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Citation for final published version:

Clare, Elizabeth L., Symondson, William Oliver Christian, Broders, Hugh, Fabianek, François, Fraser, Erin E., MacKenzie, Alistair, Boughen, Andrew, Hamilton, Rachel, Willis, Craig K. R., Martinez-Nuñez, Felix, Menzies, Allyson K., Norquay, Kaleigh J. O., Brigham, Mark, Poissant, Joseph, Rintoul, Jody, Barclay, Robert M. R. and Reimer, Jesika P. 2014. The diet of *Myotis lucifugus* across Canada: assessing foraging quality and diet variability. *Molecular Ecology* 23 (15), pp. 3618-3632. 10.1111/mec.12542

Publishers page: <http://dx.doi.org/10.1111/mec.12542>

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1 **The diet of *Myotis lucifugus* across Canada: assessing foraging quality and diet**
2 **variability**

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45
46

47 **Key words:** molecular diet analysis, species' interactions, spatial-temporal variation,
48 resource use

49 **Running Head:** Diet of little brown bats across Canada

50

For Review Only

51 **Abstract**

52 Variation in prey resources influences the diet and behaviour of predators. When
53 prey become limiting, predators may travel farther to find preferred food or adjust to
54 existing local resources. When predators are habitat limited, local resource abundance
55 | impacts foraging success. We analyzed the diet of *Myotis lucifugus* (little brown bats)
56 | from Nova Scotia (eastern Canada) to the Northwest Territories (north western Canada).
57 This distribution includes extremes of season length and temperature and encompasses
58 colonies on rural monoculture farms, and in urban and unmodified areas.

59 | We ~~identified~~recognized nearly 600 distinct species of prey, of which $\approx 30\%$
60 | could be identified using reference sequence libraries. We found a higher-than-expected
61 use of lepidopterans, which comprised a range of dietary richness from $\approx 35\%$ early in the
62 summer to $\approx 55\%$ by late summer. Diptera were the second largest prey group consumed,
63 representing $\approx 45\%$ of dietary diversity early in the summer. We observed extreme local
64 dietary variability and variation among seasons and years. Based on the species of insects
65 | that ~~we recorded in the diet~~consumed, we suggest that two locations support prey species
66 | with extremely low pollution and acidification tolerances, suggesting that these are areas
67 without environmental contamination. We conclude there is significant local population
68 variability in little brown bat diet which is likely driven by seasonal changes in insect
69 diversity and may be a good indicator of environment quality.

70

71 Introduction

72 Molecular techniques are increasingly used to identify species, particularly
73 ~~morphology~~ morphologically cryptic taxa. This has generated databases of taxonomically
74 validated reference sequences (e.g. BOLD, Ratnasingham & Hebert 2007) to quantify
75 biodiversity (e.g. Hebert *et al.* 2003), detect food market substitutions (e.g. Wong &
76 Hanner 2008; Hanner *et al.* 2011) and improperly labelled food (e.g. Cohen *et al.* 2009).
77 Characterizing ecological connections is more complicated than indexing species'
78 presence (McCann 2007) and the use of reference databases to document interactions
79 (e.g. Smith *et al.* 2006, 2007) has expanded greatly. Molecular techniques provide a
80 powerful means to unravel food webs (Symondson 2002; King *et al.* 2008; Pompanon *et*
81 *al.* 2012) which cannot be observed. These techniques developed from monoclonal
82 antibody methods (e.g. Symondson & Liddell 1993) to cloning (e.g. Zeale *et al.* 2011;
83 Alberdi *et al.* 2012), and next generation sequencing (NGS) (Pompanon *et al.* 2012).
84 NGS now dominates these analyses and has been applied to marine systems (Deagle *et al.*
85 2009, 2010), herbivores (Soininen *et al.* 2009; Valentini *et al.* 2009) and terrestrial
86 insectivores (Bohmann *et al.* 2011; Brown *et al.* 2013). Next generation sequencing is
87 particularly effective when applied to generalists.

88 One hypothesis to explain food web stability is that increased species richness is
89 related to food-web complexity (the number of interactions). When richness is coupled
90 with functional redundancy and behavioural flexibility, food webs become more stable
91 (Solé & Montoya 2001; Kondoh 2003; Dunne *et al.* 2004). Generalism provides the
92 opportunity for flexibility in prey choice and its importance is documented e.g. stabilizing
93 both predator and prey population demography (Singer & Bernays 2003) or indirectly

94 controlling lower food web links (Rosenheim & Corbett 2003). The main prediction of
95 this hypothesis is that, when resources become limited, flexible consumers become more
96 general in resource use. Dietary flexibility can be driven by limited high quality food, and
97 the necessity to diversify to achieve nutrition, to avoid toxins, to follow resources, or
98 minimize foraging risks (Singer & Bernays 2003). Some generalists switch between
99 specialized resources (e.g. omnivory, Clare *et al.* 2013) while others consume food in
100 ratios based on abundance (Rosenheim & Corbett 2003; Bastille-Rousseau *et al.* 2011).

101 Bats are an ideal group to study dietary flexibility as they occupy multiple trophic
102 levels (carnivores, sanguivores, frugivores, nectarivores, insectivores) and niches (e.g.,
103 active hunting, passive listening for prey, fishing, trawling). They are frequently top
104 predators and may consume resources at different trophic levels (e.g. Clare *et al.* 2013).

105 However, they consume resources cryptically (~~They are~~ active at night, using high-
106 frequency echolocation) and are thus difficult to observe. Molecular methods provide a
107 solution and are particularly useful in insectivores where thorough mastication of prey
108 limits traditional morphological analyses of faeces (guano) (Kunz & Whitaker 1983) or
109 culled prey remains (e.g. *Nycteris grandis* Fenton *et al.* 1981, 1990). In both cases
110 identification of prey is limited to order or family and small, soft bodied prey may be
111 overlooked (Clare *et al.* 2009). Molecular analysis permits us to identify prey to species
112 (Clare *et al.* 2009) particularly when coupled with reference libraries (Hebert *et al.* 2003;
113 Ratnasingham & Hebert 2007) increasing precision.

114 Carter *et al.* (2006) showed a proof of the concept by amplifying chicken DNA
115 from the faeces of white-winged vampire bats (*Diaemus youngi*). The first full molecular
116 analysis of bat diet assessed predator-prey relationships between *Lasiurus borealis* and

117 Lepidoptera (Clare *et al.* 2009) by sequencing DNA directly from residual prey
118 fragments. Cloning and prey-specific primers were developed (Zeale *et al.* 2011) and
119 used to uncover a novel hunting strategy of *Barbastella barbastellus* (Goerlitz *et al.*
120 2010) and the diet of *Plecotus macrobullaris* (Alberdi *et al.* 2012). These methods have
121 rapidly been replaced by NGS (Bohmann *et al.* 2011; Razgour *et al.* 2011; Clare,
122 Symondson, *et al.* 2013; Emrich *et al.* 2013) which are faster and more cost effective.

123 *Myotis lucifugus*, the little brown bat, was one of the most common and
124 widespread bats in North America, though populations are in decline due to white nose
125 syndrome (Frick *et al.* 2010). They have a distribution from Alaska, through southern
126 Northwest Territories, the prairies, Ontario, Quebec and the Maritime provinces in
127 Canada, and south through the continental United States and northern Mexico (Fenton &
128 Barclay 1980). Arthropod consumption by bats (including *Myotis lucifugus*) varies by
129 species and season (tied to lack of many prey early and late in the year and reproductive
130 cycle) (Kunz *et al.* 2011), and by age (Fraser & Fenton 2007). At peak metabolic demand
131 during lactation, little brown bats may consume more than their body mass in prey each
132 night (Kurta *et al.* 1989) and thus potentially provide a significant ecosystem service
133 through insect consumption (Boyles *et al.* 2011). They are generalists consuming insects
134 of low prey hardness (Freeman 1981) mostly emerging from aquatic systems e.g. Diptera
135 and Trichoptera (Belwood & Fenton 1976; Freeman 1981; Ober & Hayes 2008), although
136 adult females consume more Lepidoptera and Trichoptera (Belwood & Fenton 1976).

137 *Myotis lucifugus*' tendency to forage over water provides a means to assess
138 foraging location quality. In this context, our reference to foraging habitat/location
139 quality refers to both type of habitat (such as moving or still water) and also to the

140 | potential acid and pollution content of the aquatic system. Benthic macro-invertebrates
141 | are frequently used as environmental indicators. Their pollution tolerance (e.g. organic
142 | pollutants, acidification) and habitat requirements have been documented (Hilsenhoff
143 | 1988). If we consider bats as a sampling mechanisms, species-level diet analysis provides
144 | data for assessing the quality of foraging areas without complicated, potentially invasive
145 | methods such as radio tracking bats to locate foraging followed by mass insect sampling.
146 | Thus, while bats may not be used as a method of general habitat assessment (their
147 | sampling is biased by perceptual characters and preferences etc.), their diet can provide
148 | us which information on specific areas they have visited.

149 | Clare *et al.* (2011) performed the first molecular analysis of little brown bat diet in
150 | three locations in Southern Ontario. They identified 66 prey species and noted a shift
151 | from consumption of Diptera early in the summer to Ephemeroptera in mid and late
152 | summer. There was evidence of local diet variation which allowed inferences about
153 | foraging-location quality. There is evidence that diet diversity is a function of location;
154 | populations in northern Ontario have greater dietary variability than those in southern
155 | areas (Belwood & Fenton 1976). The range of little brown bats in Canada includes areas
156 | of high and low insect species richness. If prey themselves are a limited (and limiting)
157 | resource, as prey richness decreases, the null hypothesis is that predators should similarly
158 | consume a lower species richness; however, if abundance is high, diet may change little
159 | or predators may adopt a more general strategy and consume a wider variety of prey
160 | (higher values of Simpson's diversity index, Simpson 1949).

161 | Our study had two objectives. First, we assessed variability of little brown bat
162 | diets across Canada, over the summer and between years, and tested the hypothesis that

163 they have high degree of dietary variability across location and time. Second, we used the
164 identity of prey to make inferences about habitat, based on known habitat requirements
165 and pollution tolerances of the prey. We tested four predictions about diet: 1) latitude has
166 an effect on diet, 2) temporal patterns of prey exploitation across the summer are stable
167 from year to year, 3) there is a significant shift from the consumption of species of
168 Diptera to Ephemeroptera associated with phases of the reproductive cycle and 4)
169 species-level analysis of prey provides criteria for assessing foraging location-area
170 quality and yields quantitatively different insights than ordinal level analysis.

171

172 **Methods:**

173 *Sample Collection:*

174 We collected guano under maternity roosts of *M. lucifugus* across Canada (Figure
175 1) during three periods, including pregnancy (early summer = May to mid-June),
176 lactation (middle summer = mid-June to mid-July) and post lactation (late summer = mid-
177 July to September). Collections in Ontario were performed in 2009 (at Clinton, the
178 Pinery), 2009 and 2011 (Lake St. George) and in 2011 for all other locations. Sampling
179 was performed weekly in Ontario throughout the summer (fine grained analysis), and
180 during the three established periods in other locations (see Figure 1 for details).

181 Additional material was collected at two locations in Quebec but due to sampling
182 differences and difficulties with molecular analysis we include this only as a supplement
183 (see details in Supplemental Files 1 and 2) for comparison. We adopted the definitions of
184 seasons from Clare *et al.* (2011) (see Supplemental File 3 for collection dates and
185 locations). We froze samples or preserved them in high-percentage ethanol (70-100%).

186 Because we collected samples from colonies rather than individuals, the volume of
187 material was substantial (exceeding half a liter per week by volume in some cases) and
188 reflected deposition by many individuals (potentially exceeding a thousand in some
189 locations), we analyzed a random subset of the pellets from each collection (volume
190 c.1.5ml of guano or c.50 pellets, hereafter a “sample”).

191

192 *DNA Extraction, Amplification and Sequencing:*

193 We extracted DNA from homogenized samples using the QIAmp DNA Stool
194 Mini Kit (Qiagen, UK) following manufacturer’s instructions with modifications from
195 Zeale *et al.* (2011), further modified as follows: 1) to encompass more individuals and
196 thus greater prey diversity we used approximately 50x more starting material and 2) we
197 extended the first centrifuge step (Zeal step 4) to 3 minutes to aid in pelleting the
198 particulate material. Extracted DNA was stored at -20 °C prior to amplifications.

