1	Urban ponds as an aquatic biodiversity resource in modified landscapes
2	Running head: Macroinvertebrate biodiversity in urban aquatic ecosystems
3	Type of paper: Primary Research Article
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- 25 Keywords: urban, city, ecology, freshwater, aquatic, biodiversity, biotic homogenisation,
- 26 conservation, invertebrate.

27 Abstract

28 Urbanization is a global process contributing to the loss and fragmentation of natural habitats. Many studies have focused on the biological response of terrestrial taxa and habitats to 29 urbanization. However, little is known regarding the consequences of urbanization on freshwater 30 31 habitats, especially small lentic systems. In this study we examined aquatic macroinvertebrate diversity (family and species level) and variation in community composition between 240 urban 32 and 782 non-urban ponds distributed across the UK. Contrary to predictions, urban ponds 33 supported similar numbers of invertebrate species and families compared to non-urban ponds. 34 Similar gamma diversity was found between the two groups at both family and species 35 36 taxonomic levels. The biological communities of urban ponds were markedly different to those of non-urban ponds and the variability in urban pond community composition was greater than 37 that in non-urban ponds, contrary to previous work showing homogenisation of communities in 38 39 urban areas. Positive spatial autocorrelation was recorded for urban and non-urban ponds at 0-50 km (distance between pond study sites) and negative spatial autocorrelation was observed at 100-40 150 km, and was stronger in urban ponds in both cases. Ponds do not follow the same ecological 41 patterns as terrestrial and lotic habitats (reduced taxonomic richness) in urban environments; in 42 contrast they support high taxonomic richness and contribute significantly to regional faunal 43 diversity. Individual cities are complex structural mosaics which evolve over long periods of 44 time and are managed in diverse ways, promoting the development of a wide-range of 45 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.

51 Introduction

Land use change has been predicted to be the greatest driver of biodiversity change in the 21st 52 century (Sala *et al.*, 2000). The conversion of natural landscapes to urban areas represents a 53 common land use transition, and is a significant process contributing to the loss of freshwater 54 55 habitats and the degradation of those that remain, placing considerable pressure on native flora and fauna (McKinney, 2002). The fragmentation of natural habitats and development of uniform 56 landscapes in urban areas has been demonstrated to cause the biotic homogenization of flora and 57 fauna through the decline and exclusion of native species by land use modification (and 58 associated anthropogenic pressures) and the establishment and spread of non-native invasive 59 60 species through habitat disturbance and human introductions (McKinney, 2006; Grimm et al., 2008; Shochat et al., 2010). Previous research has demonstrated that high levels of urbanization 61 reduce macroinvertebrate and macrophyte species richness (e.g. in urban streams, Roy et al., 62 63 2003; Walsh et al., 2005) to the point where urban environments are viewed as 'ecological deserts'; although at moderate levels of urbanization greater diversity has been recorded for plant 64 communities (McKinney et al., 2008). In recent decades, significant improvements to the 65 physical, chemical and ecological quality of urban freshwater ecosystems have been made in 66 economically developed nations reflecting the decline in industrial developments, improved 67 waste water treatment, and more effective environmental legislation (e.g., The Water Framework 68 Directive in Europe; EC, 2000 and The Water Act 2007 in Australia; Commonwealth of 69 Australia, 2007). Although there have been significant improvements to the quality of many 70 71 urban aquatic habitats, the number of water bodies in urban areas has declined over the past century (Wood et al., 2003; Vaughan & Ormerod, 2012; Thornhill, 2013). Commercial and 72 73 residential developments are expanding in urban areas to keep pace with population growth (66%

of global urban population are predicted to live in urban areas by 2050; United Nations, 2014) at
the expense of urban green spaces (Dallimer *et al.*, 2011). Such losses of green/blue space are
likely to place significant pressure on remaining urban freshwaters to support native flora and
fauna and may lead to substantial shifts in the diversity and composition of species in urban areas
(Fitzhugh & Richter, 2004; McKinney, 2006).

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Ponds are ubiquitous habitat features in both urban and non-urban landscapes. In non-urban 80 landscapes ponds have been demonstrated to support greater regional diversity of flora and fauna 81 compared to rivers and lakes (Davies et al., 2008). This biodiversity value may result from 82 spatial and temporal diversity in pond environmental variables (Hassall et al., 2011; Hassall et 83 al., 2012), which create a highly heterogeneous "pondscape" of habitats that provide a diverse 84 array of ecological niches. Ponds have been acknowledged as providing important network 85 connectivity across landscapes, acting as "stepping stones" that facilitate dispersal (Pereira et al., 86 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 ponds commonly have concrete margins, a synthetic base, reduced vegetation cover, lower 94 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).

While the definition of a "pond" versus a "lake" is still very much debated, a general rule is that 97 ponds are standing water bodies <2ha in size. Urban waterbodies are frequently much smaller 98 (closer to $1-5m^2$ for garden ponds) but show a large variation in size (>10ha for park lakes). For 99 100 a discussion of the definitions of ponds and lakes, we refer the reader elsewhere (Hassall, 2014; Appendix 1 in Biggs et al., 2005). Despite the considerable anthropogenic pressures on urban 101 ponds, recent studies have demonstrated that ponds located within an urban matrix can provide 102 important habitats for a wide range of taxa including macroinvertebrates (Hassall, 2014; 103 Goertzen & Suhling, 2015; Hill et al., 2015) and amphibians (Hamer et al., 2012). In addition, 104 105 many support comparable diversity to surrounding non-urban ponds (Hassall & Anderson, 2015) and also provide a wide range of ecosystems services in urban areas to offset the negative 106 impacts of urbanization (Hassall, 2014). However, these patterns are inconsistent, and other 107 108 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 practices designed for purposes other than biodiversity (e.g., emergent vegetation removal, 110 111 Noble & Hassall, 2014).

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While there has been increasing research interest in the biodiversity and ecosystem services of urban ponds across Europe (Hassall, 2014; Jeanmougin *et al.*, 2014; Goertzen & Suhling, 2015), the question remains as to whether urban ponds can provide similar levels of biodiversity to that recorded in ponds in the wider landscape. Few studies have compared urban pond faunal communities with non-urban pond communities (see Hassall & Anderson, 2015) and no known studies have examined urban pond macroinvertebrate diversity at a national scale. Furthermore, 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 tested in aquatic ecosystems. This study provides a comparative analysis of environmental 122 123 characteristics and macroinvertebrate communities contained within >1000 UK ponds, including ponds located in a number of cities and towns across the UK and non-urban ponds that cover a 124 wide range of non-urban habitats including; nature reserves, agricultural land (pasture and crop), 125 meadows, woodland and other wetlands. We test the following hypotheses (i) urban ponds 126 support lower macroinvertebrate richness and diversity (family and species level) than non-urban 127 ponds, as would be predicted from the greater anthropogenic stressors in urban areas; (ii) urban 128 macroinvertebrate communities would be more homogeneous than non-urban communities at a 129 family and species scale, due to the greater similarity of urban habitats as has been reported for 130 terrestrial taxa; and (iii) urban pond communities demonstrate stronger spatial structuring at 131 smaller scales than non-urban communities, through reduced connectivity, dispersal and gene 132 flow. 133

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

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

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

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

- from 12 previous studies (Table 1). The spatial distribution of the studied urban and non-urban
- 143 ponds is displayed in Figure 1.

