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1 **SPATIAL AND TEMPORAL VARIATION IN MACROPARASITE**

2 **COMMUNITIES OF THREE-SPINED STICKLEBACK.**

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7   **SUMMARY**

8   Patterns in parasite community structure are often observed in natural systems and an important

9   question in parasite ecology is whether such patterns are repeatable across time and space. Field

10   studies commonly look at spatial or temporal repeatability of patterns, but they are rarely

11   investigated in conjunction. We use a large data set on the macroparasites of the three-spined

12   stickleback, *Gasterosteus aculeatus* L., collected from 14 locations on North Uist, Scotland

13   over an eight year period to investigate: 1) repeatability of patterns in parasite communities

14   among populations and whether variation is consistent across years, 2) whether variation

15   between years can be explained by climatic variation and progression of the season and 3)

16   whether variation in habitat characteristics explain population differences. Differences in

17   relative abundance and prevalence across populations were observed in a number of parasites

18   investigated indicating a lack of consistency across years in numerous parasite community

19   measures, however differences between populations in the prevalence and abundance of some

20   parasites were consistent throughout the study. Average temperature did not affect parasite

21   community and progression of the season was only significant for two of 13 community

22   measures. Two of the six habitat characteristics investigated (pH and calcium concentration)

23   significantly affected parasite presence.

24   **Key words:** stickleback; parasite community; repeatability;

25

26 **KEY FINDINGS:**

27 • Infections with some parasites differ between populations in three-spined sticklebacks

28 • Some parasite infections are consistent across years in three-spined sticklebacks

29 • Temperature showed little effect on parasites present

30 • Calcium and pH affected cestode infections

31

32

INTRODUCTION

A key goal of many scientific disciplines is the identification of general laws or principles based upon recurring and predictable patterns (Poulin, 2007). Such patterns can be used not only to formulate laws explaining observations in nature and their underlying mechanisms, but also as a basis for testable hypotheses (Lawton, 1999; Poulin, 2007). However, finding laws which can be applied in all cases is difficult in ecology as the complexity of natural systems results in identification of circumstantial patterns which are not applicable in all situations (Poulin, 2007). Many ecologists, including parasite ecologists, continue to search for repeatable patterns across time, geographical area and taxa (Poulin, 2007; Kennedy, 2009; de Roij and MacColl, 2012). There has been much uncertainty about the extent to which parasite communities are structured, as well as whether observed relationships are sustained or transient (Behnke *et al.* 2008a). Identifications of patterns in parasite occurrences may provide valuable insights into the shaping of parasite communities and interactions, as well as the dynamics of host-parasite relationships (Behnke, 2008; de Roij and MacColl, 2012).

The organization of parasite communities infecting a species is hierarchical and can be looked at on a number of levels, ranging from infracommunities, through to component communites and finally the total parasite fauna (as defined by Bush *et al.* 1997). The different ecological processes acting at different levels influence how dynamic community structure is, with the lowest levels being most subject to temporal and spatial variation (Behnke *et al.* 2008a). At the component community level, numerous factors, both extrinsic (location, year and season) and intrinsic (host age, sex and resistance), can be important contributors to fluctuations commonly observed (Abu-Madi *et al.* 1998; Behnke *et al.* 2008a). Extrinsic factors contributing to community variation have been the focus of numerous studies looking at the effects of season (Bolek and Coggins, 2000), year (Kennedy *et al.* 2001) and population heterogeneities (Calvete *et al.* 2004). Despite a large body of work looking at temporal and

58   spatial variation, it has been less common for these effects to be investigated in conjunction

59   with each other (de Roij and MacColl, 2012). Patterns observed when looking at population or

60   year/season alone provide a snapshot of community composition and, whilst they may succeed

61   in uncovering patterns in community structure, these patterns are rarely consistent when

62   spatially or temporally replicated communities are observed (González and Poulin, 2005).

63   Thus, such patterns are likely to describe characteristics of a certain population at one time and

64   place, rather than reflect the host's parasite community as a whole (Vidal-Martínez and Poulin,

65   2003). Kennedy (1997) emphasises the importance of long-term data sets in furthering our

66   understanding of parasite ecology; such data sets facilitate much needed investigation of

67   repeatability of observed patterns across space and time.

68   Factors affecting parasite distribution may be viewed at two levels: the host and the

69   environment in which the host resides (Cardon *et al.* 2011). Effects at the host level include

70   intrinsic variables, such as age, body size, genetic susceptibility and sex (Behnke *et al.* 2001;

71   Blanchet *et al.* 2009) although relative significance of each of these factors is currently unclear

72   (Wilson *et al.* 2002). To get a full understanding of parasite community dynamics, it is

73   important to consider also biotic and abiotic factors correlated with observed variation, which

74   can strongly affect community dynamics (Lively *et al.* 2014). These environmental

75   contributors relate to the habitat in which hosts and parasites live: for example, host density,

76   diet and climate (Cardon *et al.* 2011; de Roij and MacColl, 2012). These factors are suggested

77   to play a role in shaping component communities either directly, by affecting free-living

78   parasite stages, or indirectly, by affecting survival of intermediate hosts (Pietrock and

79   Marcogliese, 2003). Previous spatial and temporal studies have incorporated abiotic factors

80   into their work to determine whether they could explain variation in species richness,

81   prevalence and abundance across study sites (Marcogliese and Cone, 1996; Goater *et al.* 2005;

82   de Roij and MacColl, 2012). In this study we use the spatiotemporal variation in parasite

83 communities infecting three-spined sticklebacks, *Gasterosteus aculeatus* L (hereafter referred

84 to as stickleback), in 14 freshwater lochs on the Scottish island of North Uist to try to give

85 insight into factors contributing to this variation. It continues from work started by de Roij and

86 MacColl (2012), who found that parasite communities in 12 of these lochs remained constant

87 over a two year period (2007 and 2008), but found that these patterns could not be explained

88 by effects of limnological, physiochemical and geomorphological variation (pH, calcium

89 concentration, chlorophyll A concentration, dissolved organic carbon and loch surface area) on

90 occurrences of parasites.

91   There are numerous benefits to using the North Uist study system in assessing parasite

92 spatiotemporal variation and environmental effects. Firstly, the island has a large network of

93 lochs which, due to their geographic isolation, can be considered to contain separate

94 populations of sticklebacks, typically with high population densities making it easy to collect

95 sufficient sample sizes (de Roij and MacColl, 2012). Also, unlike many studies of spatial and

96 temporal repeatability, this system is confined to a small spatial scale. This allows comparison

97 of a number of different populations within a small geographic area, and thus a greater focus

98 on the impact of local factors (de Roij and MacColl, 2012). Since the work of de Roij and

99 MacColl 2012, further data have been collected from these populations in 2011, 2013 and 2014,

100 resulting in a large data set which will be used to investigate i) parasite community composition

101 and repeatability, ii) possible explanations behind between-year variation, based on year-to-

102 year temperature variation and seasonal impacts, and iii) whether environmental variables can

103 explain between-site variation. By considering these factors in models of parasite community

104 measures we hope to be able to identify possible mechanisms explaining patterns of variation

105 observed when looking at spatial and temporal variation.

