to the reference database in BOLD (www.
223 barcodinglife.org) and extracted identifications based on four criteria modified from (Razgour *et*
224 *al.* 2011). Confidence 1a = match to one species or several species in a genus (100% sequence
225 similarity) most conservative taxonomy kept; confidence 1b = good match (>98% sequence
226 similarity) but could belong to a congener if the database is updated with something with a
227 higher sequence match; confidence 2 = match to more than one species (>98.5%) only one of
228 which is known to be present in sampling range (that taxonomy kept) and confidence 3 = close
229 match (as above) to several species from different genera, or to reference sequence which itself
230 lacks a full taxonomic record. In these cases, the most conservative taxonomy (normally family)
231 was kept (Note: this is not the same as an identification to higher level taxonomy, but an
232 acknowledgement of a match meeting the criteria of 1b but retaining an ambiguity in the proper
233 assignment due to either multiple similar matches or incomplete data in the reference collection).

234 In addition, we estimated the identity of all prey (including unidentified MOTU) using the
235 methods of Emrich et al. (THIS ISSUE) and the programme MOTU. See Emrich et al. (THIS
236 ISSUE) for details of that procedure and a brief discussion.

237

238 *Ecological Analysis*

239 We divided our collections into three temporal periods: early (May 13th - June 20th)
240 middle (June 20th - Aug 6th) and late (Aug 6th - September 9th) (periods in 2011 varied by one or
241 two days to equalize time periods and based on colony establishment) following (Clare *et al.*
242 2011) to coincide with the observed periods of pregnancy, lactation and post lactation for this
243 species in Ontario. Ecological analyses were conducted using the program PAST (Hammer *et al.*
244 2001) on species and order level data with p-values estimated by permutation. We compared the
245 Shannon and Simpson diversity indices for identified prey between years and between early, middle
246 middle and late summer within years and estimated the magnitude of the effect where differences
247 were statistically significant following Jost (2006). We computed rarefaction curves for these
248 data with 95% confidence intervals.

249 We compared the frequency of consumption for each order (number of species consumed
250 and the frequency of each species consumption) and each species (with its frequency) between
251 years and between early, middle and late summer within years using a χ^2 test with p-values
252 computed using a Monte Carlo simulation with 2000 replicates in R 2.15.1 ("R Development
253 Core Team: R: A language and environment for statistical computing" 2008).

254

255 **Results**

256 *Prey Identification and Dietary Composition*

257 We recovered ~3.5 million DNA sequences from the Ion Torrent 316 chip of which
258 ~50% were produced for this study (the chip was shared). Of these, processing for quality
259 (sequence length, recoverable MIDs and primers), collapsing to unique haplotypes and splitting
260 files by MID left a total of 32,212 unique haplotypes from all 25 weeks of collection (Data
261 archived in [Dryad-Dryad:....](#)). Processing through jMOTU reduced these to 221 molecular
262 operational taxonomic units (MOTU) at the 4 bp threshold.

263 Using representative sequences of the 221 MOTU (species of prey) we identified 158
264 (71%), with most identifications at the species level, through comparison to existing reference
265 databases of known insect DNA barcodes for the area. Of these, species of Lepidoptera, Diptera
266 and Coleoptera dominated the diet, with species of Coleoptera representing the highest frequency
267 of consumption while Lepidoptera were present in the highest taxonomic richness (Table 1).

268

269 *Seasonal and Annual Variation in Prey Consumption by order*

270 Using order level taxonomy (~~ignoring genus and species~~only), the consumption of
271 species showed strong seasonal variation in 2008 ($\chi^2 = 20.6$, $p=0.005$) and in 2011 ($\chi^2 = 23.2$,
272 $p=0.004$) (Figure 2) although the pattern of variation was inconstant between years and the
273 overall consumption of prey differed significantly between the two years ($\chi^2 = 19.7$, $p=0.04$;

274 Figure 3). In 2008 the early summer diet was dominated by species of Diptera (43% of prey
275 consumed) while the importance of Coleoptera increased throughout the year peaking at 32% in
276 the late summer. In 2011, Coleoptera were dominant in both early (39%) and mid (38%) summer
277 diet while Diptera increased from 20-26% over the year. Consumption of Lepidoptera was
278 relatively constant within and between years varying between ~18-29% of the diet at all times
279 (Figure 2, Figure 3). Similarly the consumption of Ephemeroptera~~ns~~ was low but consistent
280 across the summer and years representing 8-13% of the identified prey diet at all times (Figure 2,
281 Figure 3).

282 | Despite variation ~~in how prey resources are used~~in the consumption of prey orders, we
283 found no change in the diversity of diet between years (Shannon index 2008=1.57, 2011= 1.60,
284 $p=0.55$, Simpson index 2008=0.78, 2011= 0.77 $p=0.41$; Figure 4 and 5). Within years, we
285 detected a significant increase in the diversity of prey consumed between early and late summer
286 in 2008 (Shannon index early=1.4, late= 1.5, $p=0.036$, Simpson index early=0.71, late= 0.77
287 $p=0.007$) though significance was lost in the Shannon index comparison after a sequential
288 Bonferroni correction (Figure 4, Figure 5). The magnitude of the difference in the effective
289 number of species consumed was close to 10% between the early summer and either middle or
290 late summer. In 2011 the diversity of prey consumed was significantly higher in late summer
291 than in either early (Shannon index early=1.5, late= 1.7, $p=0.009$, Simpson index early=0.75,

292 late= 0.81 p=0.014) or mid (Shannon index early=1.4, late= 1.7, p=0.001, Simpson index
293 early=0.73, late= 0.81 p=0.003) summer (Figure 4, Figure 5) with the magnitude of the
294 difference estimated at 9% and 7.5% respectively. In both cases, the effective number of species
295 in the diet appears to increase by as much as 10% from early in the summer (when bats are
296 pregnant) to late summer (when bats are preparing to hibernate).