145	Data collection methodologies employed by the majority of contributing studies (Table 1)
146	broadly followed the standardized guidelines of the National Pond Survey (Biggs et al., 1998)
147	including a 3 minute sweep sample divided between the mesohabitats present (Studies 1, 2, 3, 4,
148	5, 6, 9, 10, 11 and 12; Table 1). The other studies also sampled for aquatic macroinvertebrate
149	taxa in all available mesohabitats, but sampling was undertaken until no new species were
150	recorded (studies 7 and 8). The majority of studies were sampled across two or three seasons
151	(studies 1, 3, 4, 6, 7, 10 and 11; Table 1) although five studies were only sampled during the
152	summer months (studies 2, 5, 8, 9 and 12; Table 1). Environmental data recorded from pond sites
153	varied between studies, but always included a common core of variables that were used in the
154	comparative analysis: pond area, pH, percentage coverage of emergent macrophytes, percentage
155	pond shading, and altitude. Ponds were categorized as urban or non-urban based on whether they
156	were located within developed land use areas (DLUAs) – a landscape designation used by the
157	UK-based Ordnance Survey to delineate urban and non-urban sites. We provide a comparison
158	between our binary categorisation and two other measures of 'urbanness' (proportion of urban
159	land use in a 1km buffer, and distance from urban land use areas) in the Supplementary
160	Information (Part 1). We acknowledge that the definition of an urban pond is complex. Indeed, a
161	previous attempt to define a typology of urban ponds concluded that these sites comprise a
162	diverse array of different habitat types (Hassall, 2014). However, the intention with this study is
163	to evaluate the aquatic biodiversity in urban areas, and to establish whether those urban sites are
164	deserving of protection, value, and enhancement. Hence, rather than attempting to define the
165	precise characteristics of an "urban pond", we are focusing on the much more tractable issue of
166	"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 botanical diversity, along with high inputs of nutrients and agricultural effluents. These ponds 169 170 are likely to be subject to "benign neglect" (i.e. limited management) but this will vary across the ponds in the study. Urban ponds in this study encompass a broad spectrum of urban areas, from 171 their location in densely populated cities (e.g., Birmingham: population >1 million) to smaller 172 towns (e.g., Loughborough: estimated population of 60000). The urban ponds chosen for 173 investigation included ponds in domestic gardens, industrial ponds (old mill ponds), ornamental 174 ponds located in urban parks and drainage ponds (e.g., sustainable urban drainage systems / 175 stormwater retention ponds; see Hassall, 2014). The issue of the representative nature of UK 176 cities compared to cities elsewhere (in Europe or the wider world) is less clear for ponds, since 177 178 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 incorporated in our study covers the range of different urban landscapes that are found in 180 181 European cities, from millennia-old cities with an evolving land use pattern (e.g. London), to centuries-old industrial towns (e.g. Leeds, Manchester), to 20th century towns which have been 182 designed and built de novo (e.g. Milton Keynes). 183

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The faunal dataset was converted into a presence-absence matrix to ensure data provided by the 12 constituent studies were comparable and that any sampling bias was reduced. Abundance data may yield additional insights into variation in biomass and evenness among ponds, and we might expect greater biomass and evenness in non-urban sites where stressors are reduced and nutrient supply is greater. However, our primary goal within the present study is to investigate variation

in taxonomic richness across the pond types. Two key methodological differences exist in the 12 190 191 studies. First, although most of the corresponding studies identified the majority of macroinvertebrate taxa to species level, each study also identified selected taxa (e.g., Diptera, 192 193 Oligochaeta, Copepoda and Ostracoda) at higher taxonomic levels (Table 1). The influence of a higher taxonomic resolution of identification for aquatic macroinvertebrates has been examined, 194 primarily within lotic habitats (Monk et al., 2012; Heino, 2014). However, identification of 195 macroinvertebrate taxa at family level has been shown to be appropriate to examine alpha, beta 196 and gamma diversity in lentic systems (Le Viol et al., 2009; Mueller et al., 2013; Hassall & 197 Anderson, 2015; Vilmi et al., 2016) and is the resolution used by a range of environmental 198 monitoring indices (e.g., biological monitoring working party [BMWP] and predictive system for 199 multimetrics [PSYM] scores; Environment Agency & Pond Conservation Trust, 2002) and 200 201 legislation (e.g., The Water Framework Directive; EC, 2000) across Europe. However, to assess the sensitivity of results to taxonomic resolution we performed all analyses at two taxonomic 202 levels: first, to incorporate as many sites as possible and to ensure faunal data was comparable 203 204 across all studies, aquatic macroinvertebrate data were reclassified to family level and analysis was undertaken at this higher taxonomic resolution. Second, statistical analysis was also 205 206 undertaken on a subset of urban (207 ponds) and non-urban ponds (578 ponds) where species level data was available. 207

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The second methodological variation was in the amount of sampling effort applied to the sites: sampling effort was limited to 3 minutes in 10 of the studies (following standard UK sampling protocols) but two studies used exhaustive sampling until no more species were found. A preliminary analysis showed that, in fact, the sites sampled for 3 minutes found more taxa

(average of 14.7 ± 0.4 SE families, n=392 sites; average of 30.0 ± 0.9 species, n=340) than sites 213 214 sampled exhaustively (average of 13.6 ± 0.3 SE families, n=518 sites; average of 26.8 ± 0.6 species, n=518). However, this lower number of species in exhaustive samples is likely to result 215 216 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 samples. Finally, to provide the strongest possible test of the biodiversity value of urban ponds, 218 219 urban pond communities (at a family and species level) were compared to a subset of the nonurban ponds with degraded sites excluded (leaving n=571 non-urban ponds with family level 220 data and 542 with species level data). 221

222

223 Statistical Analysis

Differences in environmental characteristics (pond area, percentage coverage of emergent 224 225 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. All analyses were carried out in the R environment (R Development Core Team, 2013). Prior to 227 statistical analysis the data was screened to remove any missing values. Estimated gamma 228 diversity was calculated using Chao2 estimator in the vegan package in R (Oksanen et al., 2015). 229 230 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 numbers of urban and non-urban sites, taxon accumulation curves were constructed by 232 233 randomized resampling of sites without replacement using the *specaccum* function in vegan with 1,000 permutations per sample size. From these curves the mean number of families and species 234 in each simulated group of sites and the standard error were calculated. Variability between 235

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 and non-urban ponds were visualized using Non-Metric Multidimensional Scaling (NMDS) with 238 239 the *metaMDS* function in the vegan package and were examined statistically using a 'Permutational Analysis of Variance' (PERMANOVA). Bray-Curtis dissimilarity was used to 240 analyse the macroinvertebrate data and Euclidean distance used for the environmental data. 241 Homogeneity of multivariate dispersions between the environmental data and macroinvertebrate 242 communities from urban and non-urban ponds were calculated using the *betadisper* function in 243 244 vegan and compared using an ANOVA. To identify indicator taxa of ephemeral and perennial ponds Indicator Value analysis (IndVal: Dufrêne & Legendre 1997) was undertaken. To test the spatial 245 patterns of community structure in urban and non-urban ponds, a Mantel correlogram was 246 247 constructed between the aquatic macroinvertebrate distance matrix (Euclidean) and the geographical distance for urban and non-urban ponds using the *mantel.correlog* function in the 248 vegan package in R. Breaks among distance classes in the Mantel correlogram were defined in 249 250 50km intervals. The Mantel correlogram enables the identification of changes in the strength of correlation between faunal distance matrices and geographic distance matrices at different spatial 251 scales (Rangel et al., 2010). 252

253

The relationship between macroinvertebrate assemblages and environmental variables (pH, percentage coverage of emergent macrophytes, percentage pond shading, altitude, location within urban area, and pond area) was examined using redundancy analysis (RDA) in the vegan package. A stepwise selection procedure (forward and backward selection) was employed to select the best model and environmental variables that significantly (p<0.05) explained the

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

- thought to influence diversity and composition showed that altitude (W=108179.5 p<0.01;
- Figure 2A) and pond shading (W=92965.5 p<0.01; Figure 2B) were significantly higher for

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

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

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

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

271 $(7.37 \pm 0.16; W=37024 p<0.05; Figure 2C)$ 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

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

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

- ponds (2207 \pm 139m²) compared to urban ponds (1546 \pm 171m²; W=75154.5 p<0.01; Figure 2D).
- Emergent macrophyte coverage was significantly higher in non-urban ponds $(33.10 \pm 1.08\%)$