106

107 **Mechanistic explanations of variation**

108 Climate has been found directly to affect the rate of parasite development and survival of

109 transmission stages (Chappell, 1969; Behnke *et al.* 2005). Sampling point in the season can

110 thus affect parasite occurrence, as observed by increased infection with diplostomid species in

111 late spring (Pennycuick, 1971). Therefore the average temperature and the point in the season

112 (Julian date) at which parasite data were collected were considered in analysis.

113 Six factors (geomorphological, biotic and abiotic), were included as correlates of spatial

114 variation: loch surface area, mean depth, calcium concentration (Ca^{2+} conc.), pH, log *Pungitius*

115 *pungitius* and stickleback catch rate. Previous work gives some indication that each of these

116 factors may be of importance to parasite communities. Due to the expected species area

117 relationship, loch surface area is of importance as larger water bodies would be expected to

118 contain a higher parasite species richness (Connor and McCoy, 1979; Ebert *et al.* 2001). Mean

119 loch depth is anticipated to be more important in determining measures of individual parasite

120 prevalence, as habitat use by intermediate hosts affects where parasites may be found, e.g.

121 diplostomids infect snails utilising the littoral zones and cestodes infect copepods in pelagic

122 zones (Marcogliese and Cone, 1991). Calcium concentration, which is strongly positively

123 correlated with pH, (MacColl *et al.* 2013) has been found to effect the presence of diplostomids,

124 perhaps because high calcium concentration is required to support snail intermediate hosts

125 (Curtis and Rau, 1981). Similarly, in more acidic reservoirs, perch, *Perca fluviatilis,* have

126 reduced species richness and an absence of all but one digenean species (Halmetoja *et al.* 2000).

127 *Pungitius pungitius* (nine-spined stickleback)*,* is a competitor of three-spined stickleback and

128 a potential alternative host for a number of parasites, including *Protecephalus filicollis* and

129 *Schistocephalus solidus* (Dartnall, 1973). *P. pungitius* is found in 10 of the 14 lochs

130 investigated in this study (see supplementary data Table S2) therefore, as host density can effect

131 parasite transmission, *P. pungitius* density (describing the density of nine-spined stickleback)

132 and stickleback catch rate (a proxy for stickleback density) are also taken into consideration

133 (Soleng *et al.* 1999; Arneberg, 2002).

134

MATERIALS AND METHODS

Fish populations, sampling and parasite identification

137 A total of 1,130 stickleback were collected from 14 geographically isolated, freshwater lochs

138 on North Uist, Scotland. Stickleback were sampled over a two week period during the breeding

139 season (April-May) in five years between 2007 and 2014 (no relevant samples were collected

140 in 2009, 2010 or 2012). Fish were collected using minnow traps (Gee traps, Dynamic Aqua,

141 Vancouver). In general, 20 to 30 traps were set overnight and lifted the following day, spread

142 out along the shoreline of the lochs and focussed on areas with vegetation: where sticklebacks

143 are more commonly found. Samples of at least 20 fish were selected haphazardly from those

144 caught although in some instances the samples were smaller if 20 fish were not caught.

145   Fish were transferred from traps into polystyrene boxes, with an air stone, for transport

146 and were stored in these boxes in lake water for a maximum of 48 hours. Within this time (and

147 normally within 24 hours), fish were killed and thoroughly inspected for macroparasites under

148 a dissection microscope. Parasites were identified (generally to species level using a key for

149 parasites of freshwater fish (Bykhovaskaya-Pavlovskaya *et al.,* 1946)) and recorded, along with

150 measurements of the standard length (to the nearest 0.1mm) and weight of the whole fish (to

151 the nearest 0.0001g). First the caudal, dorsal and anal fins were inspected, then the rest of the

152 body surface and the gills and the abundance of the ectoparasites present was recorded. In 2007

153 and 2008, only the left eye was removed and dissected: in all subsequent years, both eyes were

154 dissected and lens and retinal tissue inspected for parasites. Data for the left eye was strongly

155 correlated with that for both eyes combined for all three eye dwelling parasites (*Apatemon*

156 *gracilis R=0.940, p<0.001; Diplostomum gasterostei R=0.917, p<0.001; Diplostomum*

157   *spatheceum R=0.983, p<0.001)* so just left eye data is used in subsequent analysis. The body

158   cavity was opened and any parasites present in the peritoneal cavity were identified and

159   counted. Fish were labelled and preserved in 70% ethanol and dissection was completed after

160   returning to the lab in Nottingham where intestines were removed and thoroughly checked.

161   Where possible, parasites were identified to species level. Two cestodes found in the intestine,

162   *Bothriocephalus scorpii* and *Eubothrium crassum,* were generally immature and are very

163   difficult to differentiate at such an early stage in the life cycle (Andersen and Valtonen, 1990),

164   thus, they were combined and recorded as a single 'Cestoda gen. spp' count. It is likely that in

165   the present analysis of freshwater populations most of the cestodes in this grouping were *E.*

166   *crassum*, since identifiable *B. scorpii* were only ever found in stickleback in saltwater (A.D.C.

167   MacColl personal observations).

168

169   **Environmental data collection**

170   Samples of fish were collected at slightly different times each year between late April and late

171   May and year to year variation (probably in winter and spring weather) meant that the season

172   had progressed to varying extents between years. Such variation could alter the proportion of

173   fish in breeding condition, and the state of parasitic infections. To account for the extrinsic

174   factor of climatic variation between years the average temperature during the months before

175   each sample was collected were obtained via publically available Met Office UK climate data

176   (http://www.metoffice.gov.uk/climate/uk/stationdata/). Using historic station data from

177   Stornoway Airport, (located on the Isle of Lewis, Scotland, approximately 82 km from North

178   Uist), the average temperature for March and April was calculated for each year sampled.

179   Variation in point in the season at which data were collected was accounted for using the

180   variable Julian date; indicating the time elapsed since January 1st.

181   Two abiotic factors, representing the dominant axis of water chemistry on North Uist

182   (Waterston *et al*, 1979) were measured for each loch. Measurements of pH were taken between

183   April 2006 and May 2013 using a calibrated pH meter (Multi 340i, Semat International) and

184   an average was taken from three to six readings. To measure calcium concentration, filtered

185   water samples were collected in May 2011, and acidified with nitric acid before freezing and

186   returning to the University of Nottingham for analysis using inductively coupled plasma mass

187   spectrometry (ICP-MS, Thermo-Fisher XSeries[II]). Mean stickleback catch rate was measured

188   by 'catch per unit effort' (CPUE); the number of sticklebacks caught was divided by the

189   number of traps set per night. The average of these measurements was then taken for two to

190   four years between 2009 and 2013 to provide a mean stickleback catch rate. Density of the

191   competitor species *P. pungitius* was calculated as the percentage of nine-spined stickleback,

192   rather than three-spined stickleback, in a haphazard sample (minimum size = 100) of all

193   stickleback caught. An average for these percentages was then taken from three years (2010,

194   2011 and 2013) and the natural log of these percentages was used for comparisons. Loch

195   surface   area   was   estimated   using   web-based   planimeter   software

196   (http://www.freemaptools.com/area-calculator.htm) and Google Earth, and mean depth was

197   calculated from 30 readings of depth taken from a boat using a handheld depth sounder

198   (Platimo Echotest II) at various locations around lochs.