199 We tested DNA extractions success using the primers ZBJ-ARTF1c and ZBJ-
200 ArtR2c (Zeale *et al.* 2011). We then amplified each sample using a modified fusion-
201 primer version for the Roche FLX sequencer (Bohmann *et al.* 2011) consisting of a Lib-L
202 adaptor, the key sequence, a unique 10 bp DNA sequence (MID) and the original primer
203 sequence (ZBJ-ARTF1c or ZBJ-ArtR2c). In our design (Brown *et al.* 2013; Clare *et al.*
204 2013), MID sequences were used on both forward and reverse primers allowing fewer
205 primers to be used to resolve the same number of samples (i.e. rather than 100 unique
206 forward MID tagged primers for 100 samples, 10 unique forward and 10 unique reverse
207 MIDsd can yield the same resolution power) while reducing primer costs. We assigned

208 each sample a unique primer combination so all sequences could be identified to original
209 samples.

210 We performed PCR reactions as described by Bohmann *et al.* (2011) in a 20 μ l
211 reaction containing 1 μ l of template DNA using Qiagen multiplex PCR kits (Qiagen, UK)
212 with the following modifications. We did not use Q solution (from the kit) or BSA (as
213 suggested by Bohmann *et al.* 2011). We visualized PCR products on a 1.5% agarose gel
214 and quantified them following Brown *et al.* (2013) and mixed approximately equal molar
215 quantities of each sample. We size-selected ~~and samples products~~ using a QIAquick Gel
216 Extraction kit (Qiagen, UK) and quantified the final PCR mix using a Qubit dsDNA BR
217 Assay Kit (low sensitivity with a Qubit Fluorometer, Invitrogen life technologies).

218 We concentrated the final product to 10 μ g/1 μ l in molecular grade water.
219 Sequencing was conducted at the Liverpool Center for Genomic Research (University of
220 Liverpool) using a $\frac{1}{4}$ plate, Lib-L chemistry on a Roche 454 GS FLX+ sequencing
221 system (Roche Applied Sciences).

222

223 *Sequence Analysis:*

224 We analyzed sequences using Galaxy (<https://main.g2.bx.psu.edu/root>, Giardine
225 *et al.* 2005; Blankenberg *et al.* 2010; Goecks *et al.* 2010). We screened all recovered
226 sequences for those longer (>180 bp) or shorter (<100 bp) than expected, collapsed all
227 sequences to unique haplotypes, split the file by forward and reverse MID, removed
228 primers, MID, and adaptors and excluded rare haplotypes (<2 copies).

229 We clustered the sequences into molecular operational taxonomic units (MOTU)
230 in jMOTU (Jones *et al.* 2011) and tested thresholds from 1-10 bp. A graph of recovered

231 MOTU vs. threshold (not shown) suggests a 4 bp cut-off was most appropriate (Razgour
232 *et al.* 2011).

233 We compared representative sequences for each MOTU to the BOLD database
234 (www.barcodinglife.org) following criteria modified from Razgour *et al.* (2011):
235 1a=match to one species or several species in a genus (100% similarity), most
236 conservative taxonomy kept; 1b=good match (>98% similarity), but could belong to a
237 congener showing a higher sequence match; 2=match to more than one species (>98%),
238 only one of which is present in the sampling range (that taxonomy kept); and 3=close
239 match (as above) to several species from different genera, or to a reference sequence
240 which lacks a full taxonomic record. In these cases, the most conservative taxonomy
241 (normally family) was kept (note this is not an identification to higher level taxonomy,
242 but a match meeting criteria 1b but retaining ambiguity in the assignment due to multiple
243 similar matches or incomplete data in the reference collection).

244 In addition, we estimated the identity of all MOTU (including unidentified
245 MOTU) using the methods of Emrich *et al.* (2013) and the programme MEGAN (Huson
246 *et al.* 2011). See Emrich *et al.* (2013) for details of that procedure and a brief discussion.

247
248 *Ecological Analysis:*

249 We divided our collections into the three time periods. We conducted ecological
250 analyses in PAST (Hammer *et al.* 2001) on species and order-level data with p-values
251 estimated by permutation. We compared the Simpson's diversity indices for identified
252 prey among locations (sequential Bonferroni correction) and among summer sampling
253 periods, and estimated the magnitude of the effect (effective number of species), where

254 differences were statistically significant, following Jost (2006). We compared species
255 richness from paired weekly samples from the high-density sampling sites at Clinton
256 (rural monoculture farming area) and Lake St. George in 2009 (environmentally variable
257 conservation area). We computed rarefaction curves for all data.

258 We compared the proportion of each order in the diet (proportion = frequency of
259 occurrence of that order / total occurrences, where an occurrence is an identified MOTU
260 in a sample) among locations and among sampling periods using a χ^2 frequency test with
261 p-values computed using a Monte Carlo simulation with 2000 replicates in R 2.15.1 (“R
262 Development Core Team: R: A language and environment for statistical computing”
263 2008).

264 We use the recovered species to evaluate the foraging area-location of the
265 populations using the Hilsenhoff Biotic Index for organic pollutants developed for the
266 western Great Lakes (Hilsenhoff 1988) and the Fjellheim & Raddum (1990) index for
267 acid tolerance.

268

269 **Results**

270 *Sequence Processing:*

271 We recovered 167,562 sequences. After filtering, these were resolved into 10,792
272 unique haplotypes that could be assigned to an original sample. We clustered these into
273 molecular operational taxonomic units (MOTU) and examined a representative sequence
274 from each cluster. We removed 6 MOTU as contaminants (nearest BLAST similarity was
275 identified as a non-prey item e.g. bacteria). The remaining 566 MOTU were used in
276 further analysis and represent a mean of ≈ 9 species per sample.

277

278 *Diet of Little Brown Bats:*

279 Through comparison to the reference library, we identified 211 MOTU to species
280 using criteria 1a, 1b and 2 (Supplemental File 1), hereafter referred to as species. We also
281 identified ~~of~~ an additional group of MOTU using criteria 3 but consider them as
282 provisional identifications. Of the identified occurrences (defined above), ≈45% were
283 Lepidoptera, ≈34% Diptera, ≈11% Ephemeroptera, ≈6% Trichoptera and ≈4% Coleoptera
284 (Figure 2). An additional 9 species represented Araneae (four species), Hemiptera (one
285 species), Hymenoptera (one species), Megaloptera (two species) and Neuroptera (one
286 species). The most common prey were two species of Chironomids (Diptera):

287 *Dicrotendipes tritonus* and *Paracladopelma winnelli* found in 29% and 22% of samples,
288 respectively, and two species of Ephemeroptera: *Caenis youngi* and *Caenis amica* found
289 in 28% and 22% of samples respectively (note that *Caenis* are difficult to separate
290 morphologically or genetically and multiple cryptic species are suspected, thus the actual
291 identity of species within this genus should be considered an estimate due to taxonomic
292 limitations). A single species was identified as prey in all sampled locations, a moth,
293 *Hydriomena* (Lepidoptera, Geometridae). However, *Hydriomena* contains species with
294 overlapping DNA barcodes (shared haplotypes at COI), and thus this identification may
295 correspond to more than one species. We recovered a similar analysis of prey diversity
296 from MEGAN (Figure 8) which suggest that unidentified prey are relatively dispersed
297 among the consumed insect groups.

298 Many of the prey consumed provide specific information on the type and quality
299 of the aquatic system; the most sensitive taxa, including families Glososomatidae,

300 Ephemeroellidae and Corydalidae and genera *Lemnephilus*, *Agrypnia* and *Phryganea*,
301 were consumed in both the Northwest Territories and Lake St. George (for a site-by-site
302 analysis see Table 1).

303

304 *Spatial-Temporal Variation in Resource Use:*

305 Considering species from the five main prey groups (Ephemeroptera, Coleoptera,
306 Lepidoptera, Diptera and Trichoptera) with all data pooled, the proportion of
307 consumption varied significantly among periods ($\chi^2 = 26.89$, $p = 0.0005$, Figure 2). In early
308 summer, the diet was dominated by Diptera (45% of occurrences) though ~~the bats'~~their
309 presence decreased throughout the summer (30% in mid summer, 29% in late summer).
310 In contrast, Lepidoptera increased from 35% of occurrences in early summer, to 46% in
311 mid summer and 55% in late summer. The frequency of occurrence of Ephemeroptera,
312 Coleoptera and Trichoptera remained stable. We did not observe a switch from
313 consumption of Diptera to Ephemeroptera as previously reported (Clare *et al.* 2011).

314 Prey use varied significantly among locations ($\chi^2 = 119.69$, $p = 0.0005$, Figure 3).
315 In some locations (Northwest Territories, Lake St. George 2009), the main prey were
316 Lepidoptera and Diptera, while in other locations (e.g. Lake St. George 2011) prey
317 consumption was dominated by Lepidoptera. These differences do not appear to reflect
318 sampling intensity; the three most heavily sampled locations (Clinton, Lake St. George
319 2009 and 2011) showed different patterns of prey use.

320 Despite difference in prey consumption, Simpson Index measures did not indicate
321 a significant difference in dietary diversity among locations (Figure 4) except at Pinery
322 Provincial Park (~~Pinery~~) in Ontario. When considered at the ordinal level, diversity of

323 prey at Pinery was particularly low. This pattern was different when considering species
324 (MOTU) level resolution; diversity estimates were more even, and bats at Pinery had
325 high diversity. Saturation of rarefaction curves (Figure 5) indicates sampling reached a
326 plateau in ordinal level identifications, while species-level identifications were still
327 increasing almost linearly (Figure 5c and 5d). Diversity estimates at ordinal and species
328 level were not correlated ($r=0.27$, $p=0.18$). Latitude did not correlate with diversity at the
329 ordinal ($r=0.43$, $p=0.15$) or species ($r=-0.11$, $p=0.4$) levels.

330 Diversity estimates varied significantly among seasons (early = 0.66, mid = 0.67,
331 late = 0.60) with a nearly significant reduction in dietary diversity observed between
332 early and late season ($p=0.05$) and a significant reduction between mid and late season
333 ($p=0.031$) (Figure 6), reflecting reductions in the effective numbers of species of 14% and
334 20%, respectively.

335 We sampled the same colony at Lake St. George in 2009 and 2011. In 2009 we
336 estimated that this colony consisted of several thousand individuals, although this number
337 declined slightly in 2011 likely due to white nose syndrome (Frick *et al.* 2010). Sampling
338 at this location was done during matched weeks between the two years, but we observed
339 remarkable difference in the spatial-temporal pattern of prey use. In 2009, prey use
340 mirrored that observed across all locations (Figure 2), while in 2011, Diptera represented
341 a minority of prey, Lepidoptera dominated all seasons (91% in late season), and no
342 Coleoptera or Trichoptera were consumed.

343 The most heavily sampled locations were Clinton ($n=14$ weeks) and Lake St.
344 George in 2009 ($n=18$ weeks). Of these, 13 sampling weeks were common and could be
345 directly compared (difference reflects differential colony establishment). Although not

346 significant, there is a trend towards higher species richness at Lake St. George in 2009;
347 mean prey species richness was 20 species/sample compared to a mean of 17 in Clinton
348 (Figure 7), although the number of species was higher in only 8 of 13 weeks.

349

350 **Discussion**

351 Our goal was to examine variation in resource use by bats across Canada and to
352 use these data to infer foraging area-location quality. Our analysis suggests that prey use
353 by little brown bats at the most northern sampling location (NWT) consumed prey evenly
354 between orders, although there was no consistent pattern of consumption among
355 locations. Intensive sampling of populations in different locations in Ontario across two
356 years indicated that there was spatial-temporal variation in prey use. We did not observe a
357 seasonal shift between the consumption of Diptera and Ephemeroptera. Analyses at
358 species level showed different patterns than at ordinal level, indicating that species-level
359 resolution provides novel insights in dietary analysis.

360

361 *Spatial Variation in Diet Across Canada*

362 When we combined data from all locations, Diptera dominated the diet in the
363 early season but was replaced by Lepidoptera in the mid and late seasons. This pattern
364 was prominent at Lake St. George (2009) and the NWT, but variable at other locations.
365 The reliance on Diptera in the early season agrees with previous morphological (Belwood
366 & Fenton 1976; Freeman 1981; Ober & Hayes 2008) and molecular (Clare *et al.* 2011)
367 analyses. Diptera are an important prey group in both species richness and dietary
368 abundance. We found no evidence to support the reported heavy reliance on Trichoptera,

369 but found more species of Lepidoptera than expected. This may reflect the
370 overabundance of Lepidoptera within the reference collection, biasing the number of
371 taxonomic identities reported. It is possible that Trichoptera represent a large number of
372 the “unknowns” within our sample however our estimations using MEGAN indicate that
373 unknowns are relatively dispersed among taxonomic groups. -

374 Traditional morphological analyses are based on estimating abundance of prey
375 groups in any given sample. Lepidoptera are frequently identified from scales and small
376 morphologically cryptic species may be lumped into a single unit or overlooked. One
377 advantage of molecular analysis is the routine detection of rare prey (Clare *et al.* 2009).
378 However, as molecular analyses cannot estimate abundance, biomass or volume (e.g.
379 haplotype number \neq abundance, MID tags, primers and adaptors influence sequencing,
380 sequencing direction produces different results and biases in sequencing are not
381 consistent between runs even using the same PCR products, (Pompanon *et al.* 2012;
382 Deagle *et al.* 2013; Piñol *et al.* 2013)) within a sample, rare and common items are both
383 “present”. A large sample size may control for overrepresentation of rare prey (or
384 underrepresentation of common prey) however there is a trade-off between increasing the
385 volume of material analysed (the pooling method here) to increase our assessment of
386 biodiversity and the potential for skew with presence and absence records, though it is not
387 a correction that can be empirically assessed.