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

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 taking into account sample size that were not statistically different across the two pond types 283 284 (urban: 108.2, 95% CI: 91.4-125.0 families; non-urban: 107.5, 95% CI: 99.7-115.3 families). At an alpha scale urban ponds (median richness = 13, range = 2-44) supported significantly greater 285 macroinvertebrate family richness compared to non-urban ponds (median richness = 12, range = 286 2-38; W=20430.5 p<0.01) although median richness values were very similar between the pond 287 types. Species-level gamma diversity was lower in urban (observed 403 species) than non-urban 288 289 sites (observed 473 species), but the Chao2 estimator showed that there was no significant difference after controlling for the number of sites (urban: 496.6, 95%CI: 445.6-547.7 species; 290 non-urban: 572.9, 95%CI: 520.2-625.7 species). No significant difference in alpha diversity 291 between macroinvertebrate species was recorded between urban (median: 28) and non-urban 292 ponds (median 26; W=17310 p=0.507). 293

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 environments. Only two families of macroinvertebrates were statistically associated with non-urban 301 ponds (one family of Plecoptera, one family of Ephemeroptera), while 20 families were identified as 302 indicator taxa for urban ponds, including seven families of Diptera. Strongest associations for families are 303

presented in Table 2 (see Supplementary Material Table S10 for the full list of statistically significant
family indicator values, and Supplementary Table S11 for significant indicator values of
macroinvertebrate species).

307

When non-urban ponds designated as degraded were removed and the macroinvertebrate 308 diversity in the remaining ponds was compared to urban ponds, alpha diversity was significantly 309 greater in urban ponds (median: 13; W=18057 p<0.01) than the higher quality non-urban ponds 310 (median: 12) at a family level, although mean and median richness values were similar between 311 the pond types (see Supplementary Information Part 2). There was no significant difference in 312 alpha diversity (W=14653.5 p=0.358) at the species level between urban ponds (median: 28) and 313 314 higher quality non-urban ponds (median: 25). Estimated gamma diversity for higher quality nonurban ponds at a family (98.7) and species scale (575.1) was marginally higher compared to 315 gamma diversity when all non-urban ponds were considered. 316

317

Chironomidae, Tipulidae, Crangonyctidae and Oligochaeta had a greater frequency of 318 319 occurrence in urban ponds, whilst Gyrinidae, Hydrophilidae and Notonectidae displayed a greater occurrence in non-urban ponds (Figure 4; for complete data see Tables S8 and S9 for 320 family and species level prevalence, respectively). Macroinvertebrate families that score highly 321 within biological monitoring surveys of ponds and other waterbodies (e.g., PSYM and BMWP) 322 such as Phryganeidae, Leptoceridae, Libellulidae and Aeshnidae occurred at similar frequencies 323 in the urban and non-urban ponds (Figure 4). Crangonyctidae were present in 49.0% of urban 324 ponds and only 29.0% of non-urban ponds. All specimens of this family from the species-level 325 dataset were the North American invasive Crangonyx pseudogracilis. A similar pattern is also 326

327	seen in the species-level dataset with the invasive New Zealand mud snail, <i>Potamopyrgus</i>
328	antipodarum, being found in 21.3% of urban ponds and 9.5% of non-urban ponds.

329 *Community Heterogeneity*

Multivariate dispersion for environmental characteristics were significantly lower in non-urban 330 ponds (median distance: 1116) than urban ponds (median distance: 1978; F=5.774 p<0.05, 331 Figure 5A). PERMANOVA showed that there was a small but significant difference between 332 environmental characteristics ($R^2=0.03 p<0.001$) and faunal communities at a family ($R^2=0.09$ 333 p < 0.001) and species level ($R^2 = 0.03 p < 0.001$). A relatively clear distinction between aquatic 334 macroinvertebrate community composition in urban and non-urban ponds was observed at the 335 family and species level within the NMDS ordination (Figure 5B, C). Among faunal 336 communities, multivariate dispersion was significantly higher at the family (median distance -337 urban: 0.451, non-urban: 0.406; F=27.584 p<0.01) and species scale (median distance - urban: 338 0.579, non-urban: 0.550; F=17.626 p < 0.01) for urban ponds compared to non-urban ponds. 339

340

There was significant positive spatial autocorrelation for urban (r=0.31 p<0.01) and non-urban 341 342 ponds (r=0.17 p<0.01) at the family level for the smallest distance class (0-50 km), indicating that those ponds in close geographical proximity have similar macroinvertebrate community 343 compositions (Figure 6A). At middle distance classes (distance class three: 100-150 km) urban 344 345 and non-urban ponds demonstrated a significant negative Mantel spatial autocorrelation, although this effect was weak for non-urban ponds (urban: r=-0.18 p<0.01, non-urban: r=-0.05346 p<0.01) (Figure 6A). At larger distances spatial autocorrelation declined in strength for urban 347 348 and non-urban ponds. The same analyses carried out on species-level data showed similar spatial patterns, but with stronger positive correlation at shorter distances (0-50km, urban: r=0.45,

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

Redundancy Analysis (RDA) of the pond macroinvertebrate family community data and 354 355 environmental parameters highlighted clear differences between urban and non-urban ponds (Figure 7A). The RDA axes were highly significant (F=3.06 p < 0.001, Adjusted R²=0.02), 356 explaining 3.8% of the variation in family assemblage on all constrained axes (see 357 358 Supplementary Information Table S4). Stepwise selection of environmental parameters identified 359 four significant physicochemical variables correlated with the first two RDA axes: altitude, emergent macrophytes (all p<0.05), surface area and location within urban area (both p<0.01) 360 361 (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 ponds were characterized by a greater pond area and emergent macrophyte cover, whilst urban 364 ponds were associated with smaller surface areas and less emergent macrophytes (Figure 7). 365 366 RDA of pond macroinvertebrate species community data showed similar patterns: urban and non-urban ponds were strongly separated along the first RDA axis, with significant effects of 367 urbanisation, pond area, altitude, and shading on community structure (Figure 7B). However, in 368 369 both RDA analyses the explanatory power of the models was very low (see Supplementary Information Table S4). 370

372 Discussion

373 Urban freshwater diversity

374 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 375 with previous work on terrestrial and lotic habitats which has shown greater fragmentation, 376 377 reduction in habitat quality (e.g., pollution/contaminant build up), alterations to biogeochemical cycles, higher air surface temperatures, increased disturbance frequencies, proliferation of non-378 native taxa, biotic homogenization and an overall decline in biological richness in urban areas 379 (e.g., McKinney, 2002; McKinney, 2006; Grimm et al., 2008). The ecological consequences of 380 urbanization for ponds do not appear to follow the same patterns identified elsewhere for 381 terrestrial habitats. 382

383

384 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 385 family level, although non-urban ponds recorded higher estimated gamma diversity at a species 386 scale. These findings are consistent with a recent study of terrestrial invertebrates that showed 387 comparable levels of diversity of particular indicator groups inhabiting birch trees (Betula 388 pendula) between urban and agricultural areas (Turrini and Knop, 2015). However, an analysis 389 of the same dataset showed a homogenization of arboreal invertebrates within urban areas (Knop, 390 2016), consistent with other terrestrial ecosystem studies (McKinney, 2008) but not with our data 391 for freshwater macroinvertebrates. The lack of agreement in ecological patterns between ponds 392 393 (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) 394

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 dispersal abilities of many semi-aquatic invertebrates (Goertzen & Suhling, 2015). Despite 397 398 commonly occurring in clusters, ponds are discrete habitats with small catchment areas (Davies et al., 2008) and disturbance in one pond or its catchment has little impact on others in the 399 network cluster, whilst a single disturbance event in, for example, a river system would impact 400 an entire reach (Thornhill, 2013). Aside from rare taxa, there were few families that showed a 401 different prevalence between urban and non-urban ponds, including indicator taxa with high 402 BMWP scores (indicative of high water quality). However, there was also a higher prevalence of 403 Oligochaeta and Chironomidae in urban ponds which is consistent with historical disturbance 404 and subsequent recolonization by disturbance tolerant taxa, and higher prevalence of the invasive 405 C. pseudogracilis and P. antipodarum in urban ponds supports previous findings that urban 406 ecosystems favour the establishment of invasive species (Shochat et al., 2010). 407