199

Methods for statistical analysis

201   In analyses of the patterns in parasite occurrence, a sample of 1130 fish was used (see

202   supplementary data Table S1 for details of samples). Data analysis was carried out using

203   computer programmes GenStat (15th edition, VSN international Ltd, Hemel Hempstead, UK)

204   and Microsoft Excel, 2010 (Microsoft Corporation, Washington, USA).

205

206 *Parasite communities: general patterns:*

207 The following summary statistics were calculated for each population/year combination in

208 order to establish general patterns of community composition: species richness, abundance and

209 prevalence of parasites (as described by Bush *et al*. (1997)). Prevalence and abundance are

210 used in conjunction because, although not completely independent, nevertheless the two

211 measures contain different information about the distribution of parasites across hosts, and

212 allow contrasting inference about the likely effect of parasites on host populations (Anderson

213 & May). As well as calculating prevalence for individual populations, presence/absence data

214 were used to calculate the overall prevalence across all populations and years in order to

215 quantify how commonly parasites occur and thus, determine which should be considered for

216 further analysis. Parasites which failed to exceed an overall prevalence of 10% were not used

217 in further analysis (MacColl, 2009). Simpson's diversity index (1-D) was also used as a simple

218 measure of diversity at the component community level (Magurran, 2004).

219

220 *Variation in abundance and individual prevalence of parasites: (i) the individual level*

221 Univariate generalised linear models (GLMs) were used to analyse parasite abundance,

222 individual prevalence and species richness at the level of the individual host. Thirteen

223 dependent variables were modelled: species richness, and the prevalence and abundance of

224 each of the six key parasite groups. Species richness was modelled using normal errors and an

225 identity link function. Parasite prevalence was modelled using binomial errors and a logit link

226 function: ('1' and '0' for infected and non-infected fish respectively). Parasite abundance was

227 modelled using negative binomial errors and a logarithmic link function. Population, year and

228 sex were included as explanatory variables for all models and standard length was fitted as a

229 covariate. In the most complex model, a population x year interaction term was included. The

230 deletion approach was used to reach a minimum adequate model, whereby the most complex

231 model was tested first and non-significant terms were sequentially removed. *P*-values were

232 corrected throughout using a sequential Bonferroni correction to account for multiple

233 comparisons. Results are displayed in tables including the estimates of coefficients for

234 continuous data.

235

236 *Variation in mean abundance and prevalence of parasites: (ii) the population level*

237 Average species richness, parasite prevalence and mean parasite abundance were modelled as

238 dependent variables across all years and all populations studied in order to find mechanistic

239 explanations for any variation observed. The 13 dependent variables remained the same but

240 distributions differed when average measurements were modelled. Prevalence was normally

241 distributed, as average prevalences approximate to a normal distribution. Average abundances

242 are not integers, therefore it was no longer appropriate to use negative binomial distribution so

243 average abundance was log transformed and a normal distribution used. In all cases, average

244 length and sex ratio were included as explanatory variables as body length is commonly

245 observed in nature to correlate with parasite presence, especially in fish (Poulin, 1997) and sex

246 can affect parasite infection (Behnke, 2008)

247

248 *Temporal climatic and seasonal effects*

249 Annual averages per population were calculated for parasite community measures, temperature

250 and Julian date. GLMs were used to model annual averages of dependent variables for each

251 population against average temperature and Julian date to look for the effects of climate and

252 season, respectively.

253

254 *Spatial environmental effects*

255 To identify mechanistic explanations for variation between populations, GLMs were used to

256 model overall population averages across all years of dependent variables against

257 environmental variables. Mean pH, calcium concentration, stickleback catch rate and log

258 (relative density *P. pungitius)* were used as explanatory variables in all models and loch surface

259 area was included for community measures (species richness), whilst mean loch depth was

260 used for parasite measures (abundance and prevalence). These two different measures were

261 used because the area of water bodies has previously been shown to impact the number of

262 parasite species present (Ebert *et al.* 2001) and different parasites can be found in different

263 depths of water (Marcogliese and Cone, 1991).

264

265 **RESULTS**

266 **Parasite communities**

267 The component community of macroparasites infecting *G. aculeatus* consisted of 12 parasites

268 (Table 1) with a total of 78% of fish being infected with at least one parasite (*n*=878 out of

269 1130). Prevalence was calculated across all populations and years in order to identify

270 commonly occurring parasites (Table 1). Seven parasite taxa were found to exceed 10%

271 prevalence across samples: the crustacean *Thersitina gasterostei,* the monogenean

272 *Gyrodactylus arcuatus,* the trematodes *Diplostomum gasterostei* and *Apatemon gracilis* and

273 the cestodes *Schistocephalus solidus* and *Proteocephalus filicollis,* and the group 'Cestoda gen.

274 spp', consisting of *Bothriocephalus scorpii* and *Eubothrium crassum.*

275 Most of these parasites are described as common parasites and considered for further

276 analysis. *Thersitina gasterostei* only occurred in three populations and was found in fewer than

277 20% of 57 population samples collected from different lochs across five years, so was not

278 included in further analysis.

279

Parasite communities: general patterns

281 Overall infection levels calculated across all years for each individual population were

282 generally high: seven of the 14 populations had more than 80% of fish infected with at least

283 one parasite (Figure 1) and only one population had fewer than 60% of fish infected (Daim,

284 31.25%). Furthermore, infracommunities consisting of more than one parasite were found to

285 be very common (Figure 1): three populations showed a large proportion (>80%) of fish were

286 infected with at least two parasites (Gill, 94%; Host, 83.5% and Reiv, 94.2%) and a further

287 four had over 50% of fish infected with multiple parasites (Buai, 57.0%; Chru, 54.8%; Maga,

288 63.6% and Mora, 56.9%). Two populations (Gill, 84% and Reiv, 81.2%) showed a large

289 proportion of fish infected with at least three parasites. Mean species richness, calculated for

290 each population, ranged from 0.45±0.88 (Daim) to 3.7±1.17 (Gill) (Figure 2a). Parasite

291 diversity (1-D) did not vary significantly between years and populations (Figure 2b, F=1.19, d.

292 f.=4, *P*=0.33; F=1.30, d. f.=13, *P*=0.252, respectively).