388 While we cannot estimate sample-based abundance, molecular analysis allows us
389 to measure species richness and frequency across samples. While richness within an
390 order can be related to abundance, there are important exceptions. Mass emerging prey
391 like mayflies (Ephemeroptera) may be extraordinarily abundant but low in species

392 richness. In our analysis, Lepidoptera may appear as the most important food source
393 because they are more speciose, while mayflies may be underrepresented. The abundance
394 of Lepidoptera may also reflect previous observations that females consume more
395 Lepidoptera than males (Belwood & Fenton 1976); all of the colonies we sampled were
396 maternity groups dominated by females and their offspring. The results from Quebec
397 based on males (Supplemental File 2) recovered more Diptera which may support this
398 conclusion.

399 We observed significant spatial variation in diet. We use Simpson's Index which
400 is less sensitive to rare events that frequently occur in species-level analysis (Bohmann *et*
401 *al.* 2011; Razgour *et al.* 2011). Our estimates of diversity were not correlated with
402 latitude and not related to sample size. The Saskatchewan and Pinery colonies had the
403 lowest sample sizes (and could not be sampled in late season at all) but differ in patterns
404 of prey use. Both were low in diversity at the ordinal level, but so was Lake St. George
405 (2011) which had one of the largest sample sizes. Significant spatial variation in resource
406 use is unsurprising across such a wide geographical area, however, it was also similarly
407 variable within southern Ontario and between years. This matches previous observations
408 (Clare *et al.* 2011) supporting the view that these bats responded to local variation in
409 environment and prey. As such, predicted declines in the populations of little brown bats
410 (Frick *et al.* 2010) may have locally-specific effects on insect populations.

411 The main assumption of the correspondence between insect diversity and diet is
412 that resources themselves are limiting. Although little brown bat colonies may each
413 consume hundreds or thousands of insects in a night, it is not clear whether their
414 populations are large enough to significantly reduce local populations of insects.

415

416 *Temporal Variation in Diet*

417 We observed a significant decrease in dietary diversity in late season when the
418 effective reduction in species richness was 20%. This contrasts with a matching analysis
419 of big brown bats (*Eptesicus fuscus*) (Clare *et al.* 2013) for which dietary diversity rose
420 sharply in late season. These inverse patterns may reflect non-overlapping resource use
421 by these predators. Big brown bats are a flexible hunter that appears to forage in most
422 habitat types (Geggie & Fenton 1985; Furlonger *et al.* 1987) and consumes large
423 numbers of beetles, moths, and flies (Clare *et al.* 2013). Insect diversity falls in late
424 season just as both species must store fat for hibernation. While big brown bats may
425 compensate by exploiting a wider variety of habitats (and thus prey), increasing their
426 dietary diversity, little brown bats may simply consume a greater volume of more limited
427 prey. Habitat selection by bats strongly influences insect availability and thus diet and
428 may explain apparent resource partitioning among many species (Emrich *et al.* 2013).
429 Current or historical competition for resources is also possible, but makes the assumption
430 that resources are limiting. There is little direct evidence that competition drives patterns
431 of resource use because this cannot be assessed without controlled removal experiments,
432 which are exceedingly difficult with bats.

433 Clare *et al.* (2011) observed a significant shift from consumption of Diptera in
434 early season to Ephemeroptera in middle and late season. The same pattern was not
435 observed here in any location, including in the same samples originally analyzed by Clare
436 *et al.* (2011). This likely reflects a difference in methodology. Clare *et al.* (2011)
437 sequenced DNA directly from fragments of prey removed from guano under microscopic

438 dissection. The advantage of this technique is that the user can preferentially attempt to
439 maximize the taxonomic richness of the sample but it is likely biased towards the
440 detection of less-digestible prey (Razgour *et al.* 2011). Because Clare *et al.* (2011) took
441 efforts to sample a large number of guano pellets, they also assumed that each fragment
442 represented a different capture, and thus frequency was calculated directly from the
443 recovered sequences. NGS provides an automated method to maximize the diversity of
444 prey recovered, but does not allow for the same assumption of independence of each
445 haplotype. The fragment and sampling method employed by Clare *et al.* (2011) is a
446 hybrid between traditional morphological analysis and NGS and may be more similar to
447 abundance-based methods. This is only likely to cause significant difference when the
448 taxa are mass-emerging species found in high abundance but low species richness, such
449 as Ephemeroptera. NGS may underestimate the importance of this prey group, while the
450 fragment method may overestimate them if the assumption of independence between
451 fragments is not met. In addition, our methods used short amplified regions (157 bp)
452 compared to Clare *et al.* (2011) who used full DNA barcodes of ≈ 657 bp. Short primers
453 | may provide lower limit taxonomic resolution in some cases but increases the likelihood
454 | that degraded DNA will be amplified. Different primers will always have different
455 binding affinities and this may partially explain specific prey differences between these
456 two analyses.

457

458 *Methodological Advances and Species vs Ordinal Level Data*

459 We used two specific methodological advances in our analysis. To separate
460 samples after sequencing, NGS uses incorporated tags in primers. These tags are often

461 called MIDAs or ‘barcodes’ (although we do not use this term to avoid confusion with
462 DNA barcodes as per Hebert *et al.* (2003)). Using MIDAs on forward primers, each sample
463 can be amplified with a unique forward primer and subsequently separated. However, for
464 very large sample sizes, this becomes costly. As introduced (Brown *et al.* 2013), we
465 incorporated MIDAs in both forward and reverse primers so that each sample can be
466 assigned a unique combination of MIDAs (e.g. 10 forwards and 10 reverses = 100 unique
467 combinations). This technique significantly reduces primer costs without impacting
468 sequencing performance. Second, rather than extracting DNA from a single guano pellet
469 (or even half a pellet as in some publications) we extracted DNA from a pool of pellets
470 totalling 1-1.5 ml by volume. This roughly translated into 20-50 pellets per sample
471 (depending on size). Previous analyses have estimated a mean of 5 taxa per pellet
472 (Bohmann *et al.* 2011) while we recovered a mean of 9 per sample. In this study, each
473 “sample” is, in effect, an assay of diet in what is likely dozens of individuals. The
474 disadvantage of this method is that larger volume extractions lead to more PCR inhibitors
475 that may complicate reactions. However, this also provides two specific advantages. In
476 general it leads to greater taxonomic richness in the resulting sequencing run. More
477 specifically, insectivorous bats have a very fast gut transit time with prey passing as fast
478 as 35 minutes after ingestion (Buchler 1975). As such, any single pellet may be low in
479 prey richness. Morphological analyses normally examine many dozens of pellets to
480 estimate diet and we have incorporated this method. As discussed earlier, large sample
481 sizes may control for the potential for overrepresentation of rare prey though this may
482 explain our lower than expected measures of Ephemeroptera.

483 Molecular methods allow us to go beyond traditional ordinal-level assessments,
484 available from morphological analysis, to establish species-level taxonomic assignments
485 of prey. It is particularly interesting that when we remove these data, some dramatic
486 changes (e.g. estimates of diversity in Pinery) can be observed. This is largely due to
487 saturation of ordinal level collections, while species-level data have not reached a
488 plateau.

489

490 *Environmental Indicators and Foraging Assessment*

491 Benthic macro-invertebrates are frequently used as environmental indicators of
492 the quality of a water system (Hilsenhoff 1988; Fjellheim & Raddum 1990; Lenat 1993).
493 The analysis of diet from bats foraging over these areas-locations provides a direct (non-
494 invasive) method to infer the quality of their foraging areaslocation. This method is more
495 specific than a general insect survey as it assesses where the bat has been rather than
496 where it may have been. Insect tolerance estimates vary by season and area (see a
497 comparison of Wisconsin and North Carolina, Lenat (1993)), but we can make a number
498 of observations from our data using the Hilsenhoff Biotic Index for organic pollutants
499 developed for the western Great Lakes (Hilsenhoff 1988) and the Fjellheim & Raddum
500 (1990) index for acid tolerance (extrapolating from related species) and inferences about
501 other Canadian regions (Table 1).

502 Among the Trichoptera, Hydropsychidae, Leptoceridae and Phryganeidae have
503 moderate pollution tolerances of 4 while Helicopsychidae have a tolerance of 3 and
504 Glossosomatidae a tolerance of 0. Glossosomatidae also have a low tolerance for
505 acidification. Leptoceridae and Phryganeidae were eaten by bats in the Northwest

506 Territories, Nova Scotia, Long Point and Lake St. George (2009), while Helicopsychidae
507 occurred in the diet at Clinton. The pollution intolerant *Glososomatides* were eaten
508 in the Northwest Territories and Lake St George (2009). Diptera in the family Tipulidae
509 have a tolerance of 3 and were also found at Clinton. The Ephemeroptera family
510 Ephemerellidae has a pollution tolerance of 1. These were detected in the Northwest
511 Territories and Lake St. George (2011); the Megaloptera family Corydalidae has a
512 pollution tolerance of 0 and was detected in Lake St. George (2009). Species of *Molanna*
513 may be acid intolerant and were detected in Nova Scotia.

514 While habitat specificity of many macro-invertebrate species declines (or
515 becomes more variable) at higher latitudes (Lenat 1993), these observations suggest that
516 bats at Clinton forage in good quality habitat (Helicopsychidae and Tipulidae both have
517 tolerance =3). However, there is convincing evidence that the sites in the Northwest
518 Territories and Lake St. George have an excellent quality habitat with little apparent
519 organic pollution (species with tolerance of 0 and 1 detected frequently) or acidification.
520 This might be expected for the remote Northwest Territories locations (which are far
521 from major human modification), but is less expected for Lake St. George, which lies on
522 the edge of the greater Toronto area. The continued presence of prey with low pollution
523 tolerances at Lake St. George in 2011 demonstrates the stability of this site and may be an
524 indication of the effectiveness of small-scale conservation efforts even in areas near
525 intensive urban modification.

526 Some macro-invertebrates are relatively good indicators of habitat type. Species
527 in the Trichoptera genera *Agrypnia* and *Traenoides* were identified in Northwest
528 Territories, Long Point and Lake St. George. They are associated with pond or lake-like

529 habitats in northern parts of their range. We have previously confirmed that the Lake St.
530 George bats hunt in the vicinity of Lake St. George (a very small water body) less than
531 300 m from the roost site. It is likely that the Long Point bats are hunting along the shores
532 of Lake Erie, and the Northwest Territories population may be using any of hundreds of
533 variously sized water bodies.

534

535 *Summary*

536 In response to resource fluctuations, species may move to track prey or adapt to
537 match local variability. The little brown bat, *M. lucifugus*, occupies a broad niche,
538 foraging over aquatic systems. Species-level identifications of benthic macro-
539 invertebrate prey serve as environmental indicators and allow us to use information about
540 diet to directly measure the quality of the foraging habitat. In total, we recorded nearly
541 600 species of prey consumed by this predator and present one of the largest and most
542 geographically diverse molecular dietary analyses to date. With these data, we
543 demonstrate seasonal, regional and inter-annual variation in little brown bat diets across
544 Canada which is independent of latitude. We identify two locations where the prey
545 consumed are particularly intolerant to organic pollution or acidification and thus
546 | locations where foraging area-habitat is of high quality, even when in the vicinity of high-
547 density urban development.

548

549 **Acknowledgements:**

550

551 We thank all participating landowners for their co-operation with sample collection.
552 Professor Gareth Jones and Colin Lazarus provided laboratory support. This study was
553 made possible by funding from K.M. Molson foundation and the Natural Sciences and
554 Engineering Research Council of Canada (NSERC) through grants to ELC. RMRB and
555 JPR were funded by NSERC, Bat Conservation International, the Yukon Government,
556 and the Alberta Conservation Association. [Bat capture in Quebec were made possible by](#)
557 [support from the Fonds Québécois de la Recherche sur la Nature et les Technologies](#)
558 [\(FQRNT\) and the Ministère du Développement Durable, de l'Environnement, de la Faune](#)
559 [et des Parcs.](#) We thank many colleagues for advice and suggestions on analysis and
560 manuscript content.