297

298 *Seasonal and Annual Variation in Prey Consumption by MOTU*

299 Using MOTU as a proxy for species level taxonomy, dietary diversity also was constant
300 between years (Shannon index 2008=4.6, 2011= 4.4, p=0.54, Simpson index 2008=0.99, 2011=
301 0.95 p=0.41) (Figure 4, Figure 5). In 2008 we detected a significant increase in the diversity of
302 prey consumed between early and mid summer (Shannon index early=3.8, mid= 4.1, p=0.032,
303 Simpson index early=0.97, mid= 0.98 p=0.021) though significance is lost in the Shannon index
304 after a sequential Bonferroni correction (Figure 4, Figure 5). We also detected an increase in
305 diversity between early and late summer (Shannon index early=3.8, late= 4.1, p=0.013, Simpson
306 index early=0.97, mid= 0.98 p=0.012) (Figure 4). We detected no significant trend in diversity
307 changes in 2011 after sequential Bonferroni correction (Figure 4, Figure 5).

308 We recovered a similar analysis of prey diversity from MEGAN (Figure 6) which suggest
309 that unidentified prey are relatively dispersed among the consumed insect groups.

310

311 *Dietary Species of Interest*

312 A number of prey species consumed by big brown bats are interesting in the context of
313 “ecosystem services” provided by bats. Big brown bats ate human pests such as *Culex pipens*
314 (the common house mosquito), *Simulium* spp. (the black flies) and *Polistes* spp. (the paper
315 wasps). Paper wasp nests were observed under the same overhang as the bat roost. It is possible
316 this record represents a secondary contamination event, however the nest was not located over
317 the collection surface. Bats also ate some species such as Trichoptera that emerge from water.
318 Other aquatic insects *Caenis* spp. and *Maccaffertium mediopunctatum* are generally found over
319 moving water habitats in this area of their distribution (Clare *et al.* 2011).

320

321 **Discussion**322 *Dietary Richness and Diversity*

323 The most striking conclusion from our analysis is the confirmation that Eptesicus
324 fuscus big brown bats rely heavily on Coleoptera although, at most, beetles constituted about
325 40% of dietary richness. This contrasts strongly with the Previous analyses have reported 42-96%
326 consumption by volume in some studies (e.g. Agosta *et al.* 2003). While abundance is not
327 necessarily proportional to species richness, they are often related, particularly when a predator

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328 cannot effectively make prey choices at the species level. It is very likely that consumption
329 varies significantly between areas and years, at least in part due to local conditions. ~~In this case,~~
330 ~~We furthermore strongly~~ suspect ~~that~~ big brown bats ~~are unlikely to~~cannot obtain enough
331 information to discriminate subtle species level characteristics, but ~~likely~~ make choices about
332 prey ~~perhaps~~ based on acoustic detection of size, flight speed, flight path, and acoustic properties
333 generated by hard carapaces revealed through echolocation (see also Brigham & Saunders
334 ~~(1990)~~ and Barclay & Brigham ~~(1994)~~). It is also very probable~~y~~ that the flexibility exhibited by
335 big brown bats leads to significant regional differences in diet thus the relative importance of
336 beetles may vary greatly by location (e.g. (Brigham 1990). Agosta *et al.* (2003) argued that
337 richness of diet increases when prey themselves become more limited, presumably because
338 resource limitation provides incentive for a more generalist strategy. Our data also ~~suggested~~ that
339 dietary richness increased in the late summer. In this case, increases in dietary richness measured
340 at the species level here, and estimates made for richness measured by abundance (Agosta *et al.*
341 2003) may be in agreement that diet is responding- to local insect population fluctuations. This
342 presumes that insects themselves are a limited resource and there may be some evidence for this
343 in late summer of temperate bats' active periods. Razgour *et al.* (2011) observed increased
344 dietary partitioning between cryptic bat species late in the year and speculated that a drop in
345 insect abundance led to increased resource competition driving a temporally constrained niche

346 specialization. Records-Trends of flight periods of adult beetles in Ontario obtained from the
347 Canadian National Collection (CNC, Ottawa Ontario, Canada) for many species in this dataset
348 suggest a local flight peak in mid summer corresponding to the lactation period of bats. For
349 example, *Ecyrus dasycerus*, and *Cymatoder bicolor* peak in June and July, *Ampedus semicinctus*
350 peaks in May-July, *Graphisurus fasciatus* and *Monochamus notatus* peak in June – August, and
351 *Melanotus similis* peaks in April-August (Bruce Gill, CNC Ottawa, personal communication).
352 We hypothesize that if beetle [insect] species richness and diversity undergoes a significant local
353 decrease in late summer coinciding with increased dietary needs of bats as they approach
354 hibernation, this could drive increases in dietary richness without the bats actively choosing new
355 prey at the species level. As such, it is an effect of insect phenology, rather than predator choice.
356 Interestingly, records for a wider geographic area show peaks for these same insect species often
357 extend into late fall (Yanega 1996) in other areas thus the affect may be local to this part of the
358 bats' and beetles' range. An additional contributing factor to this pattern is the emergence of
359 juvenile bats that may be less discriminatory in their prey choice. Their appearance co-insides
360 with this drop in insect richness and abundance and both factors may cause an increase in dietary
361 richness.

362 In addition to the importance of Coleoptera in the diet, Diptera, Lepidoptera, and
363 Ephemeroptera were often eaten. While Diptera (primarily chironomids) and Coleoptera varied

364 in importance over time, Lepidoptera and Ephemeroptera were consistent components of the
365 bats' diet across years. As such, they may be an underappreciated stable resource supporting the
366 population while other insect groups fluctuate in importance.