293

Variation in abundance and individual prevalence of parasites (i) the individual host level

295 GLMs of the abundance and prevalence of parasites in individual hosts revealed some common

296 patterns. Length had a significant effect on species richness, prevalence of all parasite species,

297 apart from the three cestodes (*S. solidus, P. filicollis* and Cestoda gen. spp)*,* and abundance of

298 all parasites apart from *G. arcuatus* and *S. solidus* (Table 2). Correlations were positive for all

299 parasites, apart from *P. filicollis,* indicating a greater prevalence and higher abundance of

300 parasites in larger fish (Table 2). Sex did not generally explain a significant proportion of the

301 variance in either parasite abundance and prevalence, although it was significant in predicting

302 the prevalence and abundance of *S. solidus*, both of which are greater in males than in females

303 (Table 2). The sex ratio of samples collected ranged between 0.30 and 0.62 and all but two

304   population samples were female biased. Buai had more males (sex ratio = 0.62) and Chru had

305   equal numbers of males and females.

306   There was significant variation between populations for all response variables (Table 2).

307   Parasite species richness, abundance and prevalence also varied between years, except for the

308   prevalence and abundance of *P. filicollis* and the prevalence of *G. arcuatus* (Table 2). The year

309   x population interaction term was significant in a number of models: species richness,

310   abundance (except for *P. filicollis* which was consistently very low in the majority of

311   populations, see below (Figure 3a)) and prevalence of *D. gasterostei* and *A. gracillis* (Table 2)

312   were all significant, indicating that variation was not completely consistent within populations

313   across years in these instances. This makes the interpretation of patterns of spatiotemporal

314   variation difficult, but this can be clarified through the use of figures.

315   For example, the prevalence of *P. filicollis* was consistently below 20% in the majority

316   of populations except Host, Chru and Maga, where, despite fluctuations, prevalence was

317   constantly high (Figure 3a). Trends can also be observed in the prevalence of Cestoda gen. spp.

318   Again there are populations with consistently low prevalence (Figure 3b), but a peak in

319   prevalence can be observed in 2011 for multiple populations (Aroi, Daim and Scad). Aside

320   from a drop in Maga and Chru in 2013, prevalence of *D. gasterostei* remains consistently low

321   (below 50%) in numerous populations, whilst maintaining at high prevalence in a number of

322   others (Figure 3c). In terms of abundance, *P. filicollis* was rare in most populations with high

323   counts only observed in Host (Figure 4a). *S. solidus* was rare or absent in many populations,

324   but was consistently present in others (Bhar and Host). It showed a gradual increase across

325   years in Host and a general trend appears to be an increase in later years samples (Figure 4b).

326

327   *Variation in mean abundance and prevalence of parasites (ii) the population level climatic and*

328   *seasonal effects*

329   Species richness varied greatly between populations (Table 3), as did the prevalence and

330   abundance of all species excluding Cestoda gen. spp. Temperature (in the immediately

331   preceding March and April) had no significant effect on parasites or their overall species

332   richness. However species richness increased later in the year (Julian date, Table 3) as did

333   prevalence and abundance of *G. arcuatus*.

334

335   *Environmental effects*

336   There were few significant relationships between environmental variables and overall average

337   measures of parasite occurrence for lochs (Table 4). Prevalence and abundance of *G. arcuatus*

338   was correlated with *P. pungitius* density. *S. solidus* prevalence and both prevalence and

339   abundance of Cestoda gen. spp were significantly correlated with both calcium concentration

340   and pH. All correlations with calcium concentration were positive. Abundance of Cestoda gen.

341   spp was positively correlated with pH, whereas *S. solidus* and Cestoda gen. spp prevalence

342   were negative correlated, indicating higher prevalence of these parasites in more acidic lochs.

343   A greater mean abundance of *P. filicollis* was also observed in lochs with higher calcium levels.

344

345   **DISCUSSION**

346   **General (population)**

347   Comparison of the macroparasites communities of three-spined sticklebacks collected from 14

348   populations across five years was used to look for spatiotemporal patterns in parasite

349   occurrence and suggest possible mechanistic explanations behind observed patterns. Whilst

350   variation occurred among populations, in general, infection levels were high: in half of the

351   populations observed, more than 80% of fish examined were infected with at least one parasite

352   and only one population had fewer than 60% of fish infected. Compared to other locations,

353   North Uist sticklebacks exhibit a relatively narrow range of parasite fauna (de Roij and

354 MacColl, 2012): the average species richness in the most diverse loch was 3.7 compared to a

355 mean species richness found to be as high as 5.3 in a study of four localities in the Baltic Sea

356 (Zander, 2007). Despite this, multiple infections were fairly common and in seven of the 14

357 populations over 50% of fish harboured more than one parasite. The most frequently

358 encountered macroparasites were the monogenean *Gyrodactylus arcuatus*, the trematodes

359 *Diplostomum gasterostei* and *Apatemon gracilis,* the cestodes *Schistocephalus solidus* and

360 *Proteocephalus filicollis* and the Cestoda gen. spp group, composed of larval *Bothriocephalus*

361 *scorpii* and *Eubothrium crassum*.

362

363 *Variation in abundance and individual prevalence of parasites (i) the individual host level*

364 In many previous studies, little evidence was found for repeatability in parasite community

365 patterns across space and/or time (Behnke *et al.* 2008b; Kennedy, 2009), although there are

366 instances which demonstrate some extent of repeatability in measures of parasite community

367 composition (Kennedy, 1993; Carney and Dick, 2000; de Roij and MacColl, 2012). Two long-

368 term studies have investigated parasites communities of eels (*Anguilla Anguilla*) in two English

369 rivers; River Clyst (Kennedy, 1993) and River Otter (Kennedy, 1997). Both studies considered

370 a range of community measures including species composition, richness, dominance and

371 diversity. Considerable and erratic variation was observed between years in both studies,

372 showing a lack of predictability. However changes in community diversity and dominance in

373 River Clyst were small, suggesting an underlying stability in community structure. The

374 previous study on North Uist covered two years and showed little change in the relative

375 difference in parasite community measures across populations, demonstrating short-term

376 stability in the spatial variation of macroparasite communities (de Roij and MacColl, 2012).

377 This being said, it is important to consider long-term studies in a range of locations before

378 presuming general trends in parasite communities (Kennedy, 1997).

379   The present investigation extends the research of de Roij and Maccoll (2012) to look for

380   longer term repeatability, using data from five sampling years, spanning an eight year period.

381   The present study showed less temporal stability than de Roij and MacColl (2012), however,

382   some measures of parasite community still exhibited substantial consistency across years

383   (prevalence of *G. arcuatus, S. solidus, P. filicollis* and Cestoda gen. spp and abundance of *P.*

384   *filicollis*). The consistency observed in our study indicated that, whilst we were unable to

385   identify clear and predictable patterns in parasite distribution, parasite infections are not

386   stochastic, as concluded in Kennedy (2009). Instead certain parasites are consistently more or

387   less persistent in different locations suggesting that the occurrence of parasites in fish lies

388   somewhere between random and structured communities.