561

562 **References**

- 563 Alberdi A, Garin I, Aizpurua O, Aihartza J (2012) The foraging ecology of the mountain
564 long-eared bat *Plecotus macrobullaris* revealed with DNA mini-barcodes. *PLoS*
565 *ONE*, **7**, e35692.
- 566 Bastille-Rousseau G, Fortin D, Dussault C, Courtois R, Ouellet J-P (2011) Foraging
567 strategies by omnivores: are black bears actively searching for ungulate neonates or
568 are they simply opportunistic predators? *Ecography*, **34**, 588–596.
- 569 Belwood JJ, Fenton MB (1976) Variation in the diet of *Myotis lucifugus* (Chiroptera-
570 Vespertilionidae). *Canadian Journal of Zoology*, **54**, 1674–1678.
- 571 Blankenberg D, Von Kuster G, Coraor N *et al.* (2010) Galaxy: a web-based genome
572 analysis tool for experimentalists. *Current protocols in molecular biology*,
573 **Supplement**, 19.10.1–19.10.21.
- 574 Bohmann K, Monadjem A, Lehmkuhl Noer C *et al.* (2011) Molecular diet analysis of two
575 african free-tailed bats (molossidae) using high throughput sequencing. *PloS ONE*,
576 **6**, e21441.
- 577 Boyles JG, Cryan PM, McCracken GF, Kunz TH (2011) Economic importance of bats in
578 agriculture. *Science*, **332**, 41–42.

- 579 | Brown DS, Symondson WOC, Burger R *et al.* (~~2013~~THIS ISSUE) Dietary competition
580 | between the alien Asian Musk Shrew (*Suncus murinus*) and a reintroduced
581 | population of Telfair's Skink (*Leiopisma telfairii*). *Molecular Ecology*, (~~XXX-~~
582 | ~~XXX~~)In press.
- 583 | Buchler ER (1975) Food transit time in *Myotis lucifugus* Chiroptera: Vespertilionidae.
584 | *Journal of Mammalogy*, **56**, 252–255.
- 585 | Carter GG, Coen CE, Stenzler LM, Lovette IJ (2006) Avian host DNA isolated from the
586 | feces of white-winged vampire bats (*Diaemus youngi*). *Acta Chiropterologica*, **8**,
587 | 255–274.
- 588 | Clare EL, Barber BR, Sweeney BW, Hebert PDN, Fenton MB (2011) Eating local:
589 | influences of habitat on the diet of little brown bats (*Myotis lucifugus*). *Molecular*
590 | *Ecology*, **20**, 1772–1780.
- 591 | Clare EL, Fraser EE, Braid HE, Fenton MB, Hebert PDN (2009) Species on the menu of
592 | a generalist predator, the eastern red bat (*Lasiurus borealis*): using a molecular
593 | approach to detect arthropod prey. *Molecular Ecology*, **18**, 2532–2542.
- 594 | Clare EL, Goerlitz HR, Drapeau VA *et al.* (2013) Trophic niche flexibility in
595 | *Glossophaga soricina*: how a nectar seeker sneaks an insect snack. *Functional*
596 | *Ecology*, **In press**.
- 597 | Clare E, Symondson WOC, Fenton MB (~~2013~~THIS ISSUE) An inordinate fondness for
598 | beetles? Variation in seasonal dietary preferences of night roosting big brown bats
599 | (*Eptesicus fuscus*). *Molecular Ecology*, **In review**(~~XXX-XXX~~).
- 600 | Cohen NJ, Deeds JR, Wong ES *et al.* (2009) Public health response to puffer fish
601 | (Tetrodotoxin) poisoning from mislabeled product. *Journal of Food Protection*,
602 | **72**, 810–817.
- 603 | Deagle BE, Chiaradia A, McInnes J, Jarman SN (2010) Pyrosequencing faecal DNA to
604 | determine diet of little penguins: is what goes in what comes out? *Conservation*
605 | *Genetics*, **11**, 2039–2048.
- 606 | Deagle BE, Kirkwood R, Jarman SN (2009) Analysis of Australian fur seal diet by
607 | pyrosequencing prey DNA in faeces. *Molecular Ecology*, **18**, 2022–2038.
- 608 | Deagle BE, Thomas AC, Shaffer AK, Trites AW, Jarman SN (2013) Quantifying
609 | sequence proportions in a DNA-based diet study using Ion Torrent amplicon
610 | sequencing: which counts count? *Molecular Ecology Resources*, **13**, 620–633.
- 611 | Dunne JA, Williams RJ, Martinez ND (2004) Network structure and robustness of marine
612 | food webs. *Marine Ecology Progress Series*, **273**, 291–302.

- 613 | Emrich MA, Clare EL, Symondson WOC, Koenig SE, Fenton MB (~~2013~~**THIS ISSUE**)
614 | Resource partitioning by insectivorous bats. *Molecular Ecology*, (~~XXX-XXX~~)**in**
615 | **review**.
- 616 | Fenton MB, Barclay RMR (1980) *Myotis lucifugus*. *Mammal Species*, **142**, 1–8.
- 617 | Fenton MB, Swanepoel CM, Brigham RM, Cebek J, Hickey MBC (1990) Foraging
618 | behaviour and prey selection by large slit-faced bats (*Nycteris grandis*; Chiroptera:
619 | Nycteridae). *Biotropica*, **22**, 2–8.
- 620 | Fenton MB, Thomas DW, Sasseen R (1981) *Nycteris grandis* (Nycteridae): an African
621 | carnivorous bat. *Journal of Zoology (London)*, **194**, 461–465.
- 622 | Fjellheim A, Raddum G. (1990) Acid precipitation: biological monitoring of streams and
623 | lakes. *Science of the Total Environment*, **96**, 57–66.
- 624 | Fraser EE, Fenton MB (2007) Age and food hardness affect food handling by
625 | insectivorous bats. *Canadian Journal of Zoology*, **85**, 985–993.
- 626 | Freeman PW (1981) Correspondence of food habits and morphology in insectivorous
627 | bats. *Mammalogy Papers: University of Nebraska State Museum*, **62**, 166–173.
- 628 | Frick WF, Pollock JF, Hicks AC *et al.* (2010) An emerging disease causes regional
629 | population collapse of a common North American bat species. *Science*, **329**, 679–
630 | 682.
- 631 | Furlonger CL, Dewar HJ, Fenton MB (1987) Habitat use by foraging insectivorous bats.
632 | *Canadian Journal of Zoology*, **65**, 284–288.
- 633 | Geggie JF, Fenton MB (1985) A comparison of foraging by *Eptesicus fuscus* (Chiroptera:
634 | Vespertilionidae) in urban and rural environments. *Canadian Journal of Zoology*,
635 | **63**, 263–267.
- 636 | Giardine B, Riemer C, Hardison RC *et al.* (2005) Galaxy: a platform for interactive large-
637 | scale genome analysis. *Genome Research*, **15**, 1451–1455.
- 638 | Goecks J, Nekrutenko A, Taylor J (2010) Galaxy: a comprehensive approach for
639 | supporting accessible, reproducible, and transparent computational research in the
640 | life sciences. *Genome biology*, **11**, R86.
- 641 | Goerlitz HR, Ter Hofstede HM, Zeale MRK, Jones G, Holderied MW (2010) An aerial-
642 | hawking bat uses stealth echolocation to counter moth hearing. *Current Biology*, **20**,
643 | 1568–1572.
- 644 | Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software
645 | package for education and data analysis. *Palaeontologia Electronica*, **4**, 1–9.

- 646 Hanner R, Becker S, Ivanova N V, Steinke D (2011) FISH-BOL and seafood
647 identification: geographically dispersed case studies reveal systemic market
648 substitution across Canada. *Mitochondrial DNA*, **22**, 106–122.
- 649 Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Biological identifications
650 through DNA barcodes. *Proceedings of the Royal Society B-Biological Sciences*,
651 **270**, 313–321.
- 652 Hilsenhoff WL (1988) Rapid field assessment of organic pollution with a family-level
653 biotic index. *Journal of the North American Benthological Society*, **7**, 65–68.
- 654 Huson DH, Mitra S, Ruscheweyh H-J, Weber N, Schuster SC (2011) Integrative analysis
655 of environmental sequences using MEGAN4. *Genome Research*, **21**, 1552–1560.
- 656 Jones M, Ghoorah A, Blaxter M (2011) jMOTU and Taxonerator: turning DNA barcode
657 sequences into annotated operational taxonomic units. *PloS ONE*, **6**, e19259.
- 658 Jost L (2006) Entropy and diversity. *Oikos*, **113**, 363–375.
- 659 King RA, Read DS, Traugott M, Symondson WOC (2008) Molecular analysis of
660 predation: a review of best practice for DNA-based approaches. *Molecular Ecology*,
661 **17**, 947–963.
- 662 Kondoh M (2003) Foraging adaptation and the relationship between food-web
663 complexity and stability. *Science*, **299**, 1388–1391.
- 664 Kunz TH, Braun de Torrez E, Bauer D, Lobova T, Fleming TH (2011) Ecosystem
665 services provided by bats. *Annals of the New York Academy of Sciences*, **1223**, 1–38.
- 666 Kunz TH, Whitaker JO (1983) An evaluation of fecal analysis for determining food
667 habits of insectivorous bats. *Canadian Journal of Zoology*, **61**, 1317–1321.
- 668 Kurta A, Bell GP, Nagy KA, Kunz TH (1989) Energetics of pregnancy and lactation in
669 freeranging little brown bats (*Myotis lucifugus*). *Physiological Zoology*, **62**, 804–
670 818.
- 671 Lenat DR (1993) A biotic index for the southeastern United States: derivation and list of
672 tolerance values, with criteria for assigning water-quality ratings. *Journal of the*
673 *North American Benthological Society*, **12**, 279–290.
- 674 McCann K (2007) Protecting biostructure. *Nature*, **446**, 29.
- 675 Ober HK, Hayes JP (2008) Prey selection by bats in forests of western oregon. *Journal of*
676 *Mammalogy*, **89**, 1191–1200.

- 677 Piñol J, San Andrés V, Clare EL, Mir G, Symondson WOC (2013) A pragmatic approach
678 to the analysis of diets of generalist predators: the use of next-generation sequencing
679 with no blocking probes. *Molecular ecology resources*.
- 680 Pompanon F, Deagle BE, Symondson WOC *et al.* (2012) Who is eating what: diet
681 assessment using next generation sequencing. *Molecular Ecology*, **21**, 1931–1950.
- 682 R Development Core Team: R: A language and environment for statistical computing
683 (2008)
- 684 Ratnasingham S, Hebert PDN (2007) BOLD: the barcode of life data system
685 (www.barcodinglife.org). *Molecular Ecology Notes*, **7**, 355–364.
- 686 Razgour O, Clare EL, Zeale MRK *et al.* (2011) High-throughput sequencing offers
687 insight into mechanisms of resource partitioning in cryptic bat species. *Ecology and*
688 *Evolution*, **1**, 556–570.
- 689 Rosenheim J, Corbett A (2003) Omnivory and the indeterminacy of predator function:
690 can a knowledge of foraging behaviour help? *Ecology*, **84**, 2538–2548.
- 691 Simpson EH (1949) Measurement of Diversity. *Nature*, **163**, 688–688.
- 692 Singer M, Bernays E (2003) Understanding omnivory needs a behavioural perspective.
693 *Ecology*, **84**, 2532–2537.
- 694 Smith MA, Wood DM, Janzen DH, Hallwachs W, Hebert PDN (2007) DNA barcodes
695 affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera,
696 Tachinidae) are not all generalists. *Proceedings of the National Academy of Sciences*
697 *of the United States of America*, **104**, 4967–4972.
- 698 Smith MA, Woodley NE, Janzen DH, Hallwachs W, Hebert PDN (2006) DNA barcodes
699 reveal cryptic host-specificity within the presumed polyphagous members of a genus
700 of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of*
701 *Sciences of the United States of America*, **103**, 3657–3662.
- 702 Soininen EM, Valentini A, Coissac E *et al.* (2009) Analysing diet of small herbivores: the
703 efficiency of DNA barcoding coupled with high-throughput pyrosequencing for
704 deciphering the composition of complex plant mixtures. *Frontiers in Zoology*, **6**, 16.
- 705 Solé R V, Montoya JM (2001) Complexity and fragility in ecological networks.
706 *Proceedings of the Royal Society B-Biological Sciences*, **268**, 2039–2045.
- 707 Symondson WOC (2002) Molecular identification of prey in predator diets. *Molecular*
708 *Ecology*, **11**, 627–641.

709 Symondson WOC, Liddell JE (1993) A monoclonal antibody for the detection of arionid
710 slug remains in carabid predators. *Biological Control*, **3**, 207–214.

711 Valentini A, Miquel C, Nawaz MA *et al.* (2009) New perspectives in diet analysis based
712 on DNA barcoding and parallel pyrosequencing: the trnL approach. *Molecular*
713 *Ecology Resources*, **9**, 51–60.

714 Wong EH-K, Hanner RH (2008) DNA barcoding detects market substitution in North
715 American seafood. *Food Research International*, **41**, 828–837.