367 Several ~~One~~-important data considerations need to be taken into account. First, is that
368 within a sample, prey ~~are~~-were measured simply by their presence (quantification is not
369 possible). We used larger pools of guano to maximize potential biodiversity by increasing the
370 number of contributing predators. However, a rare item and a common item would both be
371 recorded as "present" in that sample. A large sample size may control for the potential for
372 overrepresentation of rare prey (or underrepresentation of common prey) in any one sample,
373 though it is not a correction that can be empirically assessed. Another consideration is that
374 Lepidoptera dominates the reference collection, as this has been a major campaign of the various
375 DNA barcode consortia. As such, there is likely an identification bias towards Lepidoptera which
376 must be considered, though our estimates based on MEGAN (Figure 6) did not vary greatly
377 suggesting this is not a significant factor. Finally, we have no basis to conclude whether the bats
378 have consumed prey groups (e.g. moths) in accordance with abundance, however the authors
379 have evaluated the diet of 5 sympatric species of bat (manuscript published and in preparation)
380 and none has revealed beetles as such a strong dietary component, thus we suggest this is a true

381 preference for this predator on this prey rather than a case of encountering more in the
382 environment.

383

384 *Temporal variation in diet*

385 Increases in dietary diversity as the summer progressed, suggests a relationship between
386 diet and known changes in species richness in prey availability. Seasonal limitations in prey
387 cause shifts to more generalist behaviour in several groups. For example, spiders are normally
388 unselective in their choice of prey because they are in a state of suboptimal nutrition and cannot
389 afford to be selective, however, when prey are in excess, they become more selective (Riechert
390 & Harp 1987). We may have recorded a similar effect raising implications for the relationship
391 between diet and hibernation. Hibernation and torpor success is directly influenced by dietary
392 components. For example, in marmots, diets deficient in essential fatty acids led to decreased
393 length of torpor (Florant *et al.* 1993) and a diet high in n-6 polyunsaturated fatty acids (PUFA)
394 increases the frequency and duration of torpor, decreases body temperature and decreases mass
395 loss during inactivity, and the ratio of n-6 to n-3 PUFAs may be key (Ruf & Arnold 2008).
396 However, insectivorous bat diet is naturally very low in PUFA and, though they do apparently
397 preferentially select insects which maximize PUFA intake, the goal may be to optimize rather
398 than maximize PUFA (Munro & Thomas 2004). Coleoptera and Trichoptera are very high in the

399 essential fatty acid linoleic acid (Schalk & Brigham 1995) and the richness of these in the diet of
400 big brown bats increased in the fall in 2008 and 2011 ~~respectively~~. This may reflect an increase
401 reliance on prey which can supply high quality diet in preparation for hibernation when body
402 mass may increase by as much as 50% (Beer & Richards 1956), though the relationship
403 between dietary fats and hibernation is complex.

404 Our observations of an increase in dietary diversity contradict the pattern that dominates
405 in little brown bats across Canada (Clare *et al.* ~~in review~~ THIS ISSUE). In ~~this~~ that species,
406 diversity peaked in mid summer and was significantly reduced in late summer. This difference
407 likely reflects the degree of habitat flexibility between these two predators. Little brown bats
408 hunt ~~mainly~~ more frequently over water. In ~~this~~ that case, reduced flexibility in habitat choice
409 may reduce the diversity of their diet. Little brown bats may rely on greater volume of more
410 limited prey richness, while big brown bats exploit a wider variety of prey.

411 We documented dietary variation across the summer but also between years which
412 strongly supports the importance of long term monitoring. Had our analysis been limited to a
413 single year we would not have uncovered that the seasonal patterns of prey use change between
414 years. For example, Diptera accounted for half of the dietary prey in early 2008 and decreased in
415 importance throughout the year but the opposite trend occurred in 2011 where they increased in

416 importance. Similarly, considering only inter-annual variation in diet would not have uncovered
417 the seasonal variation in prey use.

418

419 *Ordinal vs. species level analysis of diet.*

420 We compared dietary trends using data restricted to ordinal level identifications (as
421 would have been obtained using traditional fecal analysis by morphology) to species (MOTU)
422 level taxonomy available using molecular methods. There are two main advantages of molecular
423 dietary analysis. First, it automates the identification process reducing the need for specialized
424 training. ~~While some mammalogist have specific entomological qualifications, many~~ Most
425 traditional analyses of diet are conducted by ~~mammalogists~~ ~~individuals without this~~
426 ~~specialization (not entomologists) and~~ thus the skills required for proper identification of ~~these~~
427 insect fragments under a microscope must be obtained on an *ad hoc* basis for analysis. ~~While the~~
428 ~~bioinformatics of NGS analysis similarly require training, molecular analysis in general is less~~
429 ~~specialized and most molecular labs can perform these steps without additional training.~~ Second,
430 the resolution is much higher using molecular analyses (Clare *et al.* 2009) providing much more
431 information from the same samples with less effort and ~~we which are is~~ more likely to document
432 the presence of rare prey items (Clare *et al.* 2009). While these differences may be important in
433 terms of demonstrating behavioural flexibility which stabilizes ecosystem functioning, it may not

434 be important in terms of energetics when these are consumed in low frequency (Table 1). It is
435 particularly interesting that, while the trends we found are similar between the different levels of
436 analysis (Figure 4, Figure 5), our ability to detect statistical significance differed. This is largely
437 due to the variability of the data. For example, while diversity increased between early and mid
438 summer in 2008 (Figure 4) we only detected significant differences in the species-level analysis
439 (Figure 4d). Similarly, diversity increased in late 2011 but this was only significant in ordinal
440 level analysis. Rarefaction curves (Figure 5) are more revealing in species level analysis as the
441 data quickly plateaus in ordinal level analysis. The key then is to recognize the advantage of
442 species-level resolution, while keeping in mind that the bats themselves may not be acquiring the
443 same information acoustically. Concurrent analyses at both levels may be the most revealing. We
444 used both the Shannon and Simpson diversity indices though we only report data for the
445 Simpson indices in figures. The Simpson index is less sensitive to the inclusion of rare records.
446 Species-level analysis of diet almost always leads to the detection of many rare taxa (Bohmann
447 *et al.* 2011), indeed that is one of its advantages (Clare *et al.* 2009), thus the Simpson index is
448 likely more appropriate for these data, while both the Shannon and Simpson index could be used
449 at the ordinal level where the detection of new taxa has reached a plateau (Figure 5).