408

409 We propose two potential explanations, which are not mutually exclusive, for the similarity between urban and non-urban pond biodiversity. First, it has been estimated that 80% of ponds in 410 411 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 comparable biodiversity to higher quality, non-degraded non-urban ponds. Research examining 416 417 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, neglected, and at late successional stages (Hassall et al., 2012; Sayer et al., 2012), ponds in urban 420 421 areas are often managed (primarily for purposes other than biodiversity) and a wide-range of successional stages are maintained. Furthermore, in many cases local residents (e.g., pond 422 warden schemes) monitor and manage large numbers of urban ponds for the benefit of ecological 423 communities, improving their habitat/water quality and promoting high biological richness 424 (Boothby, 1995; Hill *et al.*, 2015). Results from the present study show that urban areas have the 425 potential to become reservoirs of freshwater biodiversity rather than "ecological deserts", which 426 incorporate a wide range of aquatic habitats including ponds, canals, urban reservoirs and 427 wetlands (Hassall & Anderson, 2015). However, it should be noted that diversity was highly 428 429 variable in this study at both the family and species level of taxonomic resolution and previous research has demonstrated that some urban ponds can be of low ecological quality if 430 anthropogenic stressors such as eutrophication are allowed to persist (Noble & Hassall, 2014). 431

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 achieve this (Le Viol et al., 2009). Reduced emergent macrophyte cover in urban areas may also 438 be the result of public perceptions of pond attractiveness (clean, open water and surrounding 439 440 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 result of urban ponds location within high density, built environments providing significant 443 444 additional artificial shading to that provided by trees. In addition, reduced shading of non-urban ponds may be because many non-urban ponds were located in landscapes typically free of 445 shading (trees) including wetland meadows and the low numbers of trees in British agricultural 446 landscapes where many non-urban ponds are situated (however high levels of pond shading from 447 trees has been recorded in some UK agricultural areas: Sayer et al., 2012). 448

449

450 *Community heterogeneity*

Small but significant differences in faunal communities (family and species) were observed 451 between urban and non-urban ponds in this study (reject hypothesis 2). Differences (albeit subtle) 452 453 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 heterogeneous environmental conditions (greater environmental multivariate distances than non-459 460 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 similar communities which reflect similar city-region environmental characteristics; and 2) 464 ponds at greater spatial distances from one another in different cities have increasingly dissimilar 465 communities reflecting the high variability in environmental (Heino & Alahuhta, 2015) and 466 historical factors (Baselga, 2008; Heino & Alahuhta, 2015) among cities. Spatial patterns of 467 management may influence geographical variation in community structure to a greater extent 468 than landscape connectivity, making it difficult to evaluate our third hypothesis. However, we 469 demonstrate stronger spatial structuring of urban communities at finer spatial scales, which 470 would be expected under lower connectivity. Greater connectivity in non-urban landscapes 471 enhances species movement leading to weaker spatial structuring at finer spatial scales in non-472 473 urban ponds. Hence our observations support our third hypothesis, but further work is needed to evaluate the consequences of spatial patterns for management. Historically, urban environments 474 were highly degraded (physically, chemically and biologically) but significant improvements to 475 476 urban freshwater quality have been achieved in recent decades despite urban sprawl and intensification (Vaughan & Ormerod, 2012). Therefore, it is possible that cities are still being 477 recolonized by aquatic taxa from different regional species pools using different dispersal routes, 478 479 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

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 well as other ecosystem services alongside social and political considerations. Fundamental to 487 488 the conservation of ponds is an integrated landscape approach that recognizes the need for networks of ponds (Boothby, 1997). Hence the prioritization of ponds for conservation will need 489 to take into account their location relative to other sites, requiring a complementary approach 490 that creates new habitats, improves degraded habitats, and conserves those habitats that have 491 already achieved good quality. Changes in the management of ponds more generally has led to 492 493 change in the environmental conditions within and around these habitats, such as the reduction in riparian tree management around agricultural ponds which has consequences for light, oxygen, 494 and temperature (Sayer et al., 2013). Urban ponds are well suited to biodiversity enhancement as 495 496 many are sites of high diversity (Hassall, 2014) and even small changes to current management strategies in urban freshwaters (e.g., the planting of native macrophytes in amenity ponds; Hill et 497 al., 2015) are likely to significantly augment biodiversity in urban landscapes. Cities are highly 498 499 complex, multifunctional landscapes designed primarily for anthropogenic use yet they still 500 support considerable aquatic diversity and represent scientifically and ecologically important habitats. 501

502

503 Acknowledgements

The authors would like to thank the various organizations who provided resources for the datasets included in this study: the EU Life Program funded the PondLife Project. RB would like to thank the Carnegie Trust for the Universities of Scotland. MH would like to acknowledge Leicestershire County Council and the private land owners that granted access to their land. CH

- is grateful for support from a Marie Curie International Incoming Fellowship within the 7th
- 509 European Community Framework Programme. DG would like to thank Halton Borough Council
- 510 for support and access to pond sites and IT is grateful for the support from the Natural
- 511 Environment Research Council and The James Hutton Institute.

512 **References**

- 513 Baselga, A. (2008) Determinants of species richness, endemism and turnover in European
- 514 longhorn beetles. Ecography, **31**, 263-271.
- 515 Biggs, J., Fox, G., Whitfield, M. and Williams, P. (1998). A guide to the methods of the National
- 516 Pond Survey, Pond Action: Oxford.
- 517 Biggs J, Williams P, Whitfield M, Nicolet P, and Weatherby A. (2005) 15 years of pond
- assessment in Britain: results and lessons learned from the work of Pond Conservation. Aquatic
- 519 Conservation: Marine and Freshwater Ecosystems, 15, 693-714.
- 520 Boothby, J. (1997) Pond conservation: towards a delineation of pondscape. Aquatic
- 521 Conservation: Marine and Freshwater Ecosystems, 7, 127-132.
- 522 Boothby, J., Hull, A. P. and Jeffreys, D. A. (1995) Sustaining a threatened landscape: farmland
- ponds in Cheshire. Journal of Environmental Planning and Management, **38**, 561-568.
- 524 Briers, R. A. (2014) Invertebrate communities and environmental conditions in a series of urban
- 525 drainage ponds in Eastern Scotland: implications for biodiversity and conservation value of
- 526 SUDS. Clean Soil, Air, Water, 42, 193-200.
- 527 Commonwealth of Australia. 2007. Water Act 2007.
- 528 Dallimer, M., Tang, Z., Bibby, P. R., Brindley, P., Gaston, K. J. and Davies, Z. G. (2011)
- 529 Temporal changes in green space in a highly urbanized region. Biology Letters, 7, 763-766.
- 530 Davies, B, R., Biggs, J., Williams, P., Whitfield, M., Nicolet, P., Sear, D., Bray, S. and Maund, S.
- 531 (2008) Comparative biodiversity of aquatic habitats in the European agricultural landscape.
- 532 Agriculture, Ecosystems and Environment, **125**, 1-8.

- 533 Dufrêne, M. and P. Legendre. 1997. Species assemblages and indicator species: The need for a flexible
 534 asymmetrical approach. Ecological Monographs 67: 345-366.
- EC (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October
- 536 2000 establishing a framework for Community action in the field of water policy, 22/12/2000.
- 537 Official Journal **327/1**: 1-73.
- 538 Environment Agency and Ponds Conservation Trust. (2002) A guide to monitoring the
- ecological quality of ponds and canals using PSYM. PCTPR, Oxford.
- 540 Fitzhugh, T. W. and Richter, B. D. (2004) Quenching urban thirst: growing cities and their
- 541 impacts on freshwater ecosystems. BioScience, 54, 741-754.
- Gledhill, D. G., James, P. and Davies, D. H. (2008) Pond density as a determinant of aquatic
 species richness in an urban landscape. Landscape Ecology, 23, 1219-1230.
- 544 Goertzen, D. and Suhling, F. (2015) Central European cities maintain substantial dragonfly
- species richness a chance for biodiversity conservation. Insect Conservation and Diversity, 8,
 238-246.
- Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X. and Briggs, J. M.
 (2008) Global change and the ecology of cities. Science, **319**, 756-760.
- Hamer, A. J., Smith, P. J. and McDonnell, M. J. (2012) The importance of habitat design and
- aquatic connectivity in amphibian use of urban stormwater retention ponds. Urban Ecosystems,
- **15**, 451-471.
- Hassall, C. and Anderson, S. (2015) Stormwater ponds can contain comparable biodiversity to
 unmanaged wetlands in urban areas. Hydrobiologia, 745, 137-149.