716 Zeale MRK, Butlin RK, Barker GLA, Lees DC, Jones G (2011) Taxon-specific PCR for
717 DNA barcoding arthropod prey in bat faeces. *Molecular Ecology Resources*, **11**,
718 236–244.

719 **Data Accessibility:**

720 All DNA sequencing reads and an explanatory “read me” file along with BLAST scores
721 for figure 8 have been placed in Dryad:

722 <http://datadryad.org/submit?journalID=MolEcol&manu=MEC-13-0701>

723

724 **Author’s contributions:** ELC, HB, FF, EF, AM, AB, RH, CW, FM, AM, KN, MB, JP,
725 JR, RMRB, JPR designed and conducted field research. ELC conducted the molecular
726 analysis. WOCS contributed to molecular protocols. All authors contributed to
727 manuscript production.

728

729 **Figure Legends:**

730

731 Figure 1: Distribution of sampling sites across Canada. Samples in Northwest Territories
732 (n=5) were collected at sites in Kakisa (1) and Salt River (2) (considered as one unit in
733 statistical analysis). Samples in the prairies (n=3) were collected between Medicine Hat
734 (Alberta) and Swift Current (Saskatchewan) (3). Samples in Ontario were collected in
735 Clinton (4) (n=14), Long Point (5) (n=7), Lake St. George (6) (2009 n=18, 2011 n=7) and
736 Pinery Provincial Park (7) (n=4). Samples in Nova Scotia (n=8) were collected at sites in
737 Martock (8) and Tatamagouche (9) (considered as one unit in statistical analysis).

738 [Samples in Quebec were collected at Jacques-Cartier and Aiguebelle National Parks \(10\)](#)
739 [and Montmorency Forest Station \(11\).](#)

740 (Map Modified from: *Canada Outline Map. St. Catharines, Ontario: Brock University Map Library.*

741 *Available: Brock University Map Library Controlled Access*

742 http://www.brocku.ca/maplibrary/maps/outline/North_America/canadaNONAMES.pdf (Accessed April 2,
743 2013.)

744

745 Figure 2: Seasonal diversity in prey consumed by *M. lucifugus*. The proportion of each
746 prey group in the diet varied significantly across seasons. Diptera dominated the early
747 season diet while Lepidoptera become more important in the middle and late seasons.

748 Proportion = frequency of occurrence of that order / total occurrences, where an

749 occurrence is an identified MOTU in a sample.

750

751 Figure 3: Seasonal diversity in prey consumed by *M. lucifugus* at 8 locations across
752 Canada. The proportion of each prey group composing the diet varied significantly across
753 seasons and with location. Proportion = frequency of occurrence of that order / total
754 occurrences, where an occurrence is an identified MOTU in a sample.

755

756 Figure 4: Estimates of *M. lucifugus*' dietary diversity with 95% confidence intervals,
757 based on the Simpson diversity index on data restricted to ordinal-level taxonomy (A)
758 and using MOTU as a proxy for species (B).

759

760 Figure 5: A comparison of rarefaction curves for operational taxonomic units at the order
761 (A, B) and species (C, D) level. Lines are mean estimates (A, B, C) or mean with 95%
762 confidence levels (D) based on permutations.

763

764 Figure 6: Estimates of *M. lucifugus*' dietary diversity with 95% confidence intervals
765 based on the Simpson diversity index from three seasons. Early season=females are
766 pregnant, middle season=females are lactating, late season=young are independent.

767

768 Figure 7: Weekly species richness in the diet of *M. lucifugus* for the two most heavily
769 sampled sites, at Clinton and Lake St. George in 2009, showing a trend of higher mean
770 species richness with 95% confidence intervals in bats at Lake St. George, which is also
771 an area where prey have a lower pollution tolerance suggesting higher quality habitat.

772

773 Figure 8: A schematic of prey species consumed including all MOTU (including those
774 that could not be identified using a reference database). Identifications have been made
775 by BLAST score and are limited to hypothesis at the order level. Values at nodes or tips
776 represent the number of MOTU assigned. Node size is scaled to the number of
777 assignments. See Emrich et al. (2013) for additional details.

778

779

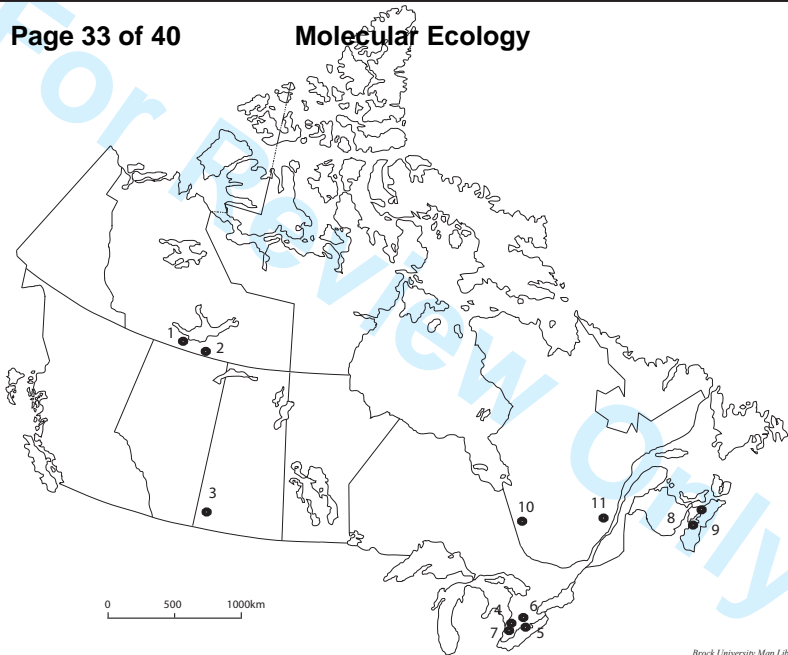
780 Table 1: Approximate habitat assessments based on the lowest scoring (least tolerant to pollution or
 781 acidification) taxa identified in the diet of bats at each location.

Location	Example Taxa	Pollution Tolerance	Acid Tolerance	Maximum Quality
NWT	Glososomatidae	0	low	Low organic pollution No acidification
	Ephemerellidae	1	Low-med	
	<i>Heptagenia sp.</i>			
Lake St. George (Ontario)	Glososomatidae	0	low	Low organic pollution No acidification
	Ephemerellidae	1	high	
	Corydalidae	0		
Clinton (Ontario)	Helicopsychidae	3		Trace organic pollution
	Tipulidae	3		
	Isonychia	3		
Long Point (Ontario)	Leptoceridae	4		Some organic pollution
	Phryganeidae	4		
Nova Scotia	Leptoceridae	4	high	Some organic pollution
	Phryganeidae	4		
	<i>Stenacron</i>	4	low	No Acidification
	<i>Molanna sp.</i>			
Pinery (Ontario)	Chironomidae	6	high	Some organic pollution Possibly acidified
	Psychodidae	10		
	Phryganeidae	4		
Saskatchewan	Chironomidae	6		Likely organic pollution*

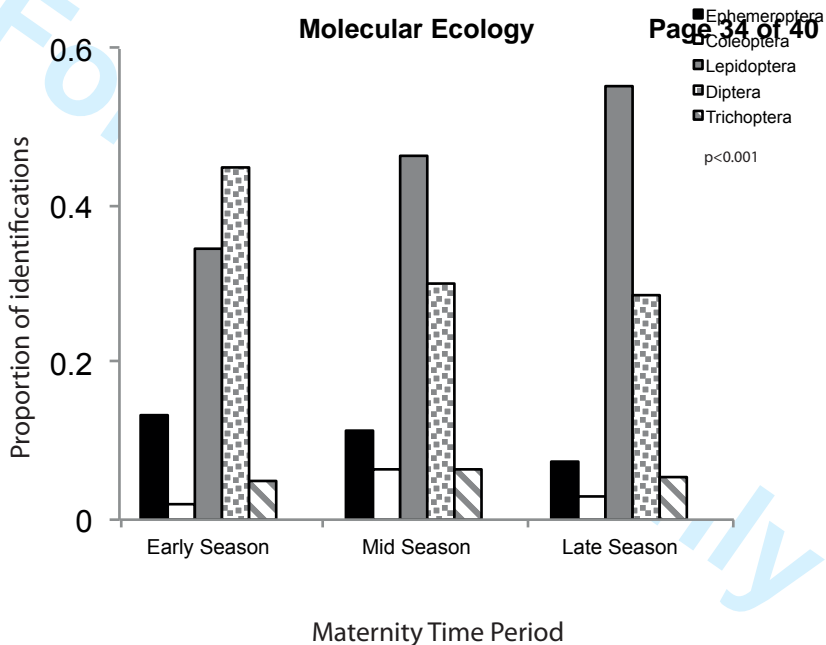
782 * Little Data Available

783 Hilsenhoff index goes from 1(low) to 10 (high) tolerance

784



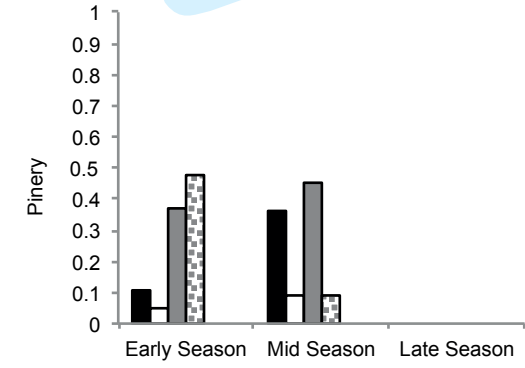
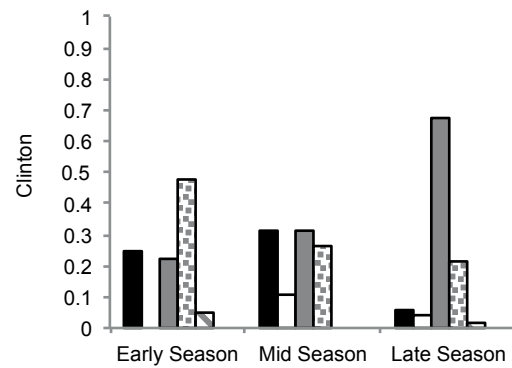
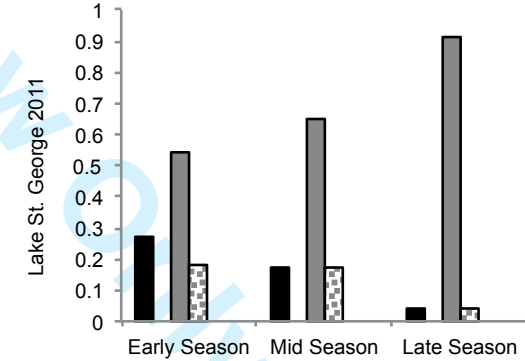
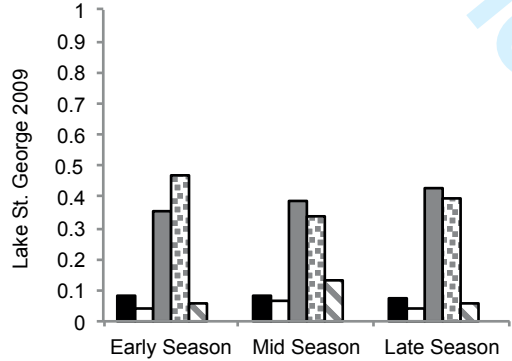
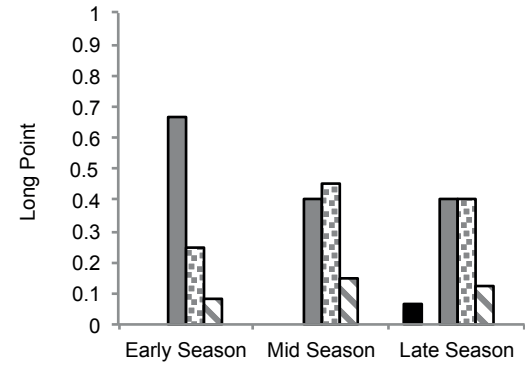
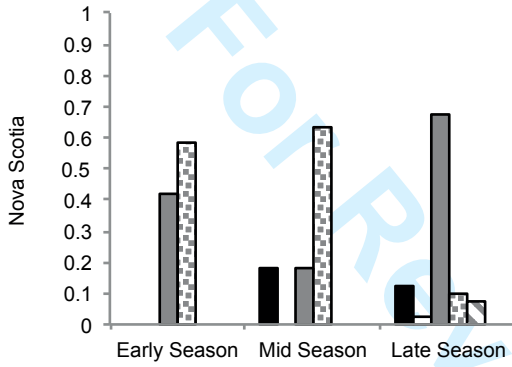
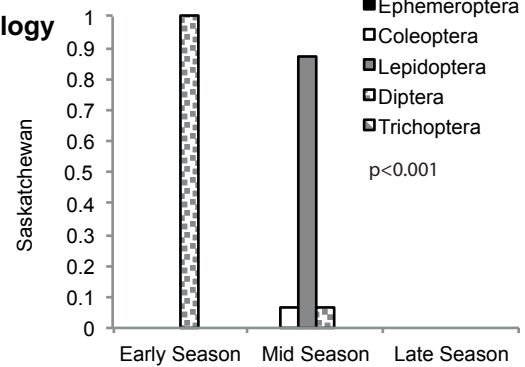
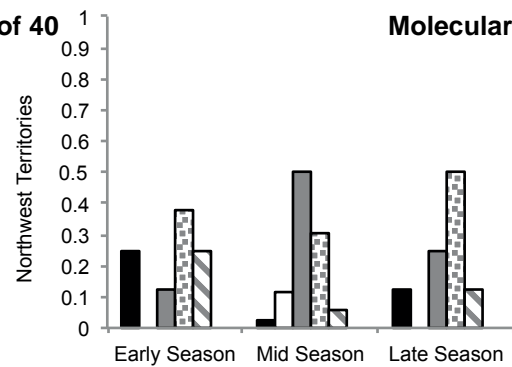
Molecular Ecology