450

451 *Habitat and foraging behaviour inferred from dietary analysis*

452 | The species richness of prey consumed by big brown bats included both terrestrial insects
453 | and those that emerge from water (e.g., mayflies and caddisflies) supporting the view that *E.*
454 | *fuscus* is a generalist. This reflects the fact that there is little evidence of preference for habitat(s)
455 | (Geggie & Fenton 1985; Furlonger *et al.* 1987). Species level dietary analysis provides a unique
456 | opportunity to infer habitat parameters non-invasively without radio tracking (Clare *et al.* 2011,
457 | [Clare et al. THIS ISSUE](#)). Consumption of *Caenis* and *Maccaffertium mediopunctatum* suggest
458 | that these bats hunt over a small, fast-moving stream located 100 m of the night roost. Similarly
459 | the bats appear to have eaten paper wasps whose nests were adjacent to the night roost providing
460 | a direct benefit to the homeowners who actively tried to discourage the wasps. The strong
461 | reliance on beetles and moths indicates that most hunting is not over water (unlike little brown
462 | bats) although some insects (e.g., Coleoptera genus *Agonum*) prefer damp habitats.

463

464 *Implications of resource competition*

465 | Resource partitioning is thought to be common and may result from previous (or
466 | ongoing) competition between consumers. This rests on the assumption that resources such as
467 | food are limited and therefore limiting. While it is obvious that many bats eat different insects
468 | (e.g., eastern red bats and Lepidoptera (Clare *et al.* 2009), little brown bats and emerging aquatic
469 | insects (Clare *et al.* 2011), big brown bats beetles + a variety of other prey) it is not clear why

470 these divisions occur. The alternative hypothesis is that habitat preferences based on morphology
471 (~~e.g.~~ Freeman 1981) lead to partitioning of insect resources in the absence of any food resource
472 limitation or current competition (~~Emrich et al. THIS ISSUE~~). For a true test of these hypotheses,
473 at least one potential competitor must be excluded to determine whether the behaviour of the
474 others changes. The continuing spread of white nosed syndrome (WNS) (Cryan *et al.* 2010) and
475 its lethal impact on bats that hibernate underground may provide a natural test of this hypothesis.
476 Although ~~*Eptesicus fuscus*~~ big brown bats appear to be somewhat resilient to infection and
477 mortality, several sympatric species of *Myotis* species are not and are predicted to be locally
478 extirpated by 2020 (Frick *et al.* 2010). Our study represents an important baseline of information
479 about diet based on a pre-WNS population (2008) and ~~effected~~ affected (but not post-WNS)
480 population (20101). If resource competition exists between bat species in this community, these
481 measures may be used to assess both competition between predators and population responses of
482 prey as predators are lost from the ecosystem, particularly if measured across a broad geographic
483 region.

484

485 *Conclusions*

486 Our observations confirm that the flexible foraging strategy of big brown bats
487 corresponds to a generalist diet. Extreme seasonal and inter-annual fluctuations in diet highlight

488 | the importance of continuous monitoring for accurate dietary characterization. We confirm the
489 | importance of beetles in the diet, but also highlight that, while this resources appears to fluctuate,
490 | Lepidoptera and Ephemeroptera are stable dietary components and may be an important buffer in
491 | times of resource limitation. Our observation that, in response to resource fluctuations, these bats
492 | become even more flexible and increase taxonomic diversity of prey, highlights their importance
493 | as a highly connected ecosystem components promoting stability in response to disturbance.

494

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625

626 **Data Accessibility:** Sequence files are [a read me file are](#) available in Dryad~~.... (accession/doi~~
627 ~~available on publication);~~ [doi:10.5061/dryad.t30bh](https://doi.org/10.5061/dryad.t30bh)

628

629 **Author Contributions:** ELC and MBF conceived of the project and provided funding. ELC
630 conducted all field, molecular and bioinformatics analysis. ELC and WOCS contributed to
631 molecular protocol designs. ELC, WOCS and MBF wrote the manuscript.

632

633 **Figure Legends**

634 Figure 1: Primer design used in this study for uni-directional sequencing on the Ion Torrent
635 sequencing platform. Our forward primer included a “key” region. The key is the only difference
636 between the design of the forward primer in Roche 454 Lib-L chemistry (Roche, Basel,
637 Switzerland) and the Ion Torrent platform primer A (Ion Torrent, Life Technologies). Including
638 this “key” permits us to use the same primers on both platforms and does not interfere with
639 amplification or sequencing thus it was included here but is not required.

640

641 Figure 2: Seasonal variation in dietary prey consumption. The proportion of each prey group
642 composing the diet varied significantly across the summer and between years. A) In 2008
643 Diptera dominated early while Coleoptera become more important in the late summer. B) In
644 2011 Coleoptera were more important earlier in the year but decreased towards late summer.

645

646 Figure 3: Overall consumption of prey groups was similar in both 2008 and 2011. Coleoptera,
647 Diptera and Lepidoptera dominated the diet of *Eptesicus fuscus*. Consumption is calculated as
648 presence or absence within a sample x frequency among samples.

649

650 Figure 4: Estimates of dietary diversity based on the Simpson diversity index. Data restricted to
651 ordinal level taxonomy shows variation between early, middle and late summer in A) 2008 and
652 B) 2011 though overall diversity C) was the same between years. Similarly trends are observed
653 in species-level data D), E) and F).

654

655 Figure 5: A comparison of rarefaction curves for operational taxonomic units at the order (A, B,
656 C) and species (D, E, F) level. Red lines indicate mean estimates while blue lines indicate the
657 95% confidence level from permutations.

658

659 Figure 6: A schematic of prey species consumed including all MOTU (including those that could
660 not be identified using a reference database). Identifications have been made by BLAST score
661 and are limited to hypothesis at the order level. Values at nodes or tips represent the number of
662 MOTU assigned. Node size is scaled to the number of assignments. See Emrich et al. (in press)
663 for additional details.

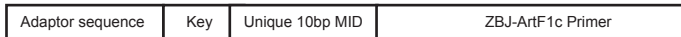
664

665 Table 1: Prey species identified in the diet of night roosting *Eptesicus fuscus*. Frequency refers to
 666 presence or absence in each weekly sample.

