Article:

1   **Urban ponds as an aquatic biodiversity resource in modified landscapes**

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27 **Abstract**

28 Urbanization is a global process contributing to the loss and fragmentation of natural habitats.

29 Many studies have focused on the biological response of terrestrial taxa and habitats to

30 urbanization. However, little is known regarding the consequences of urbanization on freshwater

31 habitats, especially small lentic systems. In this study we examined aquatic macroinvertebrate

32 diversity (family and species level) and variation in community composition between 240 urban

33 and 782 non-urban ponds distributed across the UK. Contrary to predictions, urban ponds

34 supported similar numbers of invertebrate species and families compared to non-urban ponds.

35 Similar gamma diversity was found between the two groups at both family and species

36 taxonomic levels. The biological communities of urban ponds were markedly different to those

37 of non-urban ponds and the variability in urban pond community composition was greater than

38 that in non-urban ponds, contrary to previous work showing homogenisation of communities in

39 urban areas. Positive spatial autocorrelation was recorded for urban and non-urban ponds at 0-50

40 km (distance between pond study sites) and negative spatial autocorrelation was observed at 100-

41 150 km, and was stronger in urban ponds in both cases. Ponds do not follow the same ecological

42 patterns as terrestrial and lotic habitats (reduced taxonomic richness) in urban environments; in

43 contrast they support high taxonomic richness and contribute significantly to regional faunal

44 diversity. Individual cities are complex structural mosaics which evolve over long periods of

45 time and are managed in diverse ways, promoting the development of a wide-range of

46 environmental conditions and habitat niches in urban ponds which can promote greater

47 heterogeneity between pond communities at larger scales. Ponds provide an opportunity for

48 managers and environmental regulators to conserve and enhance freshwater biodiversity in

49   urbanized landscapes whilst also facilitating key ecosystem services including storm water

50   storage and water treatment.

Introduction

51

52   Land use change has been predicted to be the greatest driver of biodiversity change in the 21st

53   century (Sala *et al*., 2000). The conversion of natural landscapes to urban areas represents a

54   common land use transition, and is a significant process contributing to the loss of freshwater

55   habitats and the degradation of those that remain, placing considerable pressure on native flora

56   and fauna (McKinney, 2002). The fragmentation of natural habitats and development of uniform

57   landscapes in urban areas has been demonstrated to cause the biotic homogenization of flora and

58   fauna through the decline and exclusion of native species by land use modification (and

59   associated anthropogenic pressures) and the establishment and spread of non-native invasive

60   species through habitat disturbance and human introductions (McKinney, 2006; Grimm *et al.,*

61   2008; Shochat *et al*., 2010). Previous research has demonstrated that high levels of urbanization

62   reduce macroinvertebrate and macrophyte species richness (e.g. in urban streams, Roy *et al.,*

63   2003; Walsh *et al.,* 2005) to the point where urban environments are viewed as 'ecological

64   deserts'; although at moderate levels of urbanization greater diversity has been recorded for plant

65   communities (McKinney *et al*., 2008). In recent decades, significant improvements to the

66   physical, chemical and ecological quality of urban freshwater ecosystems have been made in

67   economically developed nations reflecting the decline in industrial developments, improved

68   waste water treatment, and more effective environmental legislation (e.g., *The Water Framework*

69   *Directive* in Europe; EC, 2000 and *The Water Act 2007* in Australia; Commonwealth of

70   Australia, 2007). Although there have been significant improvements to the quality of many

71   urban aquatic habitats, the number of water bodies in urban areas has declined over the past

72   century (Wood *et al*., 2003; Vaughan & Ormerod, 2012; Thornhill, 2013). Commercial and

73   residential developments are expanding in urban areas to keep pace with population growth (66%

5

74   of global urban population are predicted to live in urban areas by 2050; United Nations, 2014) at

75   the expense of urban green spaces (Dallimer *et al*., 2011). Such losses of green/blue space are

76   likely to place significant pressure on remaining urban freshwaters to support native flora and

77   fauna and may lead to substantial shifts in the diversity and composition of species in urban areas

78   (Fitzhugh & Richter, 2004; McKinney, 2006).

79

80   Ponds are ubiquitous habitat features in both urban and non-urban landscapes. In non-urban

81   landscapes ponds have been demonstrated to support greater regional diversity of flora and fauna

82   compared to rivers and lakes (Davies *et al*., 2008). This biodiversity value may result from

83   spatial and temporal diversity in pond environmental variables (Hassall *et al*., 2011; Hassall *et*

84   *al*., 2012), which create a highly heterogeneous "pondscape" of habitats that provide a diverse

85   array of ecological niches. Ponds have been acknowledged as providing important network

86   connectivity across landscapes, acting as "stepping stones" that facilitate dispersal (Pereira *et al*.,

87   2011). Within urban areas, ponds provide a diverse array of habitats and occur in a wide range of

88   forms including garden ponds (Hill & Wood, 2014), sustainable urban drainage systems (SUDS;

89   Briers, 2014; Hassall & Anderson, 2015), industrial, ornamental and park ponds (Gledhill *et al*.,

90   2008; Hill *et al*., 2015), recreation and angling ponds (Wood *et al*., 2001), and nature reserve

91   ponds (Hassall, 2014) which typically display heterogeneous physicochemical conditions (Hill *et*

92   *al*., 2015). Urban ponds are almost always of anthropogenic origin and often demonstrate

93   different environmental characteristics to non-urban (semi-natural/agricultural) ponds; urban

94   ponds commonly have concrete margins, a synthetic base, reduced vegetation cover, lower

95   connectivity to other waterbodies, and are subject to run off from residential and industrial

96   developments which can greatly increase the concentration of contaminants (Hassall, 2014).

97   While the definition of a "pond" versus a "lake" is still very much debated, a general rule is that

98   ponds are standing water bodies <2ha in size. Urban waterbodies are frequently much smaller

99   (closer to 1-5m^2 for garden ponds) but show a large variation in size (>10ha for park lakes). For

100   a discussion of the definitions of ponds and lakes, we refer the reader elsewhere (Hassall, 2014;

101   Appendix 1 in Biggs et al., 2005). Despite the considerable anthropogenic pressures on urban

102   ponds, recent studies have demonstrated that ponds located within an urban matrix can provide

103   important habitats for a wide range of taxa including macroinvertebrates (Hassall, 2014;

104   Goertzen & Suhling, 2015; Hill *et al*., 2015) and amphibians (Hamer *et al*., 2012). In addition,

105   many support comparable diversity to surrounding non-urban ponds (Hassall & Anderson, 2015)

106   and also provide a wide range of ecosystems services in urban areas to offset the negative

107   impacts of urbanization (Hassall, 2014). However, these patterns are inconsistent, and other

108   studies have reported a lower diversity of macroinvertebrate and floral taxa in urban ponds