389

390   Fish length accounted for some variation in most parasite measures, excluding the

391   abundance of *G. arcuatus* and *S. solidus* and prevalence of *S. solidus, P. filicollis* and Cestoda

392   gen. spp. In general, length was positively correlated with measures of parasite infection, apart

393   from *P. filicollis* abundance, with which it was negatively correlated. This is consistent with

394   previous observations regarding the association between length and parasite burden. A

395   comparison of published data comparing length and parasite species richness showed that

396   correlations between them are usually positive (Poulin, 1997). Correlations have also been

397   observed between fish length and intensity of infection with larval digenes and cestodes

398   (Poulin, 2000). There are a number of potential explanations for observed correlations between

399   body length and parasite load. Firstly, the bodies of longer fish have a greater surface area and

400   thus a larger area for parasites to infect (Arneberg *et al.* 1998). Secondly, length is usually

401   associated with the age of fish, so that longer (older) fish have had more time to become

402   infected by parasites and accumulate parasite infections (Behnke *et al.* 2001). This effect of

403   age would be more important in some lochs than others as the age structure within populations

404   varies across North Uist. Many of the lochs contain annual populations, but some lochs are

405   home to individuals living up to three years (as observed in Reiv, Maga and Mora, A. R.

406   Singkam, *unpublished data*). These lochs may therefore contain fish which have accrued

407   parasites over a number of years, possibly resulting in greater burdens in longer, older fish.

408   The negative correlation observed between fish length and the abundance of *P. filicollis*

409   is supported by early work looking at the seasonal changes in this parasite which showed that

410   smaller fish exhibited higher infection intensity (Hopkins, 1959). Such variation is suggested

411   to be as a result of different feeding habits based on the observation that smaller stomachs of

412   fish under one year old contained more zooplankton (hosts for *P. filicollis*), whereas larger fish,

413   older than one year, tended to have stomachs containing algae and chironomid larvae, thus are

414   less likely to become infected with *P. filicollis* (Hopkins, 1959). Consideration of the life cycle

415   of *P. filicollis* is consistent with this observation. Once mature, *P. filicollis* migrates to the

416   posterior end of the host intestine in order to release eggs via the anus of the host (Hopkins,

417   1959). After release of eggs, empty proglottids degenerate, until the entire worm is shed

418   (Meggit, 1914). Field studies on numerous species of *Proteocephalus* have indicated that this

419   maturation and degradation of parasites occurs within a year (Scholz, 1999) after which

420   cestodes are lost from the host. Therefore, if the diet of smaller fish increases their chance of

421   infection, these infections are not persistent enough to be observed in older, larger fish.

422   The association of parasite communities with sex of fish was less consistent, only

423   explaining variation in abundance and prevalence of *S. solidus*, whereby males were more

424   highly parasitised. This may be explained by mating characteristic of males, both behaviourally

425   and chemically (Folstad *et al.* 1994). Males attract females using bright red colouration,

426   produced by carotenoids which are acquired via consumption of carotenoid rich foods, such as

427   copepods (Ostlund-Nilsson *et al.* 2010). Copepods are also an important transmitter of a

428   number of stickleback parasites, including *S. solidus* and *P. filicollis*, thus increased secondary

429 sexual colouration also increases exposure to parasites, possibly explaining the higher rate and

430 level of *S. solidus* infection in males (Folstad *et al.* 1994). Furthermore, altered androgen

431 profiles result in immunocompromised males during the breeding season (Folstad and Karter,

432 1992) thus sex can affect parasite infection and intensity.

433   An alternative explanation is that the higher infection observed in males could be a result

434 of sampling bias based on the time of year samples were collected. During the non-breeding

435 seasons, males and females move around in shoals, however, during the mating season

436 breeding males build and defend a nest (Pressley, 1981). Samples were collected using minnow

437 traps set around the borders of the lochs, which will catch only fish found in these areas. As

438 samples were collected during the breeding season, it is likely that many breeding males would

439 have been defending nests at the time, thus samples may be biased toward females and non-

440 breeding males (Bagamian *et al.*, 2004). This may also explain, at least in part, the heavily

441 female biased sex ratios observed in data samples. It is also worth noting the hypothesis

442 proposed by Lester (1971) that *S. solidus* infected fish move into shallower waters as a results

443 of oxygen stress, so could have an increased chance of being caught in minnow traps. However,

444 this is unlikely to be a problem in North Uist as lochs are shallow and movement of water by

445 the wind means the water is well oxygenated throughout (Andrew MacColl, *personal*

446 *observations*).

447

448 *Variation in mean abundance and prevalence of parasites (ii) the population level:*

449 *Climatic and seasonal effects*

450 The strong population effects observed for the majority of parasite measures (excluding

451 abundance and prevalence of Cestoda gen. spp) is consistent with our finding that infection

452 with some parasites differs between populations. Although temperature variation had no effect

453 on the parasites present, the time in the year at which samples were collected did affect the

454   species richness and both prevalence and abundance of *G. arcuatus*, all of which increased

455   when samples were collected at a later point in time between late April and late May.

456   *Gyrodactylus salaris,* a gyrodactylid infecting Atlantic salmon (*Salmo salar*), was observed by

457   Appleby and Mo (1997) to demonstrate seasonal patterns: infection levels were lowest in

458   winter and early spring (following low water temperatures) and increased throughout spring.

459   This is consistent with our findings of greater *G. arcuatus* infection later in the season.

460

461   *Environmental effects*

462   Nine-spined stickleback density was found to be positively associated with the prevalence and

463   abundance of *G. arcuatus.* This is consistent with previous findings of increased transmission

464   of gyrodactylid species, such as *G. salaris*, which are able to infect both hosts when high

465   densities of both three-spined and nine-spined stickleback are found (Soleng *et al.* 1999).

466   Alternatively, it may be that presence of nine-spined stickleback is indicative of some

467   unmeasured aspects of water chemistry which are favourable to both *Gyrodactylus* and *P.*

468   *pungitius* (MacColl et al 2013).

469   Other environmental variables which correlated with parasite occurrence were pH and

470   calcium concentration. Calcium concentration is commonly found to affect the presence of

471   digenean parasites, for example, Curtis and Rau (1980) found calcium concentration to be

472   associated with *Diplostomum* sp. distribution, as their life cycle requires snail hosts which use

473   calcium for shell production (Cribb *et al.* 2003). Marcogliese and Cone (1996) observed a

474   similar effect of pH on digenes infecting American eels (*Anguilla rostrata*) which were absent

475   from rivers with a pH too low to support their molluscan intermediate host. Therefore it is

476   surprising that the calcium concentration and pH did not explain variation of Digenea between

477   populations. However, calcium concentration was positively correlated with *S. solidus, P.*

478   *filicollis* and Cestoda gen. spp prevalence, as well as Cestoda gen. spp abundance. There was

479   little support for these findings in the literature, as calcium concentration is not commonly

480   found to affect the occurrence of cestodes. We considered the possibility that calcium may be

481   correlated with another variable which could affect the presence of cestodes, perhaps by

482   influencing the presence of copepod intermediate hosts, but this remains an area which will

483   require further study. pH was positively correlated with the abundance of Cestoda gen. spp:

484   this positive correlation for both calcium concentration and pH observed for this variable is

485   consistent with the findings of MacColl *et al.* (2013). A more surprising result was the negative

486   correlation observed between pH and the prevalence of *S. solidus* and Cestoda gen. spp.