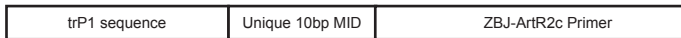
Class	Order	Family	Genus	Species	Similarity	Frequency		
Arachnida	Aranea	Theridiidae	<i>Parasteatoda</i>	<i>sp.</i>	100	1		
Insecta	Coleoptera	Carabidae	<i>Agonum</i>	<i>placidum</i>	100	1		
			<i>Amara</i>	<i>apricaria</i>	100	1		
			<i>Amphasia</i>	<i>sericea</i>	99.33	2		
			<i>Calosoma</i>	<i>frigidum</i>	99.36	1		
			<i>Harpalus</i>	<i>pennsylvanicus</i>	99.31	3		
			<i>Notiobia</i>	<i>terminata</i>	100	16		
			<i>Ophonus</i>	<i>puncticeps</i>	100	8		
			<i>Platynus</i>	<i>cincticollis</i>	100	1		
			<i>Platynus</i>	<i>sp.</i>	98.72	3		
			<i>Poecilus</i>	<i>lucublandus</i>	100	8		
			<i>Selenophorus</i>	<i>opalinus</i>	100	1		
			<i>Stenolophus</i>	<i>comma</i>	99.36	4		
			<i>Stenolophus</i>	<i>ochropezus</i>	98.08	1		
			<i>Trichotichnus</i>	<i>vulpeculus</i>	100	1		
		<i>Trichotichnus</i>	<i>sp.</i>	100	6			
				Cerambycidae	<i>Ecyrus</i>	<i>dasycerus</i>	100	1
					<i>Graphisurus</i>	<i>fasciatus</i>	100	1
					<i>Monochamus</i>	<i>notatus</i>	100	1
					<i>Monochamus</i>	<i>sp.</i>	100	2
				Cleridae	<i>Cymatodera</i>	<i>bicolor</i>	98.08	2
				Elateridae	<i>Ampedus</i>	<i>semicintus</i>	100	2
					<i>Melanotus</i>	<i>similis</i>	100	2
				Silphidae	<i>Nicrophorus</i>	<i>pustulatus</i>	100	1
				Tenebrionidae	<i>Tenebrio</i>	<i>sp.</i>	99.21	4
		Diptera	Chaboridae	<i>Unknown</i>	<i>sp.</i>	99.36	1	
				<i>Unknown</i>	<i>sp.</i>	99.36	4	
				Chironomidae	<i>Ablabesmyia</i>	<i>americana</i>	99.36	1
					<i>Dicrotendipes</i>	<i>tritonus</i>	100	7
					<i>Paracladopelma</i>	<i>winnelli</i>	99.33	3
					<i>Tanytarsus</i>	<i>mendax</i>	100	1
					<i>Tanytarsus</i>	<i>sp.</i>	98	1
					<i>Xenochironomus</i>	<i>zenolabis</i>	99.35	1
<i>Unknown</i>	<i>sp.</i>				100	1		
<i>Unknown</i>	<i>sp.</i>				100	1		
<i>Unknown</i>	<i>sp.</i>				99.31	2		
<i>Unknown</i>	<i>sp.</i>				100	1		
Culicidae	<i>Culex</i>			<i>pipiens</i>	99.07	1		
	<i>Unknown</i>			<i>sp.</i>	100	1		
Peiciidae	<i>Unknown</i>			<i>sp.</i>	100	6		
Psychodidae	<i>Unknown</i>			<i>sp.</i>	100	1		
	<i>Unknown</i>			<i>sp.</i>	100	1		
	<i>Unknown</i>			<i>sp.</i>	100	6		

	Sarcophagidae	<i>Unknown</i>	<i>sp.</i>	100	1
	Simuliidae	<i>Simulium</i>	<i>gouldingi</i>	100	1
	Tachinidae	<i>Zaira</i>	<i>sp.</i>	98.72	1
	Tipulidae	<i>Tipula</i>	<i>furca</i>	100	5
		<i>Tipula</i>	<i>sp.</i>	100	1
		<i>Unknown</i>	<i>sp.</i>	100	1
		<i>Unknown</i>	<i>sp.</i>	99.36	1
		<i>Unknown</i>	<i>sp.</i>	100	1
		<i>Unknown</i>	<i>sp.</i>	99.36	1
		<i>Unknown</i>	<i>sp.</i>	98.64	2
		Ephemeroptera Caenidae	<i>Caenis</i>	<i>youngi</i>	100
	<i>Caenis</i>		<i>sp.</i>	99.35	11
	Ephemeridae	<i>Hexagenia</i>	<i>atrocaudata</i>	100	1
		<i>Hexagenia</i>	<i>limbata</i>	100	1
	Heptageniidae	<i>Leucrocuta</i>	<i>maculipennis</i>	100	2
		<i>Maccaffertium</i>	<i>mediopunctatum</i>	98.72	9
	Isonychiidae	<i>Isonychia</i>	<i>bicolor</i>	100	1
		<i>Isonychia</i>	<i>sp.</i>	100	15
		<i>Isonychia</i>	<i>rufa</i>	99.36	6
Hemiptera	Miridae	<i>Lygus</i>	<i>lineolaris</i>	100	1
Hymenoptera	Vespidae	<i>Polistes</i>	<i>sp.</i>	100	2
Lepidoptera	Blastobasidae	<i>Holcocera</i>	<i>immaculella</i>	100	2
	Coleophoridae	<i>Coleophora</i>	<i>versurella</i>	98.72	1
	Crambidae	<i>Herpetogramma</i>	<i>phaeopteralis</i>	100	2
		<i>Petrophila</i>	<i>bifascialis</i>	100	1
	Elachistidae	<i>Agonopterix</i>	<i>robinella</i>	100	1
	Erebidae	<i>Idia</i>	<i>sp.</i>	100	2
	Gelechiidae	<i>Coleotechnites</i>	<i>sp.</i>	98.72	3
		<i>Ezoteleia</i>	<i>dodecella</i>	99.36	1
		<i>Filatima</i>	<i>pseudacaciella</i>	100	1
	Geometridae	<i>Hydriomena</i>	<i>impluviata</i>	100	6
	Lasiocampidae	<i>Malacosoma</i>	<i>disstria</i>	99.36	15
	Limacodidae	<i>Lithacodes</i>	<i>fasciola</i>	100	1
	Lyonetiidae	<i>Corythophora</i>	<i>sp.</i>	100	1
	Noctuidae	<i>Elphria</i>	<i>sp.</i>	100	1
		<i>Spodoptera</i>	<i>sp.</i>	100	3
		<i>Zale</i>	<i>galbanata</i>	100	1
	Pyralidae	<i>Sciota</i>	<i>virgatella</i>	100	2
	Tineidae	<i>Amydria</i>	<i>sp.</i>	99.36	1
	Tortricidae	<i>Acleris</i>	<i>chalybeana</i>	100	1
		<i>Clepsis</i>	<i>clemensiana</i>	99.26	1
		<i>Cnephasia</i>	<i>geitalana</i>	98.08	1
		<i>Eucosma</i>	<i>derelecta</i>	98.72	1
		<i>Eucosma</i>	<i>sp.</i>	99.31	1
		<i>Phtheochroa</i>	<i>birdana</i>	100	3