- Hassall, C. (2014) The ecology and biodiversity of urban ponds. Wiley Interdisciplinary
 Reviews: Water, 1, 187-206.
- Hassall, C., Hollinshead, J. and Hull, A. (2011) Environmental correlates of plant and
- invertebrate species richness in ponds, Biodiversity and Conservation, **20**, 3189-3222.
- Hassall, C., Hollinshead, J. and Hull, A. (2012) Temporal dynamics of aquatic communities and
- implications for pond conservation, Biodiversity and Conservation, **21**, 829-852.
- 560 Heino, J. (2014) Taxonomic surrogacy, numerical resolution and responses of stream
- 561 macroinvertebrate communities to ecological gradients: are the inferences transferable among
- regions? Ecological Indicators, **36**, 186-194.
- Heino, J. and Alahuhta, J. (2015) Elements of regional beetle faunas: faunal variation and
 compositional break points along climate, land cover and geographical gradients. Journal of
 Animal Ecology, 84, 427-441.
- Hill, M. J. and Wood, P. J. (2014) The macroinvertebrate biodiversity and conservation value of
 garden and field ponds along a rural urban gradient. Fundamental and Applied Limnology, 185,
 107-119.
- Hill, M. J., Mathers, K. L. and Wood, P. J. (2015) The aquatic macroinvertebrate biodiversity of
 urban ponds in a medium sized European town (Loughborough, UK). Hydrobiologia, 760, 225238.
- 572 Hitchings, S. P. and Beebee, T. J. C. (1997) Genetic substructuring as a result of barriers to gene
- 573 flow in urban *Rana temporaria* (common frog) populations: implications for biodiversity
- 574 conservation. Heredity, **79**, 117-127.

- 575 Jeanmougin, M., Leprieur, F., Lois, G. and Clergeau, P. (2014) Fine scale urbanization effects
- 576 Odonata species diversity in ponds of a mega city (Paris, France). Acta Oecologica, **59**, 26-34.
- 577 Knop, E. (2016) Biotic homogenization of three insect groups due to urbanization. Global
- 578 Change Biology, 22: 228–236. Le Viol, I., Mocq, J. Julliard, R. and Kerbiriou, C. (2009) The
- 579 contribution of motorway stormwater retention ponds to the biodiversity of aquatic
- 580 macroinvertebrates. Biological Conservation, **142**, 3163-3171.
- 581 McKinney, M. L. (2002) Urbanization, biodiversity and conservation. Bioscience, **52**, 883-890.
- 582 McKinney, M. L. (2006) Urbanization as a major cause of biotic homogenization. Biological
- 583 Conservation, **127**, 247-260.
- McKinney, M. L. (2008) Effects of urbanization of species richness: a review of plants and
 animals. Urban Ecosystems, 11, 161-176.
- 586 Monk, W. A., Wood, P. J., Hannah, D. M., Extence, C., Chadd, R. and Dunbar, M. J. (2012)
- 587 How does macroinvertebrate taxonomic resolution influence ecohydrological relationships in
- riverine ecosystems. Ecohydrology, **5**, 36-45.
- 589 Mueller, M., Pander, J. and Geist, J. (2013) Taxonomic sufficiency in freshwater ecosystems:
- effects of taxonomic resolution, functional traits and data transformation. Freshwater Science,
 32, 762-778.
- 592 Nassauer, J. I. (2004) Monitoring the success of metropolitan wetland restorations: cultural
- sustainability and ecological function. Wetlands, 24, 756-765.
- Noble, A. and Hassall, C. (2014) Poor ecological quality of urban ponds in northern England:
- causes and consequences. Urban Ecosystems: 1-14.

- 596 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R.B., Simpson,
- 597 G.L., Solymos, Stevens, H.H. and Wagner, H. 2015. Vegan: Community Ecology Package. R
- 598 package version 2.3-1. [Accessible at <u>http://CRAN.R-project.org/package=vegan</u>].
- 599 Pereira, M., Segurado, P. and Neves, N. (2011) Using spatial network structure in landscape
- 600 management and planning: A case study with pond turtles. Landscape and Urban Planning, **100**,
- 601 67**-**76.
- 602Pond Life Project. (2000) A landscape worth saving: Final report of the pond biodiversity survey
- of North West England. Pond Life Project: Liverpool.
- R Development Core Team. (2013) R: A Language and Environment for Statistical Computing.
- 605 R Foundation for Statistical Computing, Vienna, Austria.
- Rangel, T. F., Diniz-Filho, J. A. F. and Bini, L. M. (2010) SAM: a comprehensive application for
 spatial analysis in macroecology. Ecography, 33, 46-50.
- Roy, A. H., Rosemond, A. H., Paul, M. J., Leigh, D. S. and Wallace, J. B. 2003. Stream
- 609 macroinvertebrate response to catchment urbanization (Georgia, USA). Freshwater Biology, **48**,
- **610** 329-346.
- Sala, et al. (2000) Global biodiversity scenarios for the year 2100. Science, **287**, 1770-1774.
- 612 Sayer, C.D., Andrews, K., Shiland, E., Edmonds, N., Edmonds-Brown, R., Patmore, I., Emson,
- and D., Axmacher, J. (2012) The role of pond management for biodiversity conservation in an
- agricultural landscape. Aquatic Conservation, **22**, 626-638.
- 615 Sayer, C.D., Shilland, E., Greaves, H., Dawson, B., Patmore, I.R., Emson, E., Alderton, E.,
- 616 Robinson, P., Andrews, K., Axmacher, J.A. and Wiik, E. (2013) Managing British ponds –
- 617 conservation lessons from a Norfolk farm. British Wildlife, **25**, 21-28.

- 618 Shochat, E., Lerman, S. B., Anderies, J. M. Warren., P. S., Faeth, S. H. and Nilon, C. H. (2010)
- 619 Invasion, competition, and biodiversity loss in urban ecosystems. Bioscience, **60**, 199-208.
- 620 Thornhill, I. A. G. (2013) Water quality, biodiversity and ecosystem functioning in ponds across
- an urban land-use gradient in Birmingham, UK. PhD Thesis, University of Birmingham: UK.
- Turrini T. and Knop, E. (2015) A landscape ecology approach identifies important drivers of
- urban biodiversity. Global Change Biology, **21**, 1652-1667.
- 624 UKNEA, (2011) The UK National Ecosystem Assessment Technical Report. UNEP-WCMC,625 Cambridge.
- 626 United Nations, (2014) World Urbanization Prospects: the 2014 revision. United Nations: New627 York.
- Vaughan, I. P. and Ormerod, S. J. (2012) Large-scale, long-term trends in British river
 macroinvertebrates. Global Change Biology, 18, 2184–2194.
- 630 Vilmi, A., Maaria Karjalainen, S., Nokela, T., Tolonen, T. and Heino, J. 2016. Unravelling the
- drivers of aquatic communities using disparate organismal groups and different taxonomic
- 632 levels. Ecological Indicators, **60**, 108-118.
- Walsh, C. J., Roy, A. H., Feminella, J. W. and Cottingham, P. D. (2005) The urban stream
- 634 syndrome: current knowledge and the search for a cure. Journal of the North American
- Benthological Society, **24**, 706-723.
- 636 Williams, P., Biggs, J., Crowe, A., Murphy, J., Nicolet, P., Meatherby, A. and Dunbar, M. (2010)
- 637 Countryside survey report from 2007, Technical report No 7/07 Pond Conservation and
- 638 NERC/Centre for Ecology and Hydrology, Lancaster.