109   reflecting the greater isolation of pond habitats (Hitchings & Beebee, 1997) and management

110   practices designed for purposes other than biodiversity (e.g., emergent vegetation removal,

111   Noble & Hassall, 2014).

112

113   While there has been increasing research interest in the biodiversity and ecosystem services of

114   urban ponds across Europe (Hassall, 2014; Jeanmougin *et al*., 2014; Goertzen & Suhling, 2015),

115   the question remains as to whether urban ponds can provide similar levels of biodiversity to that

116   recorded in ponds in the wider landscape. Few studies have compared urban pond faunal

117   communities with non-urban pond communities (see Hassall & Anderson, 2015) and no known

118   studies have examined urban pond macroinvertebrate diversity at a national scale. Furthermore,

119   there are a series of ecological patterns within cities (e.g., reduced taxonomic diversity, biotic

120 homogenization, increase in non-native and invasive taxa) that have been described in terrestrial

121 systems (particularly birds, butterflies, and plants: McKinney, 2008) but these have not been

122 tested in aquatic ecosystems. This study provides a comparative analysis of environmental

123 characteristics and macroinvertebrate communities contained within >1000 UK ponds, including

124 ponds located in a number of cities and towns across the UK and non-urban ponds that cover a

125 wide range of non-urban habitats including; nature reserves, agricultural land (pasture and crop),

126 meadows, woodland and other wetlands. We test the following hypotheses (i) urban ponds

127 support lower macroinvertebrate richness and diversity (family and species level) than non-urban

128 ponds, as would be predicted from the greater anthropogenic stressors in urban areas; (ii) urban

129 macroinvertebrate communities would be more homogeneous than non-urban communities at a

130 family and species scale, due to the greater similarity of urban habitats as has been reported for

131 terrestrial taxa; and (iii) urban pond communities demonstrate stronger spatial structuring at

132 smaller scales than non-urban communities, through reduced connectivity, dispersal and gene

133 flow.

134

135 **Materials and Methods**

136 *Data Management*

137 The UK covers a total area of 242,495 km^2 and has a population of approximately 64.6 million

138 inhabitants. Over 6.8% of the UK land mass is classified as urban and approximately 80% of the

139 population resides in urban areas (defined as areas >20ha containing >20,000 people, UKNEA,

140 2011). Aquatic macroinvertebrate community data from 230 urban and 607 non-urban ponds and

141 environmental data from 240 urban ponds and 782 non-urban ponds in the UK were collated

142   from 12 previous studies (Table 1). The spatial distribution of the studied urban and non-urban

143   ponds is displayed in Figure 1.

144

Data collection methodologies employed by the majority of contributing studies (Table 1)

broadly followed the standardized guidelines of the National Pond Survey (Biggs *et al.,* 1998)

including a 3 minute sweep sample divided between the mesohabitats present (Studies 1, 2, 3, 4,

5, 6, 9, 10, 11 and 12; Table 1). The other studies also sampled for aquatic macroinvertebrate

taxa in all available mesohabitats, but sampling was undertaken until no new species were

recorded (studies 7 and 8). The majority of studies were sampled across two or three seasons

(studies 1, 3, 4, 6, 7, 10 and 11; Table 1) although five studies were only sampled during the

summer months (studies 2, 5, 8, 9 and 12; Table 1). Environmental data recorded from pond sites

varied between studies, but always included a common core of variables that were used in the

comparative analysis: pond area, pH, percentage coverage of emergent macrophytes, percentage

pond shading, and altitude. Ponds were categorized as urban or non-urban based on whether they

were located within developed land use areas (DLUAs) – a landscape designation used by the

UK-based Ordnance Survey to delineate urban and non-urban sites. We provide a comparison

between our binary categorisation and two other measures of 'urbanness' (proportion of urban

land use in a 1km buffer, and distance from urban land use areas) in the Supplementary

Information (Part 1). We acknowledge that the definition of an urban pond is complex. Indeed, a

previous attempt to define a typology of urban ponds concluded that these sites comprise a

diverse array of different habitat types (Hassall, 2014). However, the intention with this study is

to evaluate the aquatic biodiversity in urban areas, and to establish whether those urban sites are

deserving of protection, value, and enhancement. Hence, rather than attempting to define the

precise characteristics of an "urban pond", we are focusing on the much more tractable issue of

"ponds in urban areas". Similarly, the definition of a "non-urban pond" for our purposes simply

167 includes ponds outside of urban areas. Our non-urban pond dataset is concentrated in agricultural

168 landscapes which in the UK are typically characterised by low tree cover and low surrounding

169 botanical diversity, along with high inputs of nutrients and agricultural effluents. These ponds

170 are likely to be subject to "benign neglect" (i.e. limited management) but this will vary across the

171 ponds in the study. Urban ponds in this study encompass a broad spectrum of urban areas, from

172 their location in densely populated cities (e.g., Birmingham: population >1million) to smaller

173 towns (e.g., Loughborough: estimated population of 60000). The urban ponds chosen for

174 investigation included ponds in domestic gardens, industrial ponds (old mill ponds), ornamental

175 ponds located in urban parks and drainage ponds (e.g., sustainable urban drainage systems /

176 stormwater retention ponds; see Hassall, 2014). The issue of the representative nature of UK

177 cities compared to cities elsewhere (in Europe or the wider world) is less clear for ponds, since

178 there has been limited study of these habitats using standardised methods (see Hassall, 2014, for

179 a discussion and a range of biodiversity studies). It is likely that the range of urbanised areas

180 incorporated in our study covers the range of different urban landscapes that are found in

181 European cities, from millennia-old cities with an evolving land use pattern (e.g. London), to

182 centuries-old industrial towns (e.g. Leeds, Manchester), to 20th century towns which have been

183 designed and built *de novo* (e.g. Milton Keynes).

184

185 The faunal dataset was converted into a presence-absence matrix to ensure data provided by the

186 12 constituent studies were comparable and that any sampling bias was reduced. Abundance data

187 may yield additional insights into variation in biomass and evenness among ponds, and we might

188 expect greater biomass and evenness in non-urban sites where stressors are reduced and nutrient

189 supply is greater. However, our primary goal within the present study is to investigate variation

190 in taxonomic richness across the pond types. Two key methodological differences exist in the 12

191 studies. First, although most of the corresponding studies identified the majority of

192 macroinvertebrate taxa to species level, each study also identified selected taxa (e.g., Diptera,

193 Oligochaeta, Copepoda and Ostracoda) at higher taxonomic levels (Table 1). The influence of a

194 higher taxonomic resolution of identification for aquatic macroinvertebrates has been examined,

195 primarily within lotic habitats (Monk *et al.*, 2012; Heino, 2014). However, identification of

196 macroinvertebrate taxa at family level has been shown to be appropriate to examine alpha, beta

197 and gamma diversity in lentic systems (Le Viol *et al.*, 2009; Mueller *et al.*, 2013; Hassall &

198 Anderson, 2015; Vilmi *et al.*, 2016) and is the resolution used by a range of environmental

199 monitoring indices (e.g., biological monitoring working party [BMWP] and predictive system for

200 multimetrics [PSYM] scores; Environment Agency & Pond Conservation Trust, 2002) and

201 legislation (e.g., The Water Framework Directive; EC, 2000) across Europe. However, to assess

202 the sensitivity of results to taxonomic resolution we performed all analyses at two taxonomic

203 levels: first, to incorporate as many sites as possible and to ensure faunal data was comparable

204 across all studies, aquatic macroinvertebrate data were reclassified to family level and analysis

205 was undertaken at this higher taxonomic resolution. Second, statistical analysis was also

206 undertaken on a subset of urban (207 ponds) and non-urban ponds (578 ponds) where species

207 level data was available.