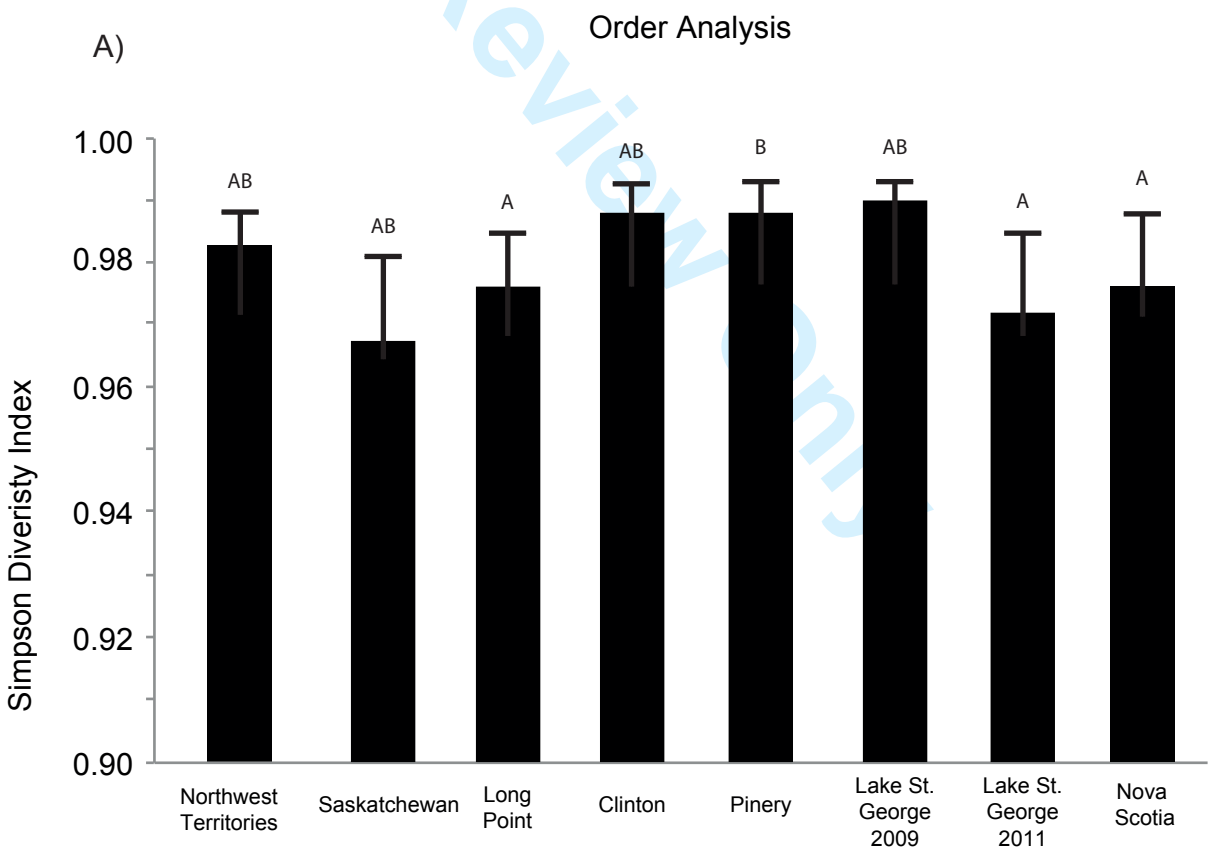
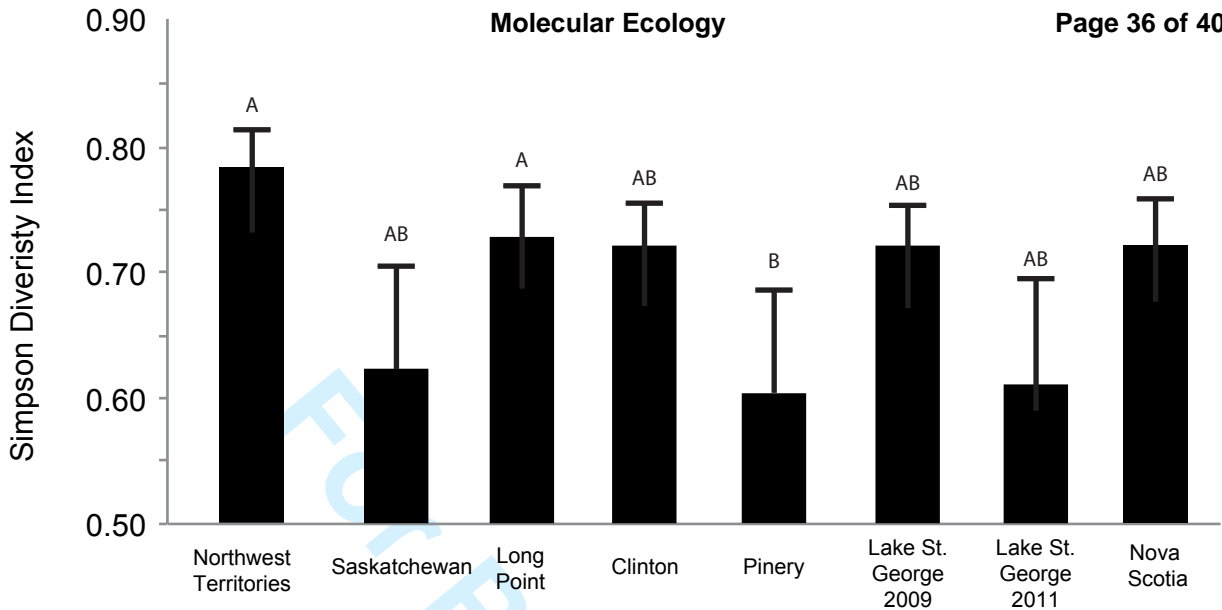
Molecular Ecology

- Ephemeroptera
 - Coleoptera
 - Lepidoptera
 - ▨ Diptera
 - Trichoptera
- p < 0.001

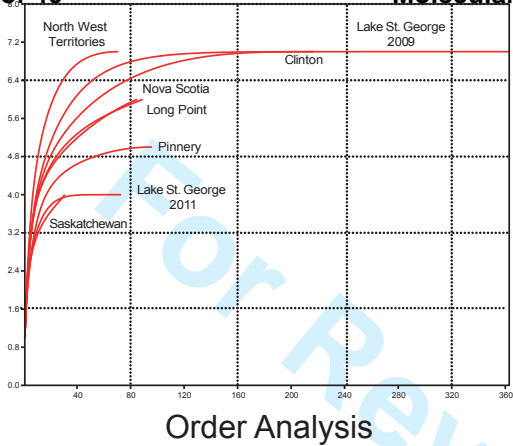
Percent of identifications at each location



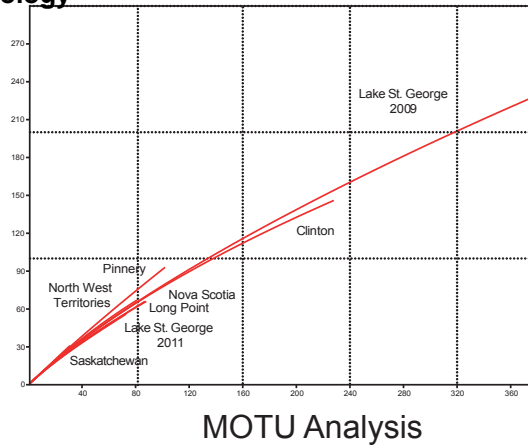
Maternity Time Period



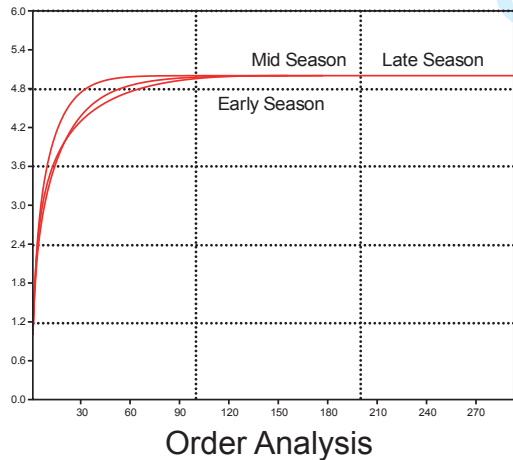
B) MOTU Analysis



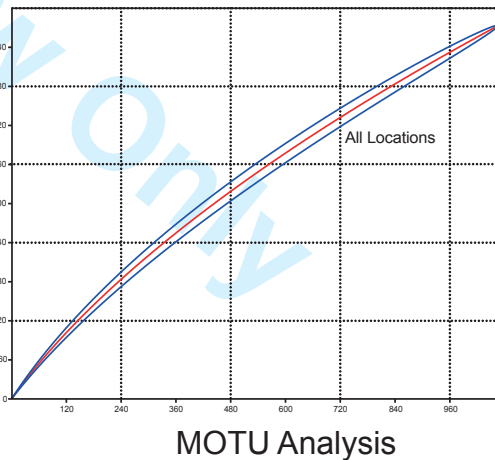
A)



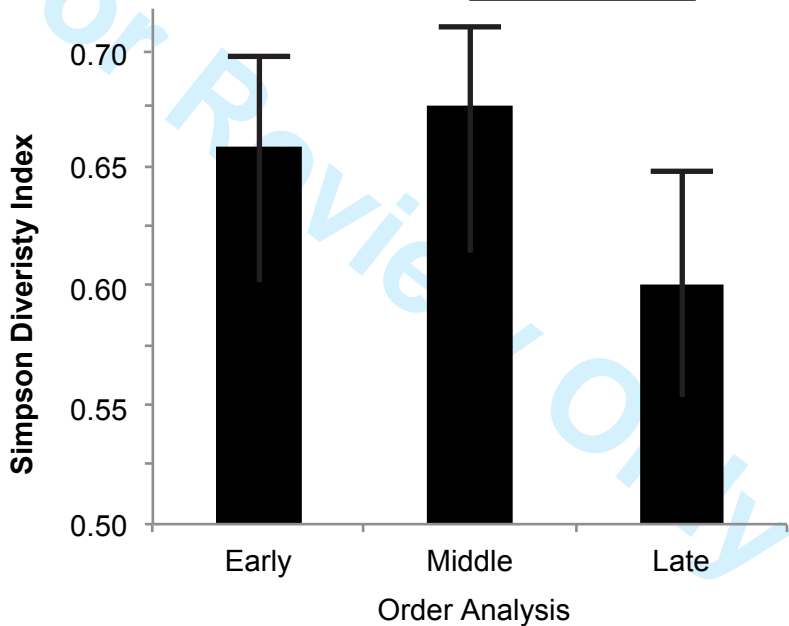
C)