- 639 Wood, P. J., Greenwood, M. T., Barker, S. A. and Gunn, J. (2001) The effects of amenity
- 640 management for angling on the conservation value of aquatic invertebrate communities in old
- industrial mill ponds. Biological Conservation, **102**, 17-29.
- 642 Wood, P.J., Greenwood, M. T. and Agnew, M. D. (2003) Pond biodiversity and habitat loss in
- 643 the UK. Area, **35**, 206-216.

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 <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et</i> <i>al.</i> , 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.

- 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

649

651 Figure legends

Figure 1 - Map of Great Britain showing the locations of the surveyed urban (light grey circles)and non-urban (dark grey circles) ponds.

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.

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

659 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).

664 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).

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

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

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

670 correlations.

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

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

37

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

Supplementary information

In this document we present additional data and analyses. **Part 1** demonstrates the differences among three different methods to describe urban ponds. **Part 2** provides the same analyses as in the main paper but for a subset of sites that exclude sites recorded as "degraded". **Part 3** contains the tables of species prevalence across urban and non-urban ponds.

Part 1: Definitions of "urban ponds"

In the main text we characterise urban ponds as those which are located within developed urban land use areas (DLUAs), areas of urban land demarcated by the UK Ordnance Survey mapping authority. However, we acknowledge that there are alternative methods to classify urban ponds and we provide a comparison with two such measures below:

- 1. Distance to urban area: The distance was calculated between each pond and the nearest urban land use area, where ponds within urban land use areas were allocated a value of 0 km.
- 2. Urban landcover in a 1 km buffer: Each pond was buffered to a distance of 1 km (a buffer area of 3.14 km²) and the proportion of that buffer containing urban land use was calculated.

Figure S1 shows the relationship between a binary categorisation of sites (as used in the main text) and these two alternative measures of urbanness. We further define additional threshold values for "urbanness" based on the distance from urban areas and the percentage of the 1 km buffer containing urban land (Table S1). To test for the sensitivity of our findings to these different definitions of "urban", we carried out supplementary sensitivity analysis which is presented below for alpha diversity and gamma diversity.

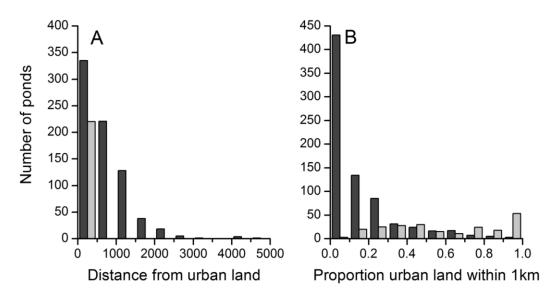


Figure S1: Comparison of three measures of pond classification. (A) shows the distance of each pond from the edge of a developed land use area (DLUA, see main text for details) for "urban" (light grey bar) and "non-urban" (dark grey bar) ponds as classified by their presence inside or outside of the DLUAs. (B) shows the proportion of urban land within a circular buffer of radius 1 km for the urban and non-urban ponds. Note that the urban ponds shown in (A) are all 0 km from urban land as they lie within the DLUAs.

		Species		Family	
Assumption	Definitions of urban pond	Urban	Non-urban	Urban	Non-urban
1	Within urban land use area	574	203	607	229
2	<500m from urban land use area	448	329	503	333
3	<1000m from urban land use area	628	149	686	150
4	100% urban land cover in 1 km buffer	23	754	28	808
5	>80% urban land cover in 1 km buffer	63	714	81	755
6	>60% urban land cover in 1 km buffer	115	662	140	696
7	>40% urban land cover in 1 km buffer	186	591	230	606
8	>20% urban land cover in 1 km buffer	328	449	379	457

Table S1: Threshold values for the definition of a pond as "urban", with sample sizes of urban and non-urban pond derived for each threshold.

Alpha diversity

<u>Methods</u>: Mann-Whitney U tests were used to test for a difference in recorded taxon number (families and species) in urban and non-urban ponds under several definitions. Spearman rank correlations were used to test for an association between alpha diversity and (i) the distance to the nearest urban land use area, and (ii) the area of

<u>*Results*</u>: There were no significant correlations between alpha diversity at the species level and the distance to urban area (rho=0.053, p=0.138) or the percentage of the 1 km containing urban land use area (rho=-0.051, p=0.156), or between alpha diversity at a family level and the distance to urban area (rho=-0.018, p=0.594) or the percentage of the 1 km containing urban land use area (rho=0.023, p=0.511). When ponds were classified as either urban or non-urban according to the criteria in Table S1, there were only two assumptions that produced a significant difference between urban and non-urban species-level richness and both results were only marginally significant (p>0.025; Table S2). One of these assumption (4) resulted in only 23 urban ponds compared against 754 non-urban ponds. None of the assumptions produced a significant difference in family-level richness.

Table S2: Sensitivity analysis showing the variation in alpha diversity in ponds categorised as "urban" or "non-urban" using different thresholds (see Table S1 for definitions of the assumptions), with results of Mann-Whitney U-tests.

Taxonomic level	Assumption	Urban alpha	Non-urban alpha	W	р
Species	1	24	27	62043	0.169
	2	26	27	72544	0.709
	3	27	26	45898	0.719
	4	17	27	10996	0.028
	5	22	27	24548	0.229
	6	23	27	39495	0.520
	7	23	27	60841	0.028
	8	25	27	78276	0.133
Family	1	13	13	65476	0.196
Panniy	2	13	13	79710	0.190
	3	13	12	46680	0.075
	4	12	13	11716	0.748
	5	13	13	29253	0.521
	6	13	13	46038	0.303
	7	13	13	68562	0.717
	8	13	13	85828	0.824

Gamma diversity

<u>Methods</u>: Gamma diversity was calculated for ponds classified according to the criteria in Table S1 using Chao's estimator from the *specpool* function in the vegan (Oskanen *et al.*, 2007) package in R (R Core Team, 2015). Significant differences were evaluated using the overlap of the 95% confidence intervals associated with the estimates of taxonomic richness.

<u>*Results*</u>: There were four assumptions that led to a significant difference (lack of overlap between 95% CIs) in species-level gamma diversity: Assumption 3 suggested a higher number of taxa in urban ponds, while Assumptions 4, 5 and 6 suggested a higher number of taxa in non-urban ponds (Table S3). In each of these cases the sample with the small number of taxa also had a far smaller number of sites (<20% of the number of sites as in the other sample; see Table S1). Indeed, even though the Chao estimator nominally controls for sample size, the Chao value correlates strongly with sample size, suggesting that the only fair comparisons occur when sample sizes are more similar (Assumptions 1, 2, 7 and 8, Figure S2). A similar pattern is also seen in the family data, but only Assumption 4 produced a significant difference between the gamma diversity estimates.

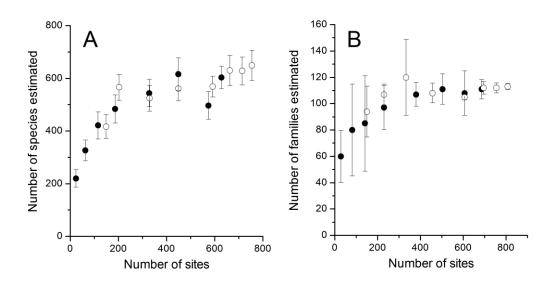


Figure S2: Chao estimates (±95% CI) for the different assumptions made concerning the definition of an "urban pond". Data are shown in relation to the number of sites included within each definition (see Table S1 for details) for gamma diversity at (A) species- and (B) family-level. Filled circles are urban pond samples, open circles are non-urban pond samples.