487   *Bothriocephalus claviceps* and *Proteocephalus microcephalus* have both previously been

488   identified in freshwater American eels (*Anguilla rostrata*) living in rivers with pH 4.7-5.0,

489   demonstrating that cestodes are suited to living in harsh water environments (Marcogliese and

490   Cone, 1996). However, these environmental results are puzzling as pH and calcium

491   concentration are usually positively correlated due to dissolved alkaline metals increasing the

492   pH of water (MacColl *et al.* 2013). Thus, one would expect calcium and pH to both be either

493   positively or negatively correlated with parasites, rather than show an inverse relationship. A

494   study by Fryer (1980) found that more acidic lakes were associated with a decreased diversity

495   of crustacean species. It is possible that species able to transmit these cestodes are more suited

496   to survival in acidic lochs than other crustaceans, increasing the chance of sticklebacks

497   consuming infected prey. This idea could be explored with analysis of zooplankton present in

498   lochs.

499   This study successfully identifies some level of repeatability in parasites infecting North

500   Uist sticklebacks. Although a number of parasites differ in relative abundance and prevalence

501   across the years, consistency was identified with regards to differences between populations in

502   the prevalence of *G. arcuatus, S. solidus, P. filicollis* and Cestoda gen. spp and abundance of

503   *P. filicollis* throughout the study, indicating that parasite occurrence is not fully stochastic.

504   Variation in temperature and season had very little effect on parasite distributions but some

505   correlation was identified between parasites and abiotic environmental factors.

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666 **TABLES AND FIGURES**

667 **Table 1:** Prevalence of all macroparasites of three-spined stickleback *(n=1130)* identified in

668 14 populations on the Scottish island of North Uist across an eight year period.

669 * parasites exceeding average 10% prevalence across all populations and years sampled

670 which were considered in further analysis

671 † This group consists of two species of cestodes found in the intestine *Eubothrium crassum*

672 and *Bothriocephalus scorpii* which were generally too immature to differentiate. In these

673 freshwater populations, the majority of these cestodes are likely to be *E. crassum*, since

674 identifiable *B. scorpii* have only been found in stickleback living in saltwater on North Uist.

Parasite	No. of fish infected	% Prevalence
Monogenea		
Gyrodactylus arcuatus	325	25.66 *
Digenea		
Diplostomum gasterostei	505	44.43 *
Diplostomum spathaceum	55	4.78
Apatemon gracilis	273	24.07 *
Podocotyle sp.	2	0.18
Arthropoda		
Thersitina gasterostei	136	12.04 *
Nematode		
Nematoda gen. sp.	7	0.62
Cestodes		
Diphyllobothrium sp.	98	8.67
Schistocephalus solidus	205	17.97 *
Proteocephalus filicollis	175	14.60 *
Cestoda gen. spp†	124	10.97 *

675

676   **Table** 2**:** Associations between measures parasite occurrence in individual three-spined sticklebacks on North Uist, and extrinsic (year, population)

677   and intrinsic (length, sex) factors, using GLM analysis. N =1130. 'Population' was associated with 13 df, 'year' with 4 df, population x year with

678   39 df, and both 'sex' and 'length' with 1 df. Probability values associated with model: ***=$P<0.001$, **=$P\leq0.01$, *=$P\leq0.05$. 'Estimate' refers to

679   1) the estimated parameter of the effect of length, as given by the GLM to reflect a coefficient of the data and 2) the estimated parameter of the

680   effect of sex, males relative to females

681

	Population		Year		Year*population		Length		Sex	
	χ^2	*P*	χ^2	*P*	χ^2	*P*	Estimate ± S.E.	*P*	Estimate ± S.E.	*P*
Parasite species richness	459.9	***	92.2	***	138.78	***	0.0446 ± 0.0659	***	-	-
G. arcuatus abundance	359.5	***	162.9	***	88.52	***	-	-	-	-
G. arcuatus prevalence	74.69	***	-	-	-	-	0.0653 ± 0.0148	***	-	-
D. gasterostei abundance	524.7	***	75.7	***	178.85	***	0. 0700 ± 0.0072	***	-	-
D. gasterostei prevalence	262.41	***	28.69	***	67.46	**	0.0868 ± 0.0156	***	-	-
A. gracilis abundance	270.2	***	84.1	***	123.48	***	0.0636 ± 0.0104	***	-	-
A. gracilis prevalence	177.37	***	30.99	***	62.39	**	0.0788 ± 0.0162	***	-	-
S. solidus abundance	328	***	99.1	***	91.78	***	-	-	0.381 ± 0.131	**

S. solidus prevalence	139.02	***	33.98	***	-	-	-	-	0.639 ± 0.205	**
P. filicollis abundance	565.6	***	-	-	-	-	-0.0494 ± 0.0142	***	-	-
P. filicollis prevalence	148.9	***	-	-	-	-	-	-	-	-
Cestoda gen. spp abundance	329.5	***	304.6	***	66.86	**	0.0653 ± 0.0184	***	-	-
Cestoda gen. spp prevalence	54.92	***	37.98	***	-	-	-	-	-	-

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683

684 **Table 3:** Associations between measures of average annual parasite occurrence in three-spined sticklebacks on North Uist, and extrinsic

685 (population, temperature, Julian date) and intrinsic (average length, sex ratio) factors, using GLM analysis for species richness, abundance and

686 prevalence of *G. arcuatus, D. gasterostei, A. gracilis, S. solidus, P. filicollis* and Cestoda gen. spp. Sample size=57 lake+year combinations, based

687 on 1130 fish. Population is associated with 13 df, all other variables are associated with 1 df. Probability values associated with model:

688 ***=$P<0.001$, **=$P\leq0.01$, *=$P\leq0.05$ before correction, significance value α ($P=0.05$) corrected using sequential Bonferroni correction (c=5).

689 'Estimate' refers to the estimated parameter of the effect of mean length, temperature and Julian date as given by the GLM.