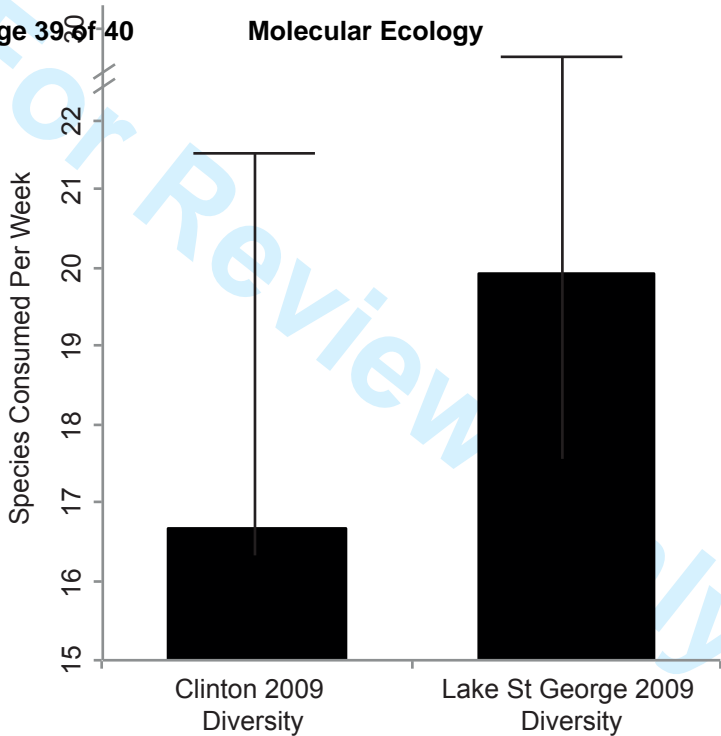


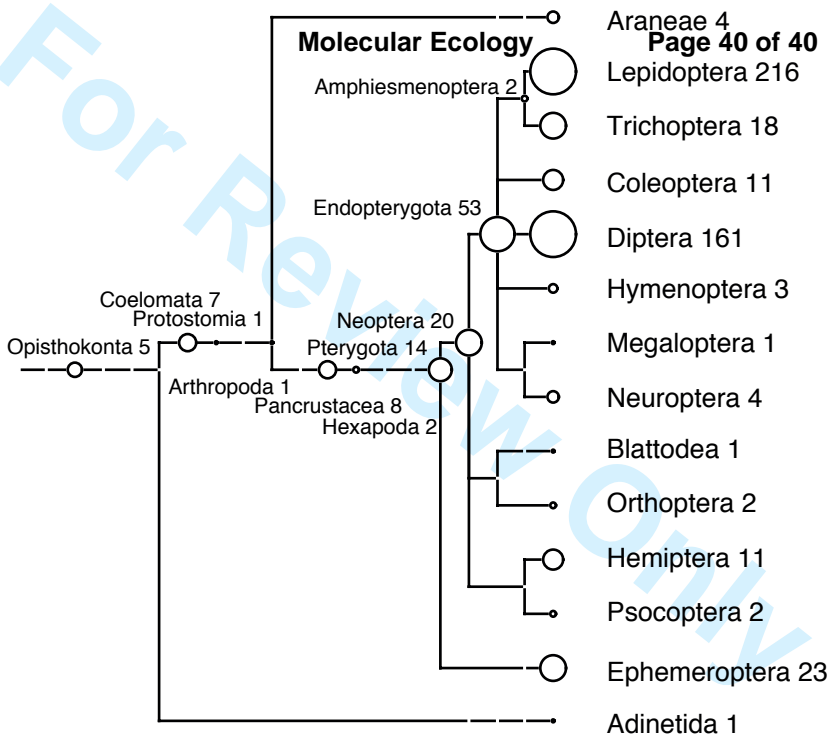
B)



D)







All taxonomic matches are 1 or 1a unless indicated with a *=level 2

Frequency	Class	Order	Family	Species	NWT	Saskatchewan	Nova Scotia	Pinnery	Long Point	Lake St. George 2011	Clinton	Lake St. George 2009
1	Arachnida	Araneae	Araneidae	<i>Anyphaena pectorosa</i>					1			
1	Arachnida	Araneae	Araneidae	<i>Larinioides cornutus</i>							1	
2	Arachnida	Araneae	Araneidae	<i>Larinioides patagiatus</i>	2							
1	Arachnida	Araneae	Araneidae	<i>Larinioides sclopetarius</i>								1
2	Insecta	Coleoptera	Carabidae	<i>Dromius piceus</i>							2	
1	Insecta	Coleoptera	Carabidae	<i>Notiobia terminata</i>								1
1	Insecta	Coleoptera	Carabidae	<i>Selenophorus sp.</i>		1						
1	Insecta	Coleoptera	Carabidae	<i>Stenolophus ochropezus</i>				1				
1	Insecta	Coleoptera	Cleridae	<i>Cymatodera bicolor</i>							1	
1	Insecta	Coleoptera	Curculionidae	<i>Hypera sp.</i>							1	
2	Insecta	Coleoptera	Curculionidae	<i>Polydrusus sericeus</i>								2
1	Insecta	Coleoptera	Dytiscidae	<i>Ilybius sp.</i>	1							
1	Insecta	Coleoptera	Elateridae	<i>Denticollis denticornis</i>							1	
1	Insecta	Coleoptera	Leiodidae	<i>Catops luridipennis</i>	1							
1	Insecta	Coleoptera	Scarabaeidae	<i>Onthophagus sp.</i>								1
7	Insecta	Coleoptera	Scirtidae	<i>Cyphon sp.</i>			1	1				5
1	Insecta	Diptera	Asilidae	<i>Dioctria sp.</i>							1	
3	Insecta	Diptera	Chironomidae	<i>Ablabesmyia americana</i>								3
1	Insecta	Diptera	Chironomidae	<i>Axarus sp.</i>				1				
3	Insecta	Diptera	Chironomidae	<i>Chironomus acidophilus</i>				1	1			1
1	Insecta	Diptera	Chironomidae	<i>Chironomus sp.</i>		1						
1	Insecta	Diptera	Chironomidae	<i>Chironomus sp.</i>	1							
1	Insecta	Diptera	Chironomidae	<i>Cryptochironomus psittacinus</i>							1	
7	Insecta	Diptera	Chironomidae	<i>Cladopelma sp.</i>								7
2	Insecta	Diptera	Chironomidae	<i>Cladopelma sp.</i>								2
2	Insecta	Diptera	Chironomidae	<i>Cricotopus bicinctus</i>				1			1	
1	Insecta	Diptera	Chironomidae	<i>Diamesa sp.</i>								1
18	Insecta	Diptera	Chironomidae	<i>Dicrotendipes tritonus</i>			1	1				16
1	Insecta	Diptera	Chironomidae	<i>Microtendipes pedellus</i>								1
6	Insecta	Diptera	Chironomidae	<i>Parachironomus tenuicaudatus</i>								6
12	Insecta	Diptera	Chironomidae	<i>Paracladopelma winnelli</i>				2			10	
1	Insecta	Diptera	Chironomidae	<i>Procladius sp.</i>								1
1	Insecta	Diptera	Chironomidae	<i>Psectrotanypus sp.</i>		1						
1	Insecta	Diptera	Chironomidae	<i>Rheopelopia ornata</i>							1	
7	Insecta	Diptera	Chironomidae	<i>Tanytarsus mendax</i>				1				6
1	Insecta	Diptera	Chironomidae	<i>Unknown</i>		1						
2	Insecta	Diptera	Culicidae	<i>Aedes implicatus</i>	2							

1	Insecta	Diptera	Culicidae	<i>Aedes sp.</i>	1				
1	Insecta	Diptera	Culicidae	<i>Aedes stimulans</i>				1*	
11	Insecta	Diptera	Culicidae	<i>Aedes vexans</i>	3	1	1	2	4
1	Insecta	Diptera	Culicidae	<i>Anopheles sp.</i>					1
1	Insecta	Diptera	Culicidae	<i>Anopheles sp.</i>	1				
3	Insecta	Diptera	Culicidae	<i>Coquillettidia perturbans</i>	2				1
6	Insecta	Diptera	Culicidae	<i>Culex sp.</i>		6			
1	Insecta	Diptera	Culicidae	<i>Culex sp.</i>	1				
1	Insecta	Diptera	Culicidae	<i>Culiseta inornata</i>	1				
1	Insecta	Diptera	Culicidae	<i>Culiseta minnesotae</i>		1			
1	Insecta	Diptera	Culicidae	<i>Culiseta sp.</i>	1				
2	Insecta	Diptera	Culicidae	<i>Ochlerotatus sp.</i>	1				1
4	Insecta	Diptera	Empididae	<i>Trichoclinocera pectinifemur</i>				4	
1	Insecta	Diptera	Limoniidae	<i>Elephantomyia westwoodi</i>			1		
4	Insecta	Diptera	Limoniidae	<i>Erioptera septemtrionis</i>			4		
2	Insecta	Diptera	Limoniidae	<i>Euphyllidorea platyphallus</i>	2				
1	Insecta	Diptera	Limoniidae	<i>Helius flavipes</i>			1		
1	Insecta	Diptera	Limoniidae	<i>Idiocera blanda</i>			1		
9	Insecta	Diptera	Limoniidae	<i>Ormosia affinis</i>		7	1		1
1	Insecta	Diptera	Limoniidae	<i>Symplecta sp.</i>				1	
1	Insecta	Diptera	Muscidae	<i>Musca autumnalis</i>				1	
1	Insecta	Diptera	Muscidae	<i>Spilogona sp.</i>	1				
2	Insecta	Diptera	Pediciidae	<i>Pedicia inconstans</i>				2	
4	Insecta	Diptera	Psychodidae	<i>Phychodid sp.</i>		2		1	1
1	Insecta	Diptera	Sepsidae	<i>Sepsis punctum</i>				1	
1	Insecta	Diptera	Tabanidae	<i>Hybomitra lurida</i>	1				
1	Insecta	Diptera	Tachinidae	<i>Cryptomeigenia sp.</i>		1			
1	Insecta	Diptera	Tachinidae	<i>Medina sp.</i>				1*	
1	Insecta	Diptera	Tachinidae	<i>Unnkown</i>	1				
1	Insecta	Diptera	Tipulidae	<i>Tipula caloptera</i>				1	
1	Insecta	Diptera	Tipulidae	<i>Tipula oleracea</i>					1
10	Insecta	Ephemeroptera	Caenidae	<i>Caenis amica sp.?</i>	1	4	1	4	
4	Insecta	Ephemeroptera	Caenidae	<i>Caenis latipennis ?</i>				3	1
1	Insecta	Ephemeroptera	Caenidae	<i>Caenis sp.</i>		1			
1	Insecta	Ephemeroptera	Caenidae	<i>Caenis sp.</i>		1			
1	Insecta	Ephemeroptera	Caenidae	<i>Caenis sp.</i>		1			
2	Insecta	Ephemeroptera	Caenidae	<i>Caenis sp.</i>		2			
6	Insecta	Ephemeroptera	Caenidae	<i>Caenis sp.</i>				6	
18	Insecta	Ephemeroptera	Caenidae	<i>Caenis youngi sp.?</i>			1	2	15
1	Insecta	Ephemeroptera	Ephemerellidae	<i>Ephemerella sp.</i>	1				

5	Insecta	Ephemeroptera	Ephemerellidae	<i>Eurylophella temporalis</i>				3		2
4	Insecta	Ephemeroptera	Ephemeridae	<i>Hexagenia sp.</i>		4				
2	Insecta	Ephemeroptera	Heptageniidae	<i>Heptagenia sp.</i>	2					
6	Insecta	Ephemeroptera	Heptageniidae	<i>Maccaffertium mediopunctatum</i>					6	
1	Insecta	Ephemeroptera	Heptageniidae	<i>Maccaffertium vicarium</i>					1	
1	Insecta	Ephemeroptera	Heptageniidae	<i>Stenacron interpunctatum</i>		1				
2	Insecta	Ephemeroptera	Isonychiidae	<i>Isonychia bicolor</i>					2	
4	Insecta	Hemiptera	Notonectidae	<i>Notonecta kirbyi</i>						4
1	Insecta	Hymenoptera	Vespidae	<i>Polistes sp.</i>						1
1	Insecta	Lepidoptera	Amphisbatidae	<i>Machimia tentoriferella</i>				1*		
3	Insecta	Lepidoptera	Amphisbatidae	<i>Psilocorsis reflexella</i>		3				
1	Insecta	Lepidoptera	Argyresthiidae	<i>Argyresthia alternatella</i>			1			
1	Insecta	Lepidoptera	Argyresthiidae	<i>Argyresthia aureoargentella</i>						1
1	Insecta	Lepidoptera	Argyresthiidae	<i>Argyresthia canadensis</i>						1
1	Insecta	Lepidoptera	Argyresthiidae	<i>Argyresthia thuiella</i>						1
2	Insecta	Lepidoptera	Batrachedridae	<i>Batrachedra praeangusta</i>	1					1
1	Insecta	Lepidoptera	Blastobasidae	<i>Asaphocrita busckiella</i>						1
1	Insecta	Lepidoptera	Blastobasidae	<i>Blastobasis floridella</i>					1	
2	Insecta	Lepidoptera	Blastobasidae	<i>Holocera chalcifrontella</i>				1		1
1	Insecta	Lepidoptera	Blastobasidae	<i>Holocera crassicornella*</i>	1					
1	Insecta	Lepidoptera	Carmbidae	<i>Herpetogramma sp.</i>						1
1	Insecta	Lepidoptera	Carmbidae	<i>Ostrinia obumbratalis</i>				1		
1	Insecta	Lepidoptera	Coleophoridae	<i>Coleophora limosipennella</i>				1		
2	Insecta	Lepidoptera	Coleophoridae	<i>Coleophora pruniella</i>		1				1
1	Insecta	Lepidoptera	Coleophoridae	<i>Coleophora sp.</i>						1
1	Insecta	Lepidoptera	Cosmopterigidae	<i>Limnaecia phragmitella</i>						1
1	Insecta	Lepidoptera	Crambidae	<i>Acentria ephemerella</i>					1	
1	Insecta	Lepidoptera	Crambidae	<i>Ostrinia penitalis</i>			1			
1	Insecta	Lepidoptera	Crambidae	<i>Thopeutis forbesellus</i>			1			
12	Insecta	Lepidoptera	Elachistidae	<i>Agonopterix robiniella</i>	1			1*	3	7
2	Insecta	Lepidoptera	Elachistidae	<i>Semioscopis packardella</i>					1	1
1	Insecta	Lepidoptera	Erebidae	<i>Ctenucha virginica</i>						1
1	Insecta	Lepidoptera	Erebidae	<i>Idia sp.</i>	1					
2	Insecta	Lepidoptera	Erebidae	<i>Unknown**</i>			1			1
2	Insecta	Lepidoptera	Erebidae	<i>Unknown**</i>						2
3	Insecta	Lepidoptera	Erebidae	<i>Unknown**</i>	1		1	1		
2	Insecta	Lepidoptera	Erebidae	<i>Unknown**</i>			1		1	
1	Insecta	Lepidoptera	Erebidae	<i>Unknown**</i>					1	
1	Insecta	Lepidoptera	Gelechiidae	<i>Carpatolechia sp.</i>		1				
1	Insecta	Lepidoptera	Gelechiidae	<i>Caryocolum cassella</i>	1					