208

209 The second methodological variation was in the amount of sampling effort applied to the sites:

210 sampling effort was limited to 3 minutes in 10 of the studies (following standard UK sampling

211 protocols) but two studies used exhaustive sampling until no more species were found. A

212 preliminary analysis showed that, in fact, the sites sampled for 3 minutes found more taxa

213   (average of 14.7 ± 0.4 SE families, n=392 sites; average of 30.0 ± 0.9 species, n=340) than sites

214   sampled exhaustively (average of 13.6 ± 0.3 SE families, n=518 sites; average of 26.8 ± 0.6

215   species, n=518). However, this lower number of species in exhaustive samples is likely to result

216   from those sites occurring in the north of England where the regional species pool may be

217   smaller. As a result, we find no evidence of bias between the exhaustive and time-limited

218   samples. Finally, to provide the strongest possible test of the biodiversity value of urban ponds,

219   urban pond communities (at a family and species level) were compared to a subset of the non-

220   urban ponds with degraded sites excluded (leaving n=571 non-urban ponds with family level

221   data and 542 with species level data).

222

223   *Statistical Analysis*

224   Differences in environmental characteristics (pond area, percentage coverage of emergent

225   macrophytes, pH, percentage pond shading and altitude) and aquatic macroinvertebrate

226   communities at a family and species level between urban and non-urban ponds were examined.

227   All analyses were carried out in the R environment (R Development Core Team, 2013). Prior to

228   statistical analysis the data was screened to remove any missing values. Estimated gamma

229   diversity was calculated using Chao2 estimator in the vegan package in R (Oksanen *et al.,* 2015).

230   Mann-Whitney U tests were used to test for differences in alpha diversity (family and species

231   richness) between urban and non-urban ponds. To account for the fact that there were different

232   numbers of urban and non-urban sites, taxon accumulation curves were constructed by

233   randomized resampling of sites without replacement using the *specaccum* function in vegan with

234   1,000 permutations per sample size. From these curves the mean number of families and species

235   in each simulated group of sites and the standard error were calculated. Variability between

236   urban and non-urban ponds in the environmental variables was tested using Mann-Whitney U

237   tests. Differences between environmental variables and faunal community composition in urban

238   and non-urban ponds were visualized using Non-Metric Multidimensional Scaling (NMDS) with

239   the *metaMDS* function in the vegan package and were examined statistically using a

240   'Permutational Analysis of Variance' (PERMANOVA). Bray–Curtis dissimilarity was used to

241   analyse the macroinvertebrate data and Euclidean distance used for the environmental data.

242   Homogeneity of multivariate dispersions between the environmental data and macroinvertebrate

243   communities from urban and non-urban ponds were calculated using the *betadisper* function in

244   vegan and compared using an ANOVA. To identify indicator taxa of ephemeral and perennial ponds

245   Indicator Value analysis (IndVal: Dufrêne & Legendre 1997) was undertaken. To test the spatial

246   patterns of community structure in urban and non-urban ponds, a Mantel correlogram was

247   constructed between the aquatic macroinvertebrate distance matrix (Euclidean) and the

248   geographical distance for urban and non-urban ponds using the *mantel.correlog* function in the

249   vegan package in R. Breaks among distance classes in the Mantel correlogram were defined in

250   50km intervals. The Mantel correlogram enables the identification of changes in the strength of

251   correlation between faunal distance matrices and geographic distance matrices at different spatial

252   scales (Rangel *et al*., 2010).

253

254   The relationship between macroinvertebrate assemblages and environmental variables (pH,

255   percentage coverage of emergent macrophytes, percentage pond shading, altitude, location

256   within urban area, and pond area) was examined using redundancy analysis (RDA) in the vegan

257   package. A stepwise selection procedure (forward and backward selection) was employed to

258   select the best model and environmental variables that significantly ($p < 0.05$) explained the

259 variance in pond macroinvertebrate assemblages using the *ordistep* function in vegan, which

260 uses permutation-based significance tests (999 permutations).

261

262 **Results**

263 *Urban and non-urban pond environmental characteristics*

264 Comparisons between specific environmental variables in urban and non-urban ponds that are

265 thought to influence diversity and composition showed that altitude (W=108179.5 p<0.01;

266 Figure 2A) and pond shading (W=92965.5 p<0.01; Figure 2B) were significantly higher for

267 urban ponds (mean altitude: 85.9 ± 3.7 masl; mean shading 22.89 ± 1.84 %) than non-urban

268 ponds (mean altitude: 78.2 ± 2.8 masl; mean shading 19.61 ± 0.95 %), but the absolute

269 differences between the pond types are small enough that they may be biologically insignificant .

270 pH was significantly higher for urban ponds (mean 7.44 ± 0.06SE) compared to non-urban ponds

271 (7.37 ± 0.16; W=37024 p<0.05; Figure 2C) although in both pond types pH was close to neutral.

272 Non-urban ponds demonstrated a greater variability in pH compared to urban ponds. A total of

273 13% of non-urban ponds (66 ponds) recorded a pH <6.5, whilst only 4% of urban ponds (10

274 urban ponds) recorded a pH <6.5. In addition, pond area was on average 43% larger in non-urban

275 ponds (2207 ± 139m^2) compared to urban ponds (1546 ± 171m^2; W=75154.5 p<0.01; Figure 2D).

276 Emergent macrophyte coverage was significantly higher in non-urban ponds (33.10 ± 1.08%)

277 compared to urban ponds (27.77 ± 1.87%; W=81695 p<0.01; Figure 2E) although the mean

278 difference was <5%.