	Population		Temperature	Julian date		
	Wald F	*p*	*p*	Estimate±S.E.	Wald F	*p*
Species richness	15.7	***	-	0.023±0.007	10.4	**
log(G. arcuatus abundance+1)	3.6	***	-	0.017±0.006-	7.5	**
G. arcuatus prevalence	6.4	***	-	0.75±0.26	8.4	**
log(D. gasterostei abundance+1)	8.6	***	-	-		-
D. gasterostei prevalence	14.3	***	-	-		-
log(A. gracilis abundance+1)	3.8	***	-	-		-
A. gracilis prevalence	6.6	***	-	-		-
log(S. solidus abundance+0.1)	6.8	***	-	-		-

S. solidus prevalence	4.6	***	-	-	-
log(P. filicollis abundance+0.1)	14.1	***	-	-	-
P. filicollis prevalence	12.7	***	-	-	-
Cestoda gen. spp abundance	-	-	-	-	-
Cestoda gen. spp prevalence	-	-	-	-	-

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Table 4: Associations between measures of annual averages of parasite occurrence in threespined sticklebacks on North Uist, and environmental factors, using GLM analysis for species richness, abundance and prevalence of *G. arcuatus, D. gasterostei, A. gracilis, S. solidus, P. filicollis* and Cestoda gen. spp. Sample size=14 populations using data from 1130 fish. All variables are associated with 1df. Probability values associated with model: Probability values associated with model: ***=$P<0.001$, **=$P\leq0.01$, *=$P\leq0.05$ before correction, significance value $\alpha(P=0.05)$ corrected using sequential Bonferroni correction (c=7). 'Estimate' refers to the estimated parameter of the effect of pH and calcium concentration, as given by the GLM. Shaded cells indicate comparisons absent from the model.

	Surface area	Mean depth		Mean pH		Ca^{2+} concentration		Stickle-back density	P. pungitius density	
	p	Estimate ± S.E	p	Estimate ± S.E	p	Estimate ± S.E.	p	p	Estimate ± S.E.	p
Log(Species richness)	-	N/A	N/A	0.40±0.15	*	-	-	-	-	-
G. arcuatus abundance	N/A	-	-	-	-	-	-	-	0.25 ± 0.09	*
G. arcuatus prevalence	N/A	-	-	-	-	-	-	-	0.07 ± 0.03	*
D. gasterostei abundance	N/A	-	-	-	-	-	-	-	-	-
D. gasterostei prevalence	N/A	-	-	-	-	-	-	-	-	-

A. gracilis abundance	N/A	-	-	-	-	-	-	-	-	-
A. gracilis prevalence	N/A	-0.10 ± 0.04	*	-	-	-	-	-	-	-
log(S. solidus abundance)	N/A	0.70±0.30	***	-	-	-	-	-	-	-
log(S. solidus prevalence)	N/A	-	-	-2.05±0.59	**	8126±2455	**	-	-	-
log(P. filicollis abundance)	N/A	-	-	-	-	-	-	-	-	-
log(P. filicollis prevalence)	N/A	-	-	-	-	3776±1409	*	-	-	-
log(Cestoda gen. spp abundance+0.1)	N/A	-	-	1.24±0.39	**	6529±1615	**	-	-	-
Cestoda gen. spp prevalence	N/A	-	-	-0.15±0.05	**	717±194	**	-	-	-

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699   **Figure 1:** Percentage of three-spined stickleback in 14 North Uist lochs infected with at least

700   one (white), two (grey) and three (black) parasites (± 95% confidence interval).

701

702   **Figure 2**:Variation in diversity of parasites of three-spined stickleback in 14 different lochs

703   on North Uist. a) Average species richness per population (±S.E.); b) Simpson's diversity

704   index.

705

706   **Figure 3**: Year to year variation in prevalence of three parasites of three-spined stickleback in

707   eight populations from North Uist: a) *P. filicollis,* b) Cestoda gen. spp and c) *D. gasterostei*.

708   For all figure components: Aroi ————— ; Bhar —×— ; Chru ····+····; Daim —◇— ; Host —▲—

709   ; Maga —□— ; Scad ━━━━; Torm ━ ● ━ . Only eight populations of the 14 studied are

710   shown, to increase clarity. These eight represent the range of variation in all 14.

711

712   **Figure 4:** Year to year variation in abundance of two parasites of three-spined stickleback in

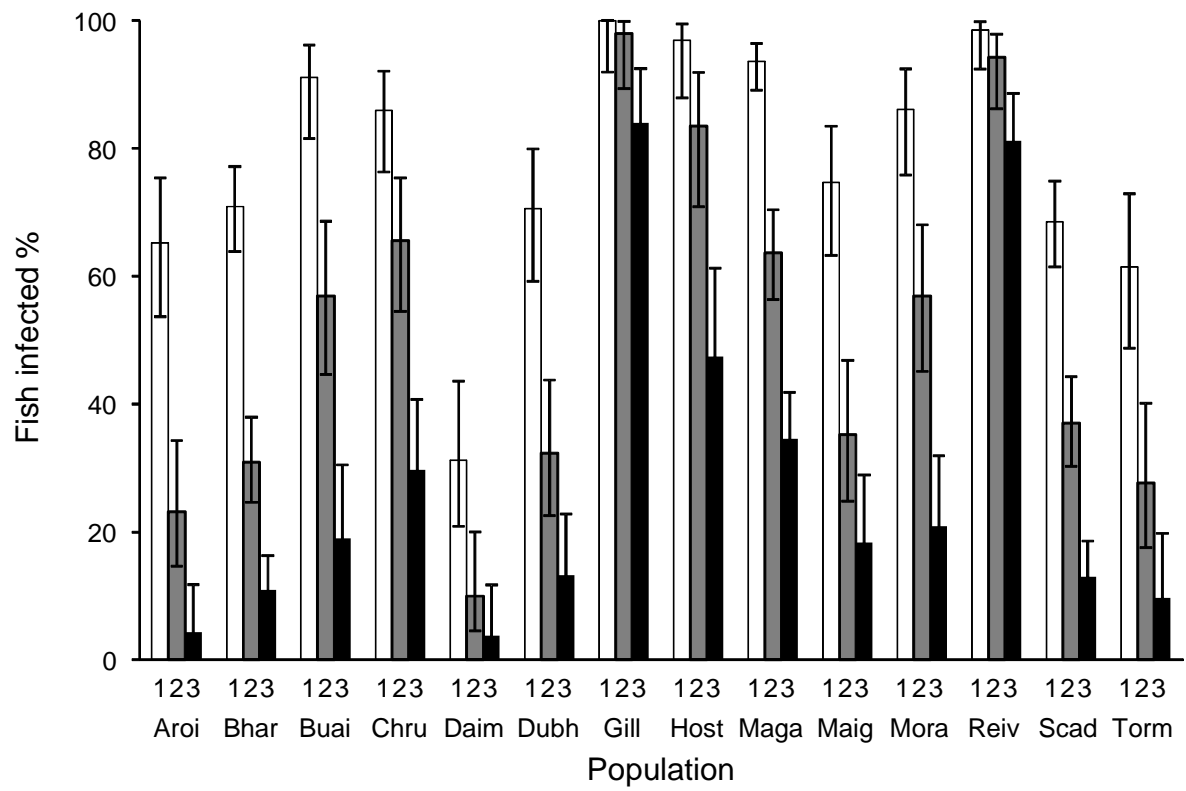713   8 populations from North Uist illustrating: (a) a rise in *S. solidus* abundance and (b)

714   consistently low abundance of *P. filicollis*. For all figure components: Aroi ————— ; Bhar —×—

715   ; Chru ····+···· ; Daim —◇— ; Host —▲— ; Maga —□— ; Scad ━━━━; Torm ━ ● ━ . Only

716   eight populations of the 14 studied are shown, to increase clarity. These eight represent the

717   range of variation in all 14.