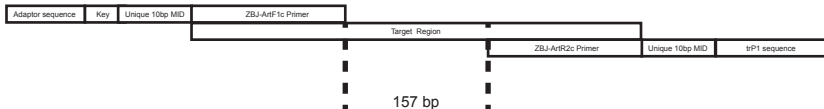
		<i>Pseudoexentera</i>	<i>cressoniana</i>	99.36	3
		<i>Pseudoexentera</i>	<i>maracana</i>	99.36	1
		<i>Sparganothis</i>	<i>pettitana</i>	100	1
Mantodea	Mantidae	<i>Mantis</i>	<i>religiosa</i>	100	3
Megaloptera	Corydalidae	<i>Chauliodes</i>	<i>sp.</i>	98.67	20
Neuroptera	Hemerobiidae	<i>Hemerobius</i>	<i>stigma</i>	100	4
		<i>Unknown</i>	<i>sp.</i>	100	1
Orthoptera	Gryllidae	<i>Gryllus</i>	<i>pennsylvanicus</i>	100	1
Trichoptera	Helicopsychidae	<i>Helicopsyche</i>	<i>borealis</i>	100	1
		<i>Cheumatopsyche</i>	<i>campyla</i>	100	1
		<i>Cheumatopsyche</i>	<i>sp.</i>	100	1
		<i>Cheumatopsyche</i>	<i>campyla</i>	99.36	5
		<i>Macrostemum</i>	<i>zebratum</i>	98.69	1
	Leptoceridae	<i>Ceraclea</i>	<i>transversa</i>	100	1
	Limnephilidae	<i>Limnephilus</i>	<i>sp.</i>	99.36	1

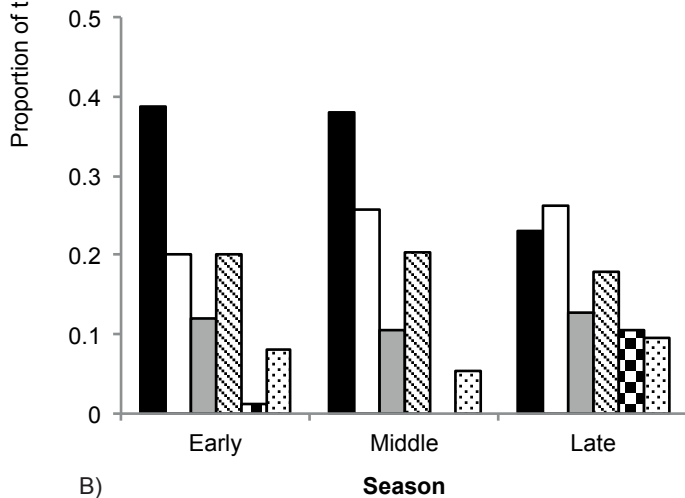
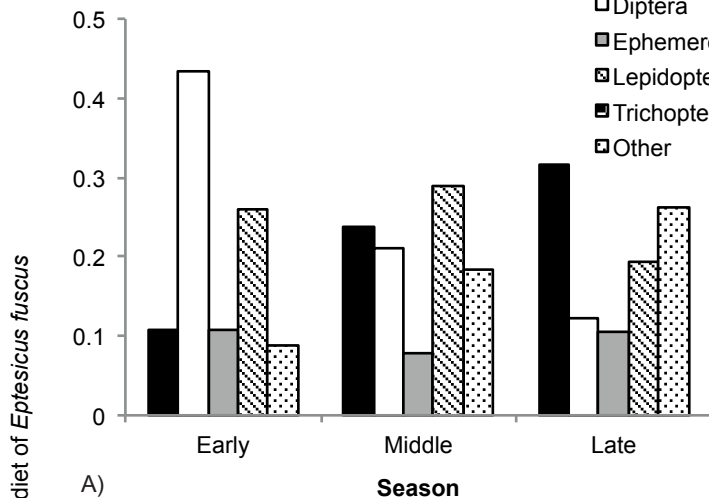
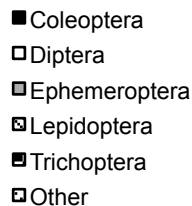