279

280 *Aquatic macroinvertebrate diversity*

281 Family-level gamma diversity was similar between urban (observed 96 families, Figure 3A) and

282 non-urban ponds (observed 103 families, Figure 3B), and the Chao2 estimator produced results

283 taking into account sample size that were not statistically different across the two pond types

284 (urban: 108.2, 95% CI: 91.4-125.0 families; non-urban: 107.5, 95% CI: 99.7-115.3 families). At

285 an alpha scale urban ponds (median richness = 13, range = 2-44) supported significantly greater

286 macroinvertebrate family richness compared to non-urban ponds (median richness = 12, range =

287 2-38; W=20430.5 p<0.01) although median richness values were very similar between the pond

288 types. Species-level gamma diversity was lower in urban (observed 403 species) than non-urban

289 sites (observed 473 species), but the Chao2 estimator showed that there was no significant

290 difference after controlling for the number of sites (urban: 496.6, 95%CI: 445.6-547.7 species;

291 non-urban: 572.9, 95%CI: 520.2-625.7 species). No significant difference in alpha diversity

292 between macroinvertebrate species was recorded between urban (median: 28) and non-urban

293 ponds (median 26; W=17310 p=0.507).

294

295 Urban ponds demonstrated a greater variability in alpha diversity among individual ponds at a

296 family and species level (Figure 3C, 3D). A total of 25 urban ponds (11% of total urban pond

297 number) supported >25 macroinvertebrate families, whilst only 9 non-urban ponds (1.5% of total

298 non-urban pond number) supported macroinvertebrate communities with >25 families. In

299 addition, the greatest number of invertebrate families recorded was from an urban pond (46 taxa)

300 and 5 of the 6 ponds with the greatest macroinvertebrate family richness were located in urban

301 environments. Only two families of macroinvertebrates were statistically associated with non-urban

302 ponds (one family of Plecoptera, one family of Ephemeroptera), while 20 families were identified as

303 indicator taxa for urban ponds, including seven families of Diptera. Strongest associations for families are

16

304 presented in Table 2 (see Supplementary Material Table S10 for the full list of statistically significant

305 family indicator values, and Supplementary Table S11 for significant indicator values of

306 macroinvertebrate species).

307

308 When non-urban ponds designated as degraded were removed and the macroinvertebrate

309 diversity in the remaining ponds was compared to urban ponds, alpha diversity was significantly

310 greater in urban ponds (median: 13; W=18057 p<0.01) than the higher quality non-urban ponds

311 (median: 12) at a family level, although mean and median richness values were similar between

312 the pond types (see Supplementary Information Part 2). There was no significant difference in

313 alpha diversity (W=14653.5 p=0.358) at the species level between urban ponds (median: 28) and

314 higher quality non-urban ponds (median: 25). Estimated gamma diversity for higher quality non-

315 urban ponds at a family (98.7) and species scale (575.1) was marginally higher compared to

316 gamma diversity when all non-urban ponds were considered.

317

318 Chironomidae, Tipulidae, Crangonyctidae and Oligochaeta had a greater frequency of

319 occurrence in urban ponds, whilst Gyrinidae, Hydrophilidae and Notonectidae displayed a

320 greater occurrence in non-urban ponds (Figure 4; for complete data see Tables S8 and S9 for

321 family and species level prevalence, respectively). Macroinvertebrate families that score highly

322 within biological monitoring surveys of ponds and other waterbodies (e.g., PSYM and BMWP)

323 such as Phryganeidae, Leptoceridae, Libellulidae and Aeshnidae occurred at similar frequencies

324 in the urban and non-urban ponds (Figure 4). Crangonyctidae were present in 49.0% of urban

325 ponds and only 29.0% of non-urban ponds. All specimens of this family from the species-level

326 dataset were the North American invasive *Crangonyx pseudogracilis*. A similar pattern is also

17

327 seen in the species-level dataset with the invasive New Zealand mud snail, *Potamopyrgus*

328 *antipodarum*, being found in 21.3% of urban ponds and 9.5% of non-urban ponds.

329 *Community Heterogeneity*

330 Multivariate dispersion for environmental characteristics were significantly lower in non-urban

331 ponds (median distance: 1116) than urban ponds (median distance: 1978; F=5.774 p<0.05,

332 Figure 5A). PERMANOVA showed that there was a small but significant difference between

333 environmental characteristics (R^2=0.03 p<0.001) and faunal communities at a family (R^2=0.09

334 p<0.001) and species level (R^2=0.03 p<0.001). A relatively clear distinction between aquatic

335 macroinvertebrate community composition in urban and non-urban ponds was observed at the

336 family and species level within the NMDS ordination (Figure 5B, C). Among faunal

337 communities, multivariate dispersion was significantly higher at the family (median distance -

338 urban: 0.451, non-urban: 0.406; F=27.584 p<0.01) and species scale (median distance - urban:

339 0.579, non-urban: 0.550; F=17.626 p<0.01) for urban ponds compared to non-urban ponds.

340

341 There was significant positive spatial autocorrelation for urban (*r*=0.31 p<0.01) and non-urban

342 ponds (*r*=0.17 p<0.01) at the family level for the smallest distance class (0-50 km), indicating

343 that those ponds in close geographical proximity have similar macroinvertebrate community

344 compositions (Figure 6A). At middle distance classes (distance class three: 100-150 km) urban

345 and non-urban ponds demonstrated a significant negative Mantel spatial autocorrelation,

346 although this effect was weak for non-urban ponds (urban: *r*=-0.18 p<0.01, non-urban: *r*=-0.05

347 p<0.01) (Figure 6A). At larger distances spatial autocorrelation declined in strength for urban

348 and non-urban ponds. The same analyses carried out on species-level data showed similar spatial

349   patterns, but with stronger positive correlation at shorter distances (0-50km, urban: r=0.45,

350   p<0.01; non-urban: r=0.27, p<0.01) and stronger negative correlation at middle distances (100-

351   150km, urban: r=-0.29, p<0.01; non-urban: r=-0.08, p<0.01; Figure 6B).

352

353   *Macroinvertebrate - environment relationships*

354   Redundancy Analysis (RDA) of the pond macroinvertebrate family community data and

355   environmental parameters highlighted clear differences between urban and non-urban ponds

356   (Figure 7A). The RDA axes were highly significant (F=3.06 p<0.001, Adjusted R^2=0.02),

357   explaining 3.8% of the variation in family assemblage on all constrained axes (see

358   Supplementary Information Table S4). Stepwise selection of environmental parameters identified

359   four significant physicochemical variables correlated with the first two RDA axes: altitude,

360   emergent macrophytes (all p<0.05), surface area and location within urban area (both p<0.01)

361   (Figure 7A). RDA indicated that urban and non-urban pond invertebrate communities were

362   separated on the first and second axes along gradients associated with pond surface area and

363   emergent macrophyte cover/their location within the urban landscape (Figure 7A). Non-urban

364   ponds were characterized by a greater pond area and emergent macrophyte cover, whilst urban

365   ponds were associated with smaller surface areas and less emergent macrophytes (Figure 7).