1	Insecta	Lepidoptera	Sphingidae	<i>Amorpha juglandis</i>					1
1	Insecta	Lepidoptera	Sphingidae	<i>Deidamia inscriptum</i>			1		
2	Insecta	Lepidoptera	Tineidae	<i>Acrolophus heppneri*</i>	1				1
1	Insecta	Lepidoptera	Tineidae	<i>Homosetia fasciella</i>					1
2	Insecta	Lepidoptera	Tortricidae	<i>Acleris chalybeana</i>			1	1	
3	Insecta	Lepidoptera	Tortricidae	<i>Acleris forsskaleana</i>			1	1	1
1	Insecta	Lepidoptera	Tortricidae	<i>Acleris negundana</i>			1		
1	Insecta	Lepidoptera	Tortricidae	<i>Adoxophyes negundana</i>			1*		
1	Insecta	Lepidoptera	Tortricidae	<i>Aethes sp.</i>				1	
1	Insecta	Lepidoptera	Tortricidae	<i>Ancylis divisana</i>			1		
1	Insecta	Lepidoptera	Tortricidae	<i>Argyrotaenia quercifoliana</i>		1			
1	Insecta	Lepidoptera	Tortricidae	<i>Argyrotaenia sp.</i>		1			
1	Insecta	Lepidoptera	Tortricidae	<i>Catastega aceriella</i>					1
1	Insecta	Lepidoptera	Tortricidae	<i>Choristoneura fumiferana</i>			1		
1	Insecta	Lepidoptera	Tortricidae	<i>Choristoneura sp.</i>	1				
1	Insecta	Lepidoptera	Tortricidae	<i>Clepsis virescana</i>	1				
4	Insecta	Lepidoptera	Tortricidae	<i>Cnephasia sp.</i>		1		3	
1	Insecta	Lepidoptera	Tortricidae	<i>Epinotia transmissana</i>			1		
2	Insecta	Lepidoptera	Tortricidae	<i>Eucosma sp.</i>			1		1
1	Insecta	Lepidoptera	Tortricidae	<i>Grapholita eclipsana</i>				1	
3	Insecta	Lepidoptera	Tortricidae	<i>Gretchena sp.</i>			1	2	
1	Insecta	Lepidoptera	Tortricidae	<i>Oecetis cinerascens</i>			1		
1	Insecta	Lepidoptera	Tortricidae	<i>Olethreutes glaciana</i>	1				
1	Insecta	Lepidoptera	Tortricidae	<i>Olethreutes sp.</i>					1
2	Insecta	Lepidoptera	Tortricidae	<i>Pandemis lamprosana</i>			2		
1	Insecta	Lepidoptera	Tortricidae	<i>Pandemis sp.</i>	1				
1	Insecta	Lepidoptera	Tortricidae	<i>Phtheochroa sp.</i>					1
1	Insecta	Lepidoptera	Tortricidae	<i>Platynota idaeusalis</i>	1				
1	Insecta	Lepidoptera	Tortricidae	<i>Platynota sp.</i>				1	
1	Insecta	Lepidoptera	Tortricidae	<i>Platynota sp.</i>			1		
4	Insecta	Lepidoptera	Tortricidae	<i>Proteoteras crescentana</i>			1	1	2
4	Insecta	Lepidoptera	Tortricidae	<i>Pseudexentera sp.</i>					4
3	Insecta	Lepidoptera	Tortricidae	<i>Pseudexentera sp.</i>			2	1	
1	Insecta	Lepidoptera	Tortricidae	<i>Pseudexentera sp.</i>			1		
1	Insecta	Lepidoptera	Tortricidae	<i>Pseudexentera sp.</i>				1	
7	Insecta	Lepidoptera	Tortricidae	<i>Sparganothis pettitana</i>			2	1	4
1	Insecta	Lepidoptera	Tortricidae	<i>Zeiraphera sp.</i>		1			
1	Insecta	Megaloptera	Corydalidae	<i>Chauliodes sp.</i>					1
1	Insecta	Megaloptera	Sialidae	<i>Sialis sp.</i>				1	
3	Insecta	Neuroptera	Hemerobiidae	<i>Hemerobius sp.</i>				1	2

3	Insecta	Tricoptera	Glossosomatidae	<i>Glossosoma intermedium</i>	2						1	
2	Insecta	Tricoptera	Helicopsychidae	<i>Helicopsyche borealis</i>						2		
1	Insecta	Tricoptera	Hydropsychidae	<i>Arctopsyche ladogensis</i>	1							
1	Insecta	Tricoptera	Hydropsychidae	<i>Cheumatopsyche sp.</i>						1		
2	Insecta	Tricoptera	Limnephilidae	<i>Limnephilus sp.</i>							2	
3	Insecta	Tricoptera	Leptoceridae	<i>Triaenodes injustus</i>							3	
1	Insecta	Tricoptera	Leptoceridae	<i>Triaenodes nox</i>							1	
5	Insecta	Tricoptera	Leptoceridae	<i>Triaenodes sp.</i>					5			
1	Insecta	Tricoptera	Leptoceridae	<i>Triaenodes sp.</i>					1			
2	Insecta	Tricoptera	Molannidae	<i>Molanna sp.</i>				2				
8	Insecta	Tricoptera	Nectopsyche	<i>Nectopsyche albida</i>							8	
2	Insecta	Tricoptera	Phryganeidae	<i>Agrypnia colorata</i>	2							
1	Insecta	Tricoptera	Phryganeidae	<i>Agrypnia deflata</i>	1							
3	Insecta	Tricoptera	Phryganeidae	<i>Phryganea cinerea</i>				1		1		
				Additional unidentiifed prey (includes level 3 identifications)	33	18	42	64	42	27	88	158

Procedures for Quebec samples:

Samples from Quebec were not included in regular statistical analyses for three reasons. First, they were collected from individuals rather than from under roosts in large “community” samples. Second, for reasons that are not clear, the DNA was difficult to amplify and so additional steps were taken to recover the data. We include these data then as a supplement to the full analysis. Third, the sample includes males rather than all females and young (as expected in maternity roosts).

Collection procedures: The sampling in Quebec was performed from 15th of June to 5th of August in 2011 (Jacques-Cartier National Park and Montmorency Research Forest) and 2012 (Aiguebelle National Park). A total of 2-5 pellets were collected directly from males.

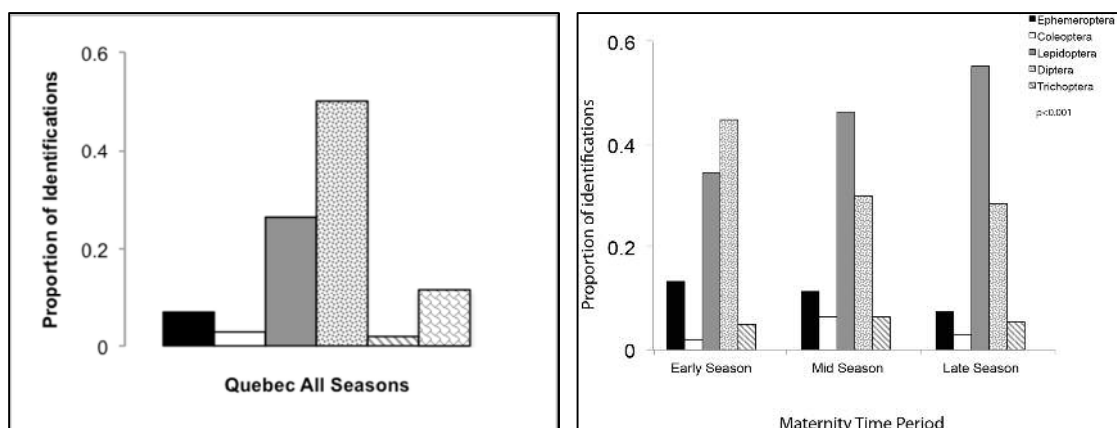
Sample preparation: We extracted DNA as described in the main manuscript. We encountered a high rate of PCR failure for these samples. Thus we treated all as “recalcitrant” and the PCR was conducted using Q-solution (provided by the Qiagen, UK multi-plex PCR kit) and modified hot start PCR programme.

PCR mixture: 12µl reactions contains 5µl of Master Mix, 1µl of Q solution, 0.5µl of each primer, 3µl H₂O and 2µl template DNA.

Thermocycler protocols: An initial denaturation period of 15 min at 95°C followed by 35 cycles of 94°C for 30s, 53°C for 90s and 72°C for 90s, with a final extension period of 10min at 72°C. Using this protocol >90% of samples provided a band on an agarose gel.

Sequencing: To maximize sequencing potential and recovery, the reverse primers were modified for the Ion Torrent platform (Clare et al. 2014) and sequencing and informatics was carried out as described in that same publication.

Results: We recovered sequences from all samples (Supplemental File 1, worksheet 2)



Supplemental Figure: A comparison of the overall diet of little brown bats at locations in Quebec (across all seasons) with the overall results from Figure 2.

Location

Long Point
Long Point
Long Point
Long Point
Long Point
Long Point
Long Point

Collection Date

June 27, 2011
July 5, 2011
July 18, 2011
August 1, 2011
August 17, 2011
June 6, 2011
June 13, 2011

Clinton
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May 20, 2009
May 27, 2009
June 3, 2009
June 11, 2009
June 17, 2009
July 8, 2009
July 15, 2009
July 22, 2009
July 29, 2009
August 5, 2009
August 12, 2009
August 19, 2009
August 26, 2009
September 9, 2009

Lake St George
Lake St George
Lake St George
Lake St George
Lake St George
Lake St George
Lake St George

June 8, 2011
June 21, 2011
July 5, 2011
July 12, 2011
July 18, 2011
Aug 1, 2011
Aug 15, 2011

Lake St George
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Lake St George

May 21, 2009
May 27, 2009
May 29, 2009
June 3, 2009
June 10, 2009
June 16, 2009
June 26, 2009
July 2, 2009
July 8, 2009
July 15, 2009
July 22, 2009
July 29, 2009
August 5, 2009
August 12, 2009
August 19, 2009
August 26, 2009

Lake St George	September 2, 2009
Lake St George	September 9, 2009
Praries	June 22, 2011
Praries	July 18, 2011
Praries	August 15, 2011
Pinery Provincial Park	June 14, 2009
Pinery Provincial Park	Exact Date Not Know
Pinery Provincial Park	Exact Date Not Know
Pinery Provincial Park	July 13, 2008
Kakisa NWT	June 28, 2011
Salt river NWT	June 23, 2011
Kakisa NWT	July 27, 2011
Salt river NWT	July 20, 2011
Salt river NWT	Sept 1, 2011
Martock, Nova Scotia	June 16, 2011
Martock, Nova Scotia	July 10, 2011
Martock, Nova Scotia	July 24, 2011
Martock, Nova Scotia	August 29, 2011
Tatamagouche Nova Scotia	May 31, 2011
Tatamagouche Nova Scotia	July 5, 2011
Tatamagouche Nova Scotia	July 1, 2011
Tatamagouche Nova Scotia	August 1, 2011