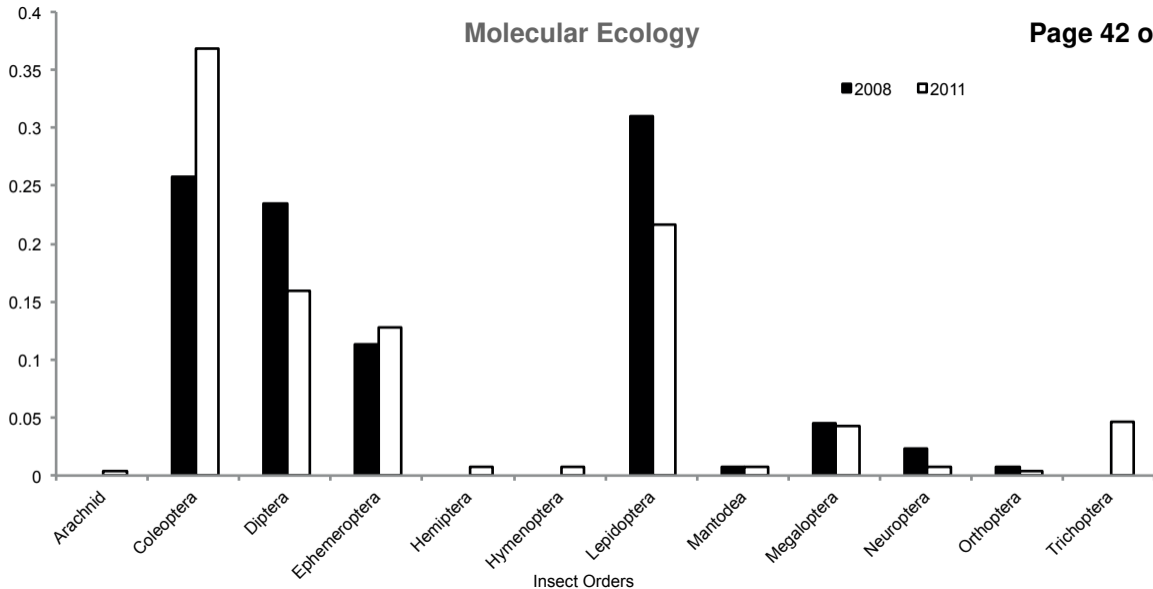
Reverse Fusion Primer

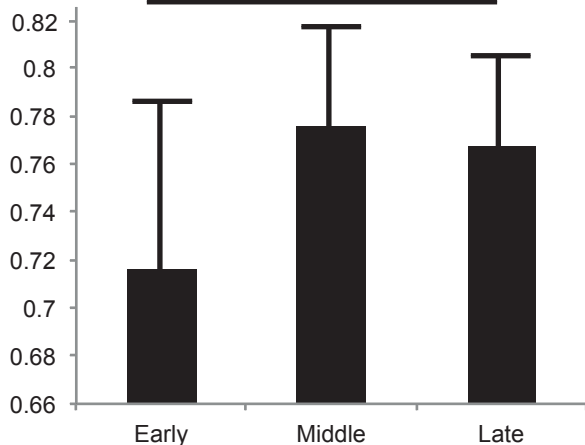


Resulting Amplicon

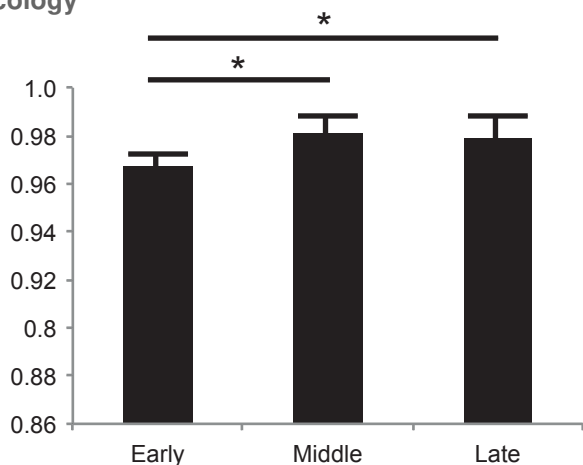




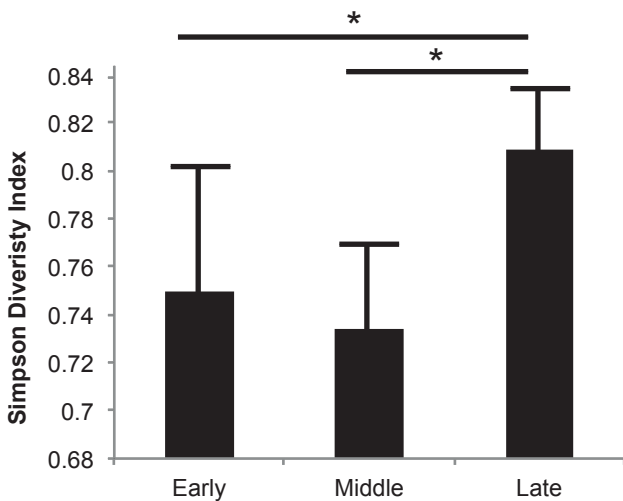
Frequency in the diet of *Eptesicus fuscus*