Taxonomic level	Assumption	Urban gamma	Urban SE	Non-urban gamma	Non-urban SE
Species	1	497	27	566	25
-	2	616	32	524	25
	3	603	22	417	23
	4	220	17	649	29
	5	326	20	628	27
	6	421	26	630	29
	7	484	27	569	20
	8	544	26	561	24
Family	1	108	8.6	107	4.0
2	2	111	5.9	120	14.7
	3	111	3.8	94	9.8
	4	60	10.1	113	1.2
	5	80	17.7	112	2.0
	6	85	18.5	112	2.5
	7	97	8.6	105	1.1
	8	107	4.6	108	3.8

Table S3: Sensitivity analysis showing the variation in relative gamma diversity in ponds categorised as "urban" or "non-urban" using different thresholds (see Table S1 for definitions of the assumptions).

Table S4 - Summary statistics for redundancy analysis of macroinvertebrate community data at (A) family-level and (B) species-level, with significant explanatory environmental parameters.

A: Eigenvalues for constrained axes in family-level RDA							
	RDA	RDA	RDA	RDA	RDA	RDA	
	1	2	3	4	5	6	
Eigenvalues	0.198	0.056	0.033	0.018	0.015	0.006	
Proportion Explained (%)	2.3	0.66	0.38	0.21	0.17	0.06	
Cumulative Proportion Explained (%)	2.3	2.96	3.34	3.55	3.72	3.78	
Adjusted R ²	0.02						
Significant Environmental							
Variables							
	Df	F	Р				
Emergent Macrophytes	1	1.62	0.02				
Altitude	1	2.03	0.015				
Pond Area	1	2.25	0.01				
In Urban	1	9.05	0.005				

B: Eigenvalues for constrained axes in species-level RDA							
	RDA 1	RDA 2	RDA 3	RDA 4			
Eigenvalues	0.250	0.128	0.076	0.064			
Proportion Explained (%)	1.02	0.55	0.32	0.28			
Cumulative Proportion Explained (%)	1.02	1.52	1.84	2.1			
Adjusted R^2	0.01						
Significant Environmental							
Variables							
	Df	F	Р				
Percentage pond shaded	1	1.37	0.04				
Area	1	1.64	0.02				
Altitude	1	2.17	0.01				
In Urban	1	3.23	0.005				

Part 2: Analysis excluding degraded ponds

As discussed in the text, this analysis follows precisely the same methods as in the main part of the study but with the exclusion of sites which were explicitly recorded as being "degraded".

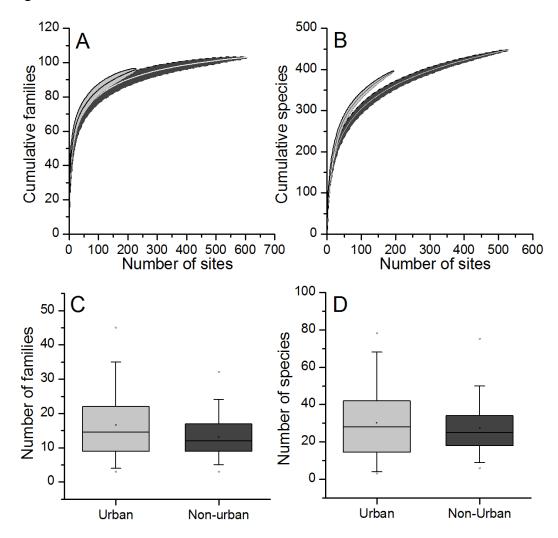


Figure S3 - Species accumulation curves of family richness (a) and species richness (b): grey area with black line = urban ponds, black area with white line = non-degraded, non-urban ponds, and median macroinvertebrate family richness (c) and species richness (d) for urban and non-degraded, non-urban ponds.

Table S5 - Homogeneity of multivariate dispersions for non-degraded, non-urban ponds at a family and species taxonomic scale.

Taxonomic scale	Median	F	p-value
Family	0.398	28.323	< 0.001
Species	0.5504	17.439	< 0.001

Table S6 - PERMANOVA results for urban and non-degraded, non-urban pond macroinvertebrate communities at a family and species level.

PERMANOVA	\mathbf{R}^2	p-value
Species	0.030	0.001
Family	0.039	0.001

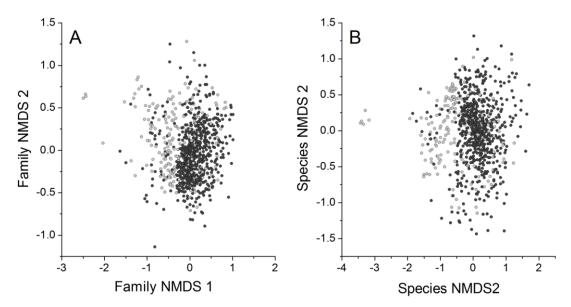


Figure S4 - Non-Metric Multidimensional scaling plots of variation in aquatic macroinvertebrate families (A) and aquatic macroinvertebrate species (B) from urban and non-degraded, non-urban ponds (dark grey symbols = non-degraded, non-urban ponds and light grey symbols = urban ponds).

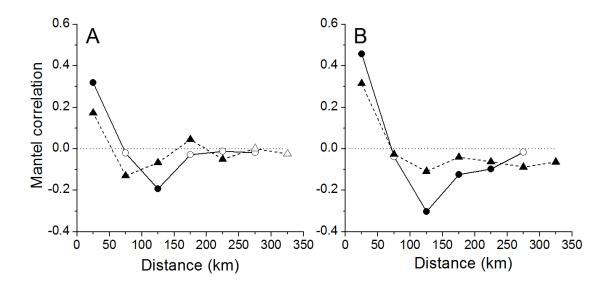


Figure S5 - Mantel correlogram for presence-absence macroinvertebrate family (A) and species (B) data along 50 km distance intervals excluding known degraded sites. Triangles = non degraded, non-urban macroinvertebrate communities, circles = urban macroinvertebrate communities. Filled symbols indicate statistically significant mantel correlations.

Table S7 – Table of summary statistics for Redundancy Analysis of macroinvertebrate family (A) and species (B) assemblage data for urban pond assemblages and non-degraded, non-urban pond assemblages (RDA axes were significant for the family (F=3.085 p<0.001) and species (F=1.70 p<0.001) models).

(A) Eigenvalues for constrained axes (Family)						
	RDA 1	RDA 2	RDA 3	RDA 4		
Eigenvalues	0.21633	0.06478	0.02835	0.01456		
Proportion Explained (%)	0.02647	0.00792	0.00347	0.00178		
Cumulative Proportion Explained (%)	2.6	3.4	3.8	4.0		
Adjusted R^2	0.03					
Significant Environmental Variables						
	Df	F	Р			
pН	1	2.58	0.005			
Area	1	2.1	0.01			
Altitude	1	1.68	0.025			
In Urban	1	8.48	0.005			
(B) Eigenvalues for constrained axes (S	pecies)					
	RDA 1	RDA 2	RDA 3	RDA 4		
Eigenvalues	0.21553	0.17987	0.07284	0.06056		
Proportion Explained (%)	0.00958	0.00800	0.00324	0.00269		
Cumulative Proportion Explained (%)	0.96	1.76	2.08	2.35		
Adjusted R^2	0.01					
Significant Environmental Variables						
	Df	F	Р			
Emergent Plants	1	1.90	0.005			
Altitude	1	2.25	0.005			

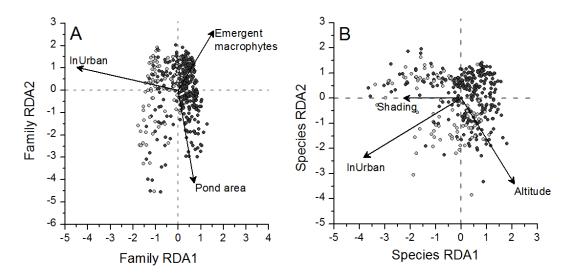


Figure S6 - RDA site plots of family (A) and species (B) macroinvertebrate communities recorded from the urban and non-degraded, non-urban pond types studied across the UK. Note - only significant environmental parameters are presented. Dark grey symbols = non-urban ponds and light grey symbols = urban ponds.