366   RDA of pond macroinvertebrate species community data showed similar patterns: urban and

367   non-urban ponds were strongly separated along the first RDA axis, with significant effects of

368   urbanisation, pond area, altitude, and shading on community structure (Figure 7B). However, in

369   both RDA analyses the explanatory power of the models was very low (see Supplementary

370   Information Table S4).

371

Discussion

Urban freshwater diversity

This is the first study to provide a large scale, inter-city approach to test the biological response of entire pond macroinvertebrate communities to urbanization. The results provide a contrast with previous work on terrestrial and lotic habitats which has shown greater fragmentation, reduction in habitat quality (e.g., pollution/contaminant build up), alterations to biogeochemical cycles, higher air surface temperatures, increased disturbance frequencies, proliferation of non-native taxa, biotic homogenization and an overall decline in biological richness in urban areas (e.g., McKinney, 2002; McKinney, 2006; Grimm *et al.*, 2008). The ecological consequences of urbanization for ponds do not appear to follow the same patterns identified elsewhere for terrestrial habitats.

Urban ponds and non-urban ponds support similar alpha diversity of aquatic macroinvertebrates at a family and species level (reject hypothesis 1) and estimated gamma diversity was similar at a family level, although non-urban ponds recorded higher estimated gamma diversity at a species scale. These findings are consistent with a recent study of terrestrial invertebrates that showed comparable levels of diversity of particular indicator groups inhabiting birch trees (*Betula pendula*) between urban and agricultural areas (Turrini and Knop, 2015). However, an analysis of the same dataset showed a homogenization of arboreal invertebrates within urban areas (Knop, 2016), consistent with other terrestrial ecosystem studies (McKinney, 2008) but not with our data for freshwater macroinvertebrates. The lack of agreement in ecological patterns between ponds (which, in this study, show similar patterns of diversity across urban boundaries) and lotic/terrestrial habitats (which tend to show reduced faunal richness with increasing urbanisation)

20

395 in cities may reflect the ability of pond communities to recover relatively quickly from

396 temporary anthropogenic disturbance (Thornhill, 2013). This resilience is supported by the high

397 dispersal abilities of many semi-aquatic invertebrates (Goertzen & Suhling, 2015). Despite

398 commonly occurring in clusters, ponds are discrete habitats with small catchment areas (Davies

399 *et al.,* 2008) and disturbance in one pond or its catchment has little impact on others in the

400 network cluster, whilst a single disturbance event in, for example, a river system would impact

401 an entire reach (Thornhill, 2013). Aside from rare taxa, there were few families that showed a

402 different prevalence between urban and non-urban ponds, including indicator taxa with high

403 BMWP scores (indicative of high water quality). However, there was also a higher prevalence of

404 Oligochaeta and Chironomidae in urban ponds which is consistent with historical disturbance

405 and subsequent recolonization by disturbance tolerant taxa, and higher prevalence of the invasive

406 *C. pseudogracilis* and *P. antipodarum* in urban ponds supports previous findings that urban

407 ecosystems favour the establishment of invasive species (Shochat et al., 2010).

408

409 We propose two potential explanations, which are not mutually exclusive, for the similarity

410 between urban and non-urban pond biodiversity. First, it has been estimated that 80% of ponds in

411 the wider UK landscape are in a degraded state (Williams *et al.,* 2010). Hence non-urban ponds

412 and urban ponds may be suffering from external pressures and mismanagement leading to the

413 similar alpha diversities recorded. With both pond types in degraded states the biodiversity value

414 of urban ponds must be treated with caution, as their richness is compared to similar degraded

415 non-urban ponds. However, our secondary analysis demonstrated that urban ponds still show

416 comparable biodiversity to higher quality, non-degraded non-urban ponds. Research examining

417 the diversity of high-quality urban and non-urban ponds is required to fully quantify the

418   biodiversity value of urban ponds. Second, intensive management in cities may actually promote

419   biodiversity. Whilst many ponds in non-urban areas (e.g., agricultural land) are left unmanaged,

420   neglected, and at late successional stages (Hassall *et al*., 2012; Sayer *et al.,* 2012), ponds in urban

421   areas are often managed (primarily for purposes other than biodiversity) and a wide-range of

422   successional stages are maintained. Furthermore, in many cases local residents (e.g., pond

423   warden schemes) monitor and manage large numbers of urban ponds for the benefit of ecological

424   communities, improving their habitat/water quality and promoting high biological richness

425   (Boothby, 1995; Hill *et al.,* 2015). Results from the present study show that urban areas have the

426   potential to become reservoirs of freshwater biodiversity rather than "ecological deserts", which

427   incorporate a wide range of aquatic habitats including ponds, canals, urban reservoirs and

428   wetlands (Hassall & Anderson, 2015). However, it should be noted that diversity was highly

429   variable in this study at both the family and species level of taxonomic resolution and previous

430   research has demonstrated that some urban ponds can be of low ecological quality if

431   anthropogenic stressors such as eutrophication are allowed to persist (Noble & Hassall, 2014).

432

433   Urban ponds were also characterized by contrasting values of some environmental parameters to

434   non-urban ponds. As expected, urban ponds were smaller than non-urban ponds reflecting the

435   high level of competition and the economic value of urban land. Lower emergent macrophyte

436   coverage was recorded in urban ponds compared to non-urban ponds which reflects their primary

437   function for flood water storage/water treatment and the management practices undertaken to

438   achieve this (Le Viol *et al*., 2009). Reduced emergent macrophyte cover in urban areas may also

439   be the result of public perceptions of pond attractiveness (clean, open water and surrounding

440   vegetation mown; Nassauer, 2004) which pond amenity managers aim to replicate, or other

441 management practices for amenity purposes such as angling or boating (Wood *et al.,* 2001).

442 Urban ponds were significantly more shaded than non-urban ponds, which is most likely the

443 result of urban ponds location within high density, built environments providing significant

444 additional artificial shading to that provided by trees. In addition, reduced shading of non-urban

445 ponds may be because many non-urban ponds were located in landscapes typically free of

446 shading (trees) including wetland meadows and the low numbers of trees in British agricultural

447 landscapes where many non-urban ponds are situated (however high levels of pond shading from

448 trees has been recorded in some UK agricultural areas: Sayer *et al.,* 2012).

449

450 *Community heterogeneity*

451 Small but significant differences in faunal communities (family and species) were observed

452 between urban and non-urban ponds in this study (reject hypothesis 2). Differences (albeit subtle)

453 in community composition found in the present study contrast with the findings of Hassall and

454 Anderson (2015) and Le Viol *et al.* (2009) and suggest that at greater spatial scales urban ponds

455 contribute as much to the regional biodiversity pool as non-urban ponds. The higher community

456 dissimilarity among urban ponds may reflect the different levels of disturbance and diverse

457 management practices (reflecting their primary function e.g., flood alleviation, biodiversity,

458 amenity), as well as general pond characteristics such as small catchments which result in highly

459 heterogeneous environmental conditions (greater environmental multivariate distances than non-

460 urban ponds) even in ponds that are in close proximity (Davies *et al*., 2008).