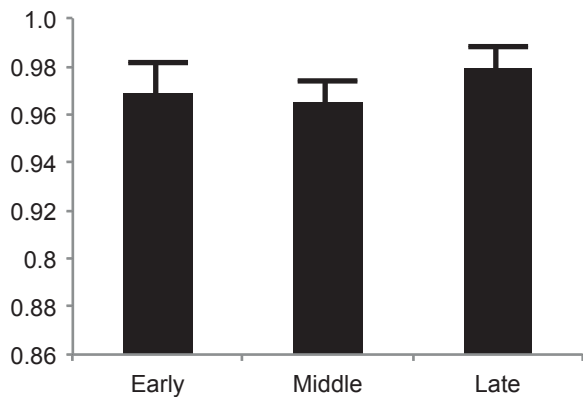
A) 2008 Season



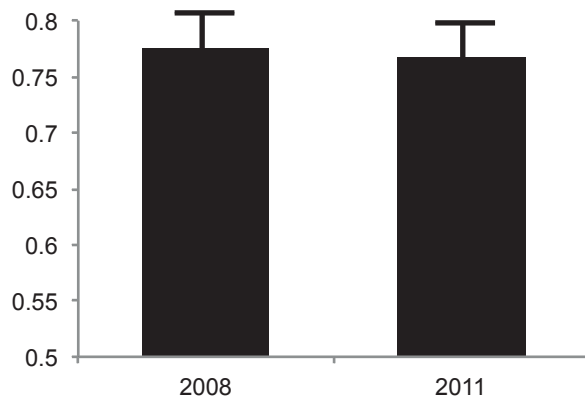
D) 2008 Season



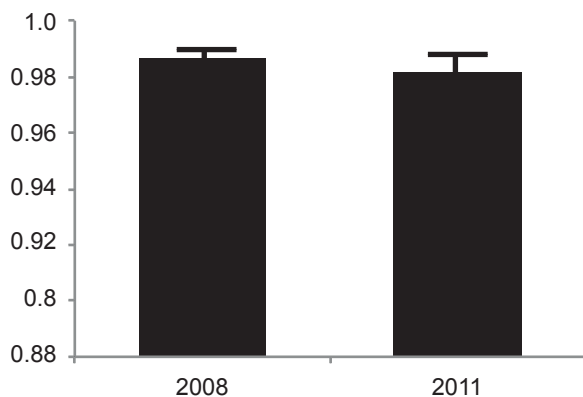
B) 2011 Season



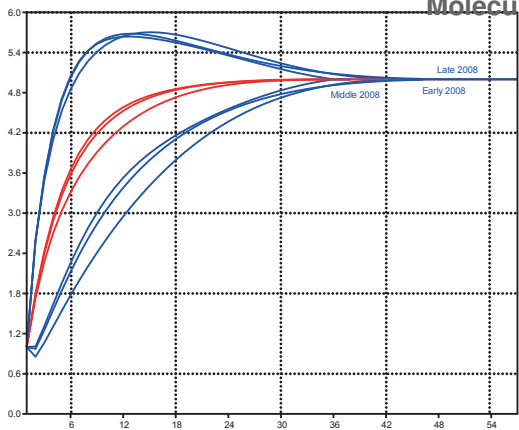
E) 2011 Season



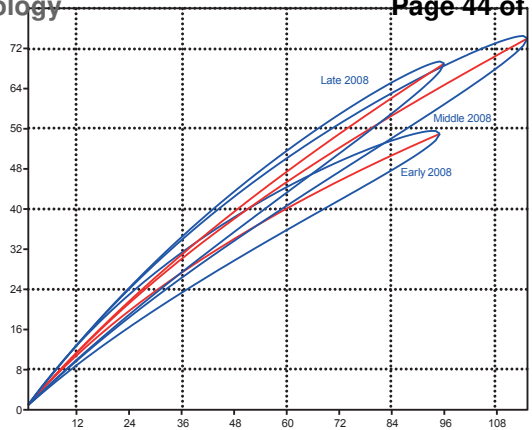
C) Year to Year Orders



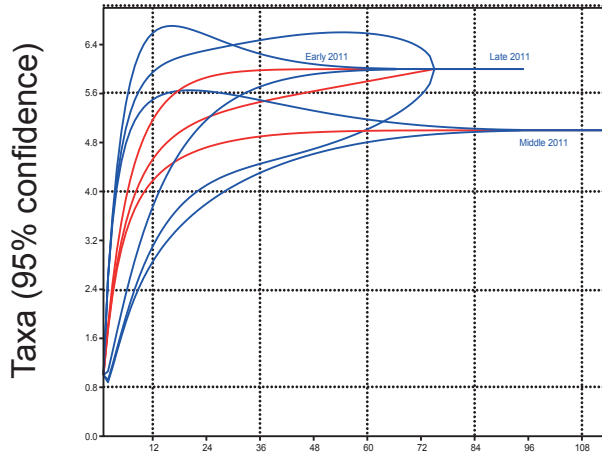
F) Year to Year MOTU



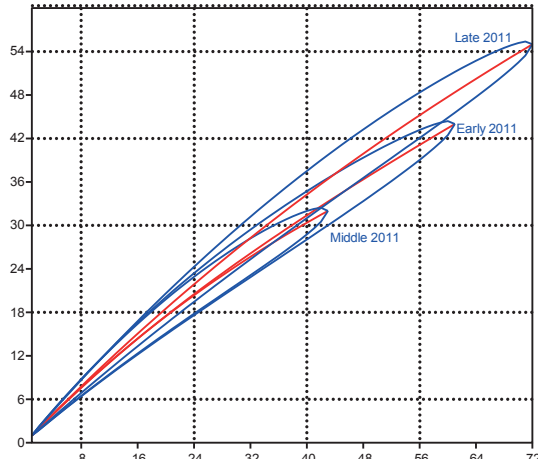
A)



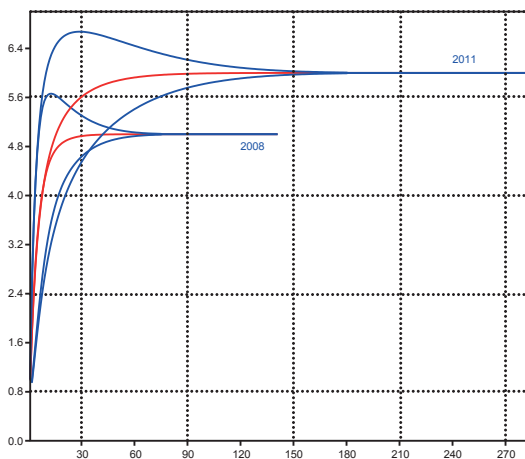
D)



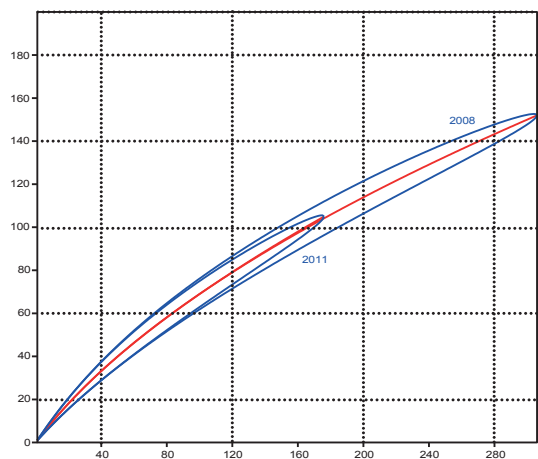
B)



E)



C)



F)

Order Analysis

MOTU Analysis