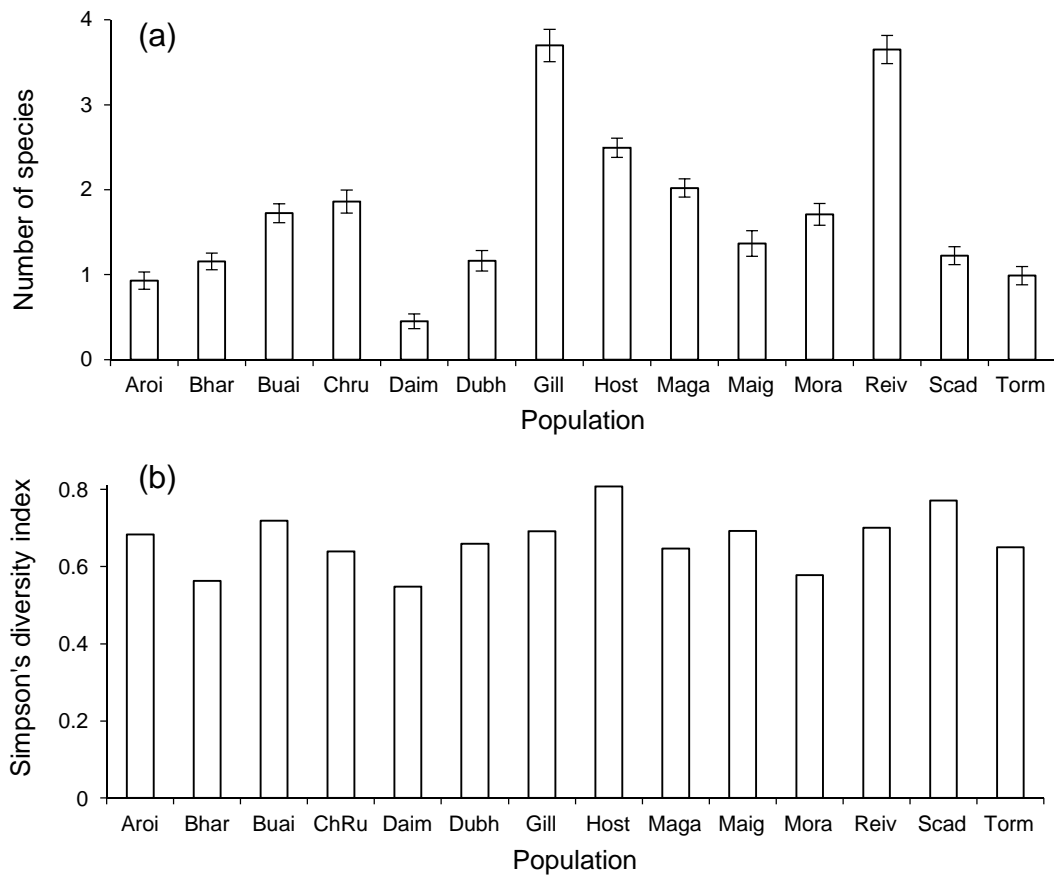718

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720   Fig. 1
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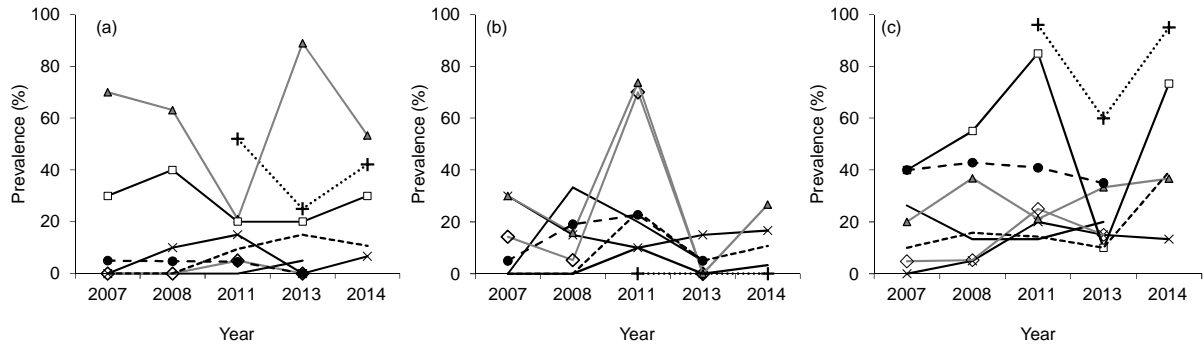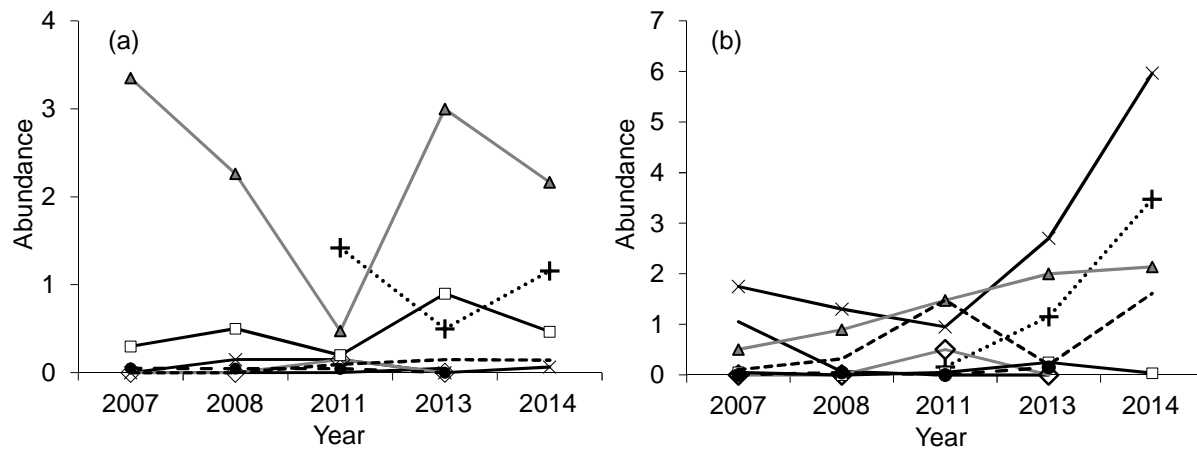
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724   Fig. 2

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730   Fig. 3

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