461

462   Significant positive spatial autocorrelation at the smallest distance class and significant negative

463   spatial autocorrelation at medium distances suggest that: 1) ponds within individual cities have

464   similar communities which reflect similar city-region environmental characteristics; and 2)

465   ponds at greater spatial distances from one another in different cities have increasingly dissimilar

466   communities reflecting the high variability in environmental (Heino & Alahuhta, 2015) and

467   historical factors (Baselga, 2008; Heino & Alahuhta, 2015) among cities. Spatial patterns of

468   management may influence geographical variation in community structure to a greater extent

469   than landscape connectivity, making it difficult to evaluate our third hypothesis. However, we

470   demonstrate stronger spatial structuring of urban communities at finer spatial scales, which

471   would be expected under lower connectivity. Greater connectivity in non-urban landscapes

472   enhances species movement leading to weaker spatial structuring at finer spatial scales in non-

473   urban ponds. Hence our observations support our third hypothesis, but further work is needed to

474   evaluate the consequences of spatial patterns for management. Historically, urban environments

475   were highly degraded (physically, chemically and biologically) but significant improvements to

476   urban freshwater quality have been achieved in recent decades despite urban sprawl and

477   intensification (Vaughan & Ormerod, 2012). Therefore, it is possible that cities are still being

478   recolonized by aquatic taxa from different regional species pools using different dispersal routes,

479   creating a dynamic pattern of communities.

480

481   *Conservation implications*

482   Urban ponds support relatively high alpha and gamma diversity comparable to non-urban ponds.

483   A lack of monitoring of urban freshwaters (particularly ponds that are excluded from the EU

484   Water Framework Directive) may be hiding considerably more diversity such that urban planners

24

485 fail to identify high biodiversity sites (Hassall, 2014). There is a need for a concerted,

486 comparative, empirical approach to freshwater management that incorporates biodiversity as

487 well as other ecosystem services alongside social and political considerations. Fundamental to

488 the conservation of ponds is an integrated landscape approach that recognizes the need for

489 networks of ponds (Boothby, 1997). Hence the prioritization of ponds for conservation will need

490 to take into account their location relative to other sites, requiring a complementary approach

491 that creates new habitats, improves degraded habitats, and conserves those habitats that have

492 already achieved good quality. Changes in the management of ponds more generally has led to

493 change in the environmental conditions within and around these habitats, such as the reduction in

494 riparian tree management around agricultural ponds which has consequences for light, oxygen,

495 and temperature (Sayer et al., 2013). Urban ponds are well suited to biodiversity enhancement as

496 many are sites of high diversity (Hassall, 2014) and even small changes to current management

497 strategies in urban freshwaters (e.g., the planting of native macrophytes in amenity ponds; Hill *et*

498 *al*., 2015) are likely to significantly augment biodiversity in urban landscapes. Cities are highly

499 complex, multifunctional landscapes designed primarily for anthropogenic use yet they still

500 support considerable aquatic diversity and represent scientifically and ecologically important

501 habitats.

502

503 **Acknowledgements**

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644

Table 1 – Summary table of the geographic scale, sampling methodology and taxonomic resolution of contributing studies.

Reference Number	Geographic Scale	Aquatic macroinvertebrate Sampling Methodology	Taxonomic Resolution	Taxa Included	Reference
1	UK wide n= 152	Individual ponds sampled for 3 minutes in spring, summer and autumn using a sweep sample technique. Sampling time was divided between the mesohabitats recorded in each pond.	Species, except for Oligochaeta, Diptera and small bivalves	Aquatic macroinvertebrates (water mites, zooplankton and other micro-arthropods were not included)	Biggs *et al.*, 1998
2	Dunfermline, Fife, Scotland n= 14	Individual ponds were sampled annually between 2007-2011 in the summer following the methods of the National Pond Survey.	Species, except for Oligochaeta, Ostracoda and Diptera	Aquatic macroinvertebrates	Briers, 2014
3	Leicestershire, UK n = 41	Individual ponds were sampled over spring, summer and autumn seasons. Sampling time was proportional to surface area, up to a maximum of three minutes. Sampling time designated to each pond was divided between the mesohabitats recorded.	Species, except for Diptera, Oligochaeta, Hydrachnidiae and Collembola	Aquatic macroinvertebrates (zooplankton and other micro arthropods were not included)	Hill *et al.*, 2015
4	West Yorkshire, UK n = 36	Individual ponds were sampled during the summer and autumn, following the guidelines of the National Pond Survey. In addition, soft benthic samples were taken using an Eckman Grab.	Species, except Ostracoda, Copepoda and Diptera	Aquatic macroinvertebrates	Wood *et al.*, 2001
5	Bradford, UK n = 21	Individual ponds were sampled for 3 minutes in the summer. Sampling time was divided between the mesohabitats present.	Family level	Aquatic macroinvertebrates (presence of fish and amphibians noted)	Noble & Hassall, 2014
6	Birmingham, UK n = 30	Individual ponds were sampled for 3 minutes in the spring and summer, following the guidelines of the National Pond Survey.	Species, except Diptera, Sphaeriidae and Oligochaeta	Aquatic macroinvertebrates	Thornhill, 2013

|					
7	Halton, UK n = 37	Individual ponds were sampled twice per year (summer and autumn) for 2 years. Samples were taken from all available mesohabitats using a standard pond net until no new species were recorded.	Species	Aquatic macroinvertebrates, Aquatic macrophytes, Amphibians	Gledhill *et al*., 2008
8	North West England n = 425	Samples were taken from all available mesohabitats using a standard pond net until no new species were recorded. Logs and debris was lifted to look for macroinvertebrates located beneath.	Species except Diptera, and Oligochaeta which were not examined.	Aquatic macroinvertebrates, Aquatic macrophytes, Amphibians	Pond life Project, 2000
9	Leeds, UK n = 11	Individual ponds were sampled for 3 minutes in the summer. Sampling time was divided between the mesohabitats present.	Family level	Aquatic macroinvertebrates	Moyers & Hassall unpub.
10	UK wide n = 169	Individual ponds were sampled for 3 minutes in spring, summer and autumn using a sweep sample technique. Sampling time was divided between the mesohabitats recorded in each pond.	Species, except for Oligochaeta, Diptera and small bivalves	Aquatic macroinvertebrates (water mites, zooplankton and other micro-arthropods were not included)	FHT Realising Our Potential Award dataset unpub.
11	UK wide n = 76	Individual ponds sampled for 3 minutes in spring, summer and autumn using a sweep sample technique. Sampling time was divided between the mesohabitats recorded in each pond.	Species, except for Oligochaeta, Diptera and small bivalves	Aquatic macroinvertebrates (water mites, zooplankton and other micro-arthropods were not included)	FHT Temporary Ponds dataset unpub.
12	Leeds, UK n = 10	Individual ponds were sampled for 3 minutes in the summer. Sampling time was divided between the mesohabitats present.	Family level	Aquatic macroinvertebrates	Barber & Hassall unpub.

645

646   Table 2 - Aquatic macroinvertebrate families identified as indicator taxa for urban (top 6 out of 20) and

647   non-urban ponds (the only two significant values) based on indicator value analysis (see text for details).

648   * = p<0.05, ** = P<0.01.

Non-Urban ponds	Stat	Urban ponds	Stat
Nemouridae**	0.34	Chironomidae**	0.72
Heptageniidae*	0.20	Oligochaeta**	0.69
		Crangonyctidae**	0.63
		Sphaeriidae**	0.51
		Certaopogonidae**	0.48
		Dixidae**	0.46

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Figure legends
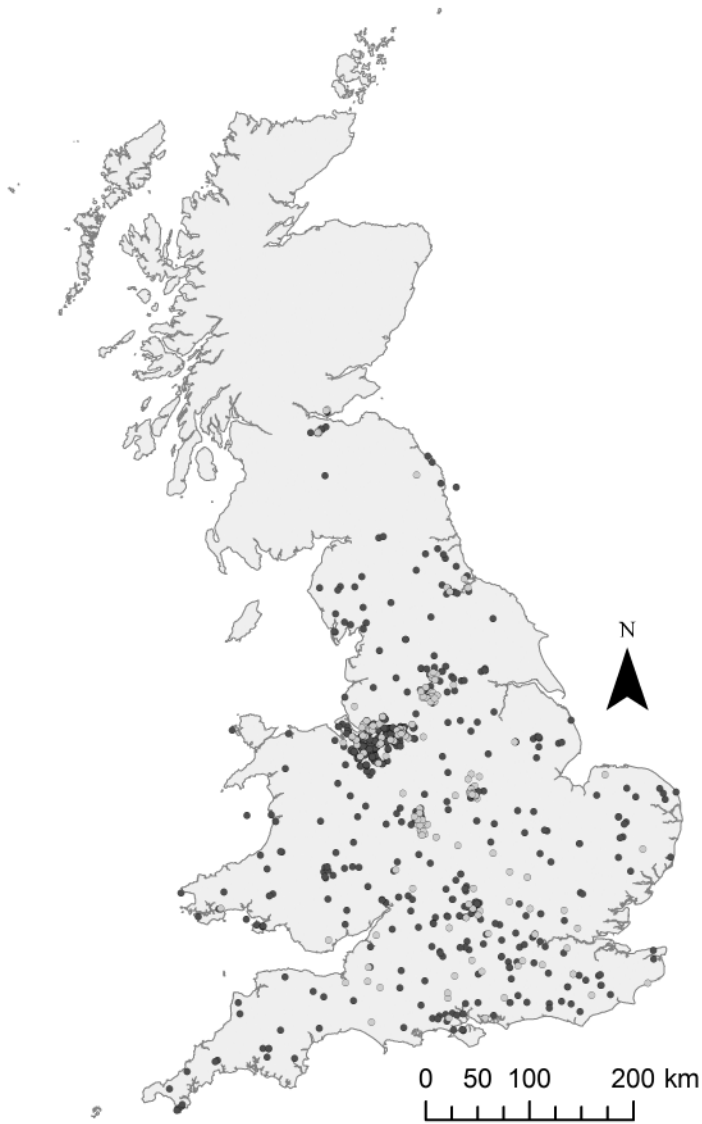
653   Figure 1 - Map of Great Britain showing the locations of the surveyed urban (light grey circles)

654   and non-urban (dark grey circles) ponds.

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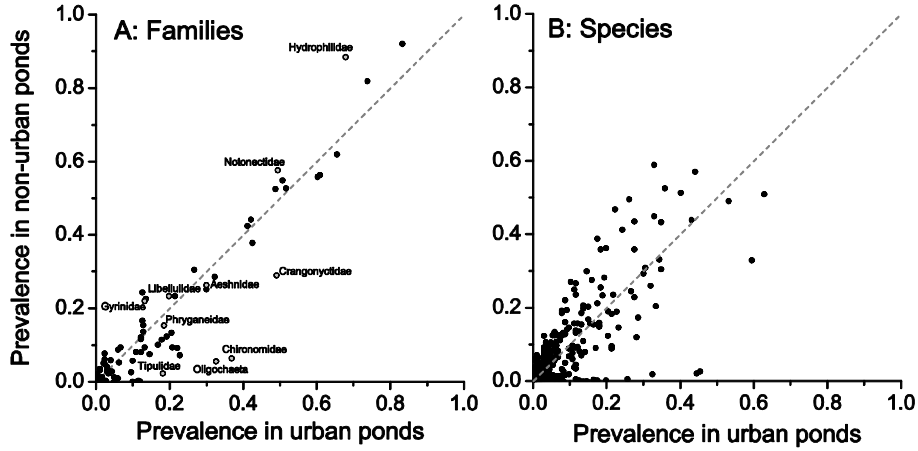
Figure 2: Comparison of environmental values between non-urban and urban ponds for (a)

altitude, (b) shading, (c) pH, (d) pond area, and (e) emergent plant cover. Each dot represents a

site, and dots are offset to illustrate multiple sites at the same value.

659
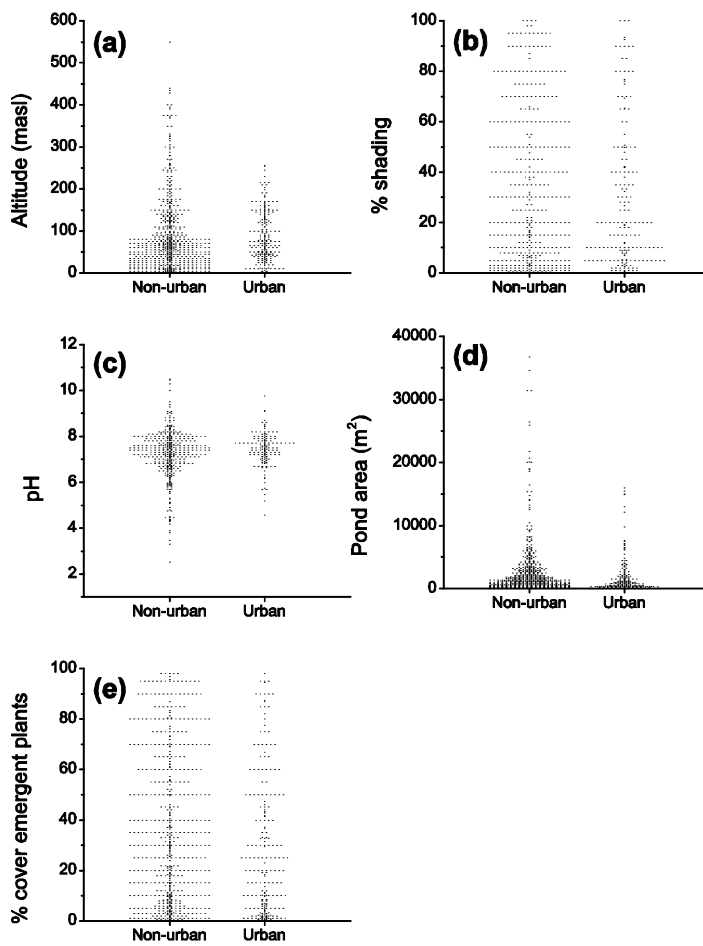
Figure 3: Species accumulation curves of family richness (a) and species richness (b): grey area

with black line = urban ponds, black area with white line = non-urban ponds, and median

macroinvertebrate family richness (c) and species richness (d) for urban and non-urban ponds.

Boxes show 25th, 50th, and 75th percentiles and whiskers show 5th and 95th percentiles.
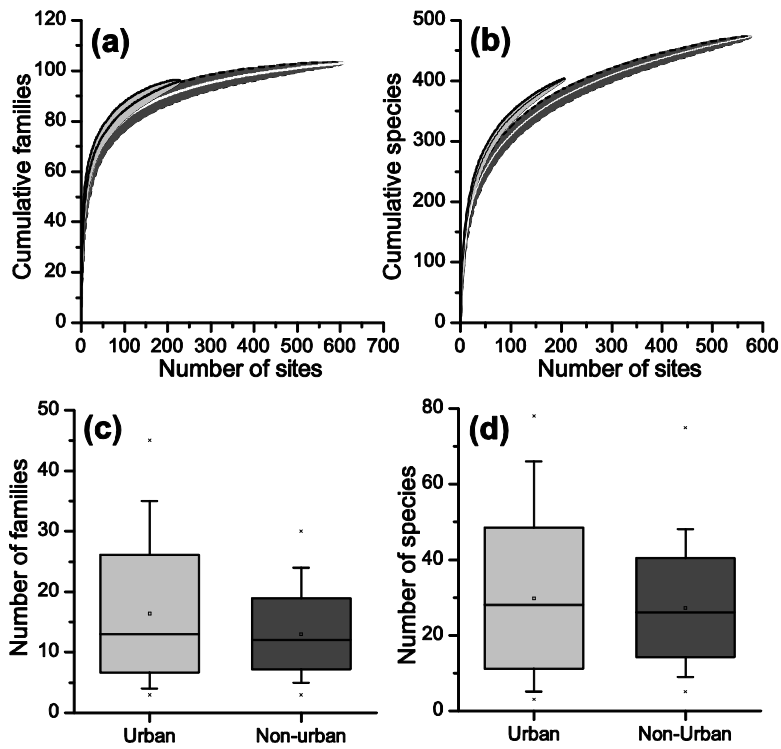
Figure 4: Prevalence of aquatic macroinvertebrate families (a) and species (b) in urban and non-urban ponds. Macroinvertebrate families listed in text are presented as grey circles and have been named (see Table S8 and Table S9 for raw data).
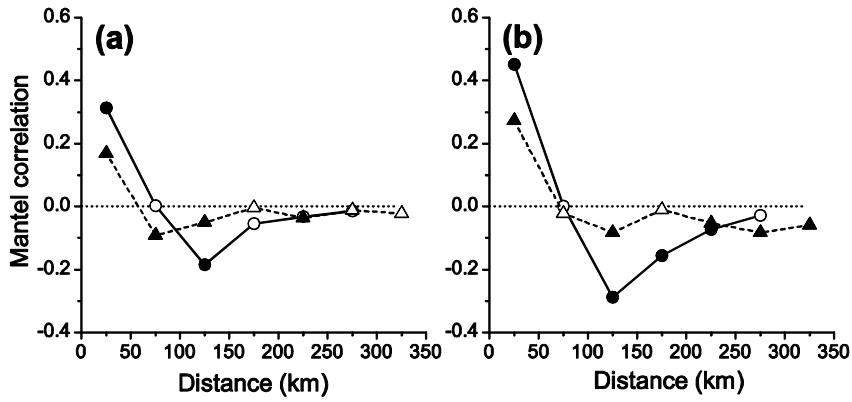
669
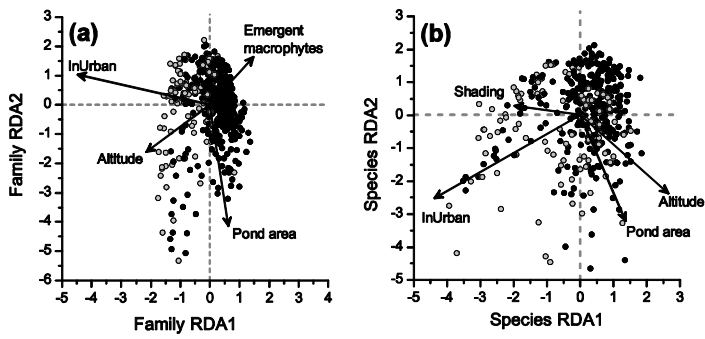
Figure 5: Non-metric multidimensional scaling plots of variation in (a) environmental variables, (b) aquatic macroinvertebrate families and (c) aquatic macroinvertebrate species from urban and non-urban ponds (light grey symbols = urban ponds and dark grey symbols = non-urban ponds).
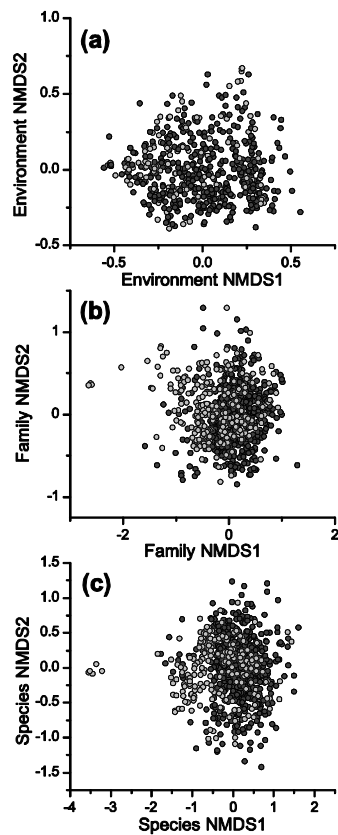
673

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676   Figure 6 - Mantel correlogram for presence-absence macroinvertebrate data at (a) family and (b)

677   species level along 50 km distance intervals (distances between pond study sites). Triangles =

678   non-urban sites, circles = urban sites. Filled symbols indicate statistically significant Mantel

679   correlations.

680

681

Figure 7 - RDA site plots of (a) family-level and (b) species-level macroinvertebrate

communities recorded from the urban and non-urban pond types studied across the UK. Only

significant environmental parameters are presented. Dark grey circles = urban ponds, light grey

circles = non-urban ponds.