Part 3: Species and family prevalence in urban and non-urban ponds

v 1 v					
	Non-urban	Urban	Non-urban	Urban	
Family	occurrence	occurrence	prevalence	prevalence	
Acroloxidae	50	33	0.082	0.109	
Aeshnidae	160	91	0.264	0.299	
Ancylidae	3	1	0.005	0.003	
Anthribidae	0	1	0.000	0.003	
Aphelocheiridae	8	5	0.013	0.016	
Araneae	22	3	0.036	0.010	
Argulidae	0	2	0.000	0.007	
Asellidae	376	199	0.619	0.655	
Astacidae	8	2	0.013	0.007	
Baetidae	333	154	0.549	0.507	
Beraeidae	2	3	0.003	0.010	
Bibionidae	1	0	0.002	0.000	
Bithyniidae	35	30	0.058	0.099	
Brachycentridae	2	0	0.003	0.000	
Caenidae	71	37	0.117	0.122	
Calopterygidae	2	1	0.003	0.003	
Carabidae	1	2	0.002	0.007	
Ceratopogonidae	1	36	0.002	0.118	
Chaoboridae	0	4	0.000	0.013	
Chironomidae	39	112	0.064	0.368	
Chloroperlidae	1	1	0.002	0.003	
Chrysomelidae	137	41	0.226	0.135	
Cladocera	1	2	0.002	0.007	
Coccinellidae	101	38	0.166	0.125	
Coenagrionidae	319	148	0.526	0.487	
Copepoda	2	3	0.003	0.010	
Cordulegasteridae	0	1	0.000	0.003	
Corixidae	497	224	0.819	0.737	
Crambidae	83	39	0.137	0.128	
Crangonyctidae	176	149	0.290	0.490	
Culicidae	1	34	0.002	0.112	
Curculionidae	19	3	0.031	0.010	
Dendrocoelidae	6	18	0.010	0.059	
Dixidae	2	35	0.003	0.115	
Dryopidae	31	6	0.051	0.020	
Dugesidae	49	37	0.081	0.122	
Dytiscidae	559	253	0.921	0.832	
Ecnomidae	6	255	0.010	0.000	
Elmidae	18	9	0.030	0.000	
Ephemeridae	4	1	0.030	0.003	
Erpobdellidae	174	98	0.007	0.003	
Euconulidae					
	5	1	0.008	0.003	
Ferrissidae	5	2	0.008	0.007	
Gammaridae	81	62	0.133	0.204	
Gastrodontidae	1	0	0.002	0.000	
Gerridae	268	128	0.442	0.421	
Glossiphoniidae	230	129	0.379	0.424	
Glossosomatiidae	1	1	0.002	0.003	
Gyrinidae	134	40	0.221	0.132	

Table S8: Occurrence of aquatic macroinvertebrate families in urban (n=304) *and non-urban* (n=607) *ponds*

Haliplidae	258	125	0.425	0.411
Hebridae	10	0	0.016	0.000
Helodidae	0	2	0.000	0.007
Heptageniidae	12	1	0.020	0.003
Heteroceridae	5	0	0.008	0.000
Hirudidae	25	9	0.041	0.030
Hydrachnidae	2	8	0.003	0.026
Hydraenidae	148	38	0.244	0.125
Hydrobiidae	57	63	0.094	0.207
Hydrometridae	70	54	0.115	0.178
Hydrophilidae	537	206	0.885	0.678
Hydropsychidae	1	3	0.002	0.010
Hydroptilidae	8	15	0.013	0.049
Hygrobiidae	53	18	0.087	0.059
Lepidostomatidae	3	2	0.005	0.007
Leptoceridae	93	56	0.153	0.184
Leptophlebiidae	17	13	0.028	0.043
Lestidae	47	7	0.077	0.023
Leuctridae	6	3	0.010	0.010
Libellulidae	142	60	0.234	0.197
Limacidae	14	10	0.023	0.033
Limnephilidae	320	157	0.527	0.516
Limnichidae	2	0	0.003	0.000
Lymnaeidae	342	185	0.563	0.609
Mesoveliidae	0	1	0.000	0.003
Microveliidae	36	12	0.059	0.039
Nabidae	75	58	0.124	0.191
Naucoridae	94	39	0.155	0.128
Nemouridae	57	20	0.094	0.066
Nepidae	16	20 29	0.026	0.095
Neuroptera	0	1	0.000	0.003
Niphargidae	2	0	0.003	0.000
Noteridae	61	51	0.100	0.168
Notonectidae	350	150	0.577	0.493
Odontoceridae	4	150	0.007	0.003
Oligochaeta	34	99	0.056	0.326
Ostracoda	2	3	0.003	0.010
Paguroidea	3	2	0.005	0.010
Phryganeidae	57	40	0.003	0.132
Physidae	56	40 67	0.094	0.132
Piscicolidae	50 16	11	0.032	0.220
Pisidiidae	142	65	0.020	0.030
Planariidae	142	81	0.234	0.214
Planorbidae	339	183		0.200
			0.558	
Pleidae	37	7	0.061	0.023
Polycentropodidae	46	44	0.076	0.145
Potamanthidae	6	2	0.010	0.007
Psychodidae	0	30	0.000	0.099
Psychomyiidae	7	5	0.012	0.016
Ptychopteridae	0	5	0.000	0.016
Pyralidae	6	5	0.010	0.016
Scirtidae	74	37	0.122	0.122
Sericostomatidae	4	1	0.007	0.003
Sialidae	153	91	0.252	0.299

Simuliidae	0	5	0.000	0.016
Siphlonuridae	6	3	0.010	0.010
Sphaeriidae	44	69	0.072	0.227
Stratiomyidae	0	15	0.000	0.049
Succineidae	30	7	0.049	0.023
Taeniopterygidae	8	0	0.013	0.000
Tipulidae	14	55	0.023	0.181
Tortricoidea	0	1	0.000	0.003
Unionidae	12	0	0.020	0.000
Valvatidae	19	10	0.031	0.033
Veliidae	32	19	0.053	0.063
Viviparidae	4	1	0.007	0.003

Table S9: Occurrence of aquatic macroinvertebrate species in urban (n=207) and non-urban (n=577) ponds

Species	Urban occurrence	Non-urban occurrence	Urban prevalence	Non-urban prevalence	
Acilius canaliculatus	0	1	0.000	0.002	
Acilius sulcatus	19	81	0.092	0.140	
Acroloxus lacustris	25	54	0.022	0.094	
Aeshna cyanea	37	86	0.121	0.149	
Aeshna cyanea Aeshna grandis	26	47	0.179	0.149	
-	20	47 20	0.120	0.081	
Aeshna juncea Aeshna mixta	6	20 0	0.003	0.000	
Aeshna mixia Agabus affinis	0 2	11	0.029	0.000	
8 10	1	11	0.010	0.019	
Agabus arcticus	74	303	0.003	0.002	
Agabus bipustulatus	2	18	0.010	0.323	
Agabus chalconatus			0.010	0.0031	
Agabus congener	1	2			
Agabus conspersus	0	1	0.000	0.002	
Agabus didymus	0	3	0.000	0.005	
Agabus guttatus	0	2	0.000	0.003	
Agabus labiatus	0	5	0.000	0.009	
Agabus melanarius	0	10	0.000	0.017	
Agabus melanocornis	5	10	0.024	0.017	
Agabus montanus	1	8	0.005	0.014	
Agabus nebulosus	21	156	0.101	0.270	
Agabus paludosus	1	5	0.005	0.009	
Agabus sturmii	40	163	0.193	0.282	
Agabus uliginosus	7	13	0.034	0.023	
Agraylea multipunctata	12	5	0.058	0.009	
Agraylea sexmaculata	5	1	0.024	0.002	
Agrypnia obsoleta	5	5	0.024	0.009	
Agrypnia pagetana	4	2	0.019	0.003	
Agrypnia varia	3	11	0.014	0.019	
Amphinemoura sulcicollis	0	1	0.000	0.002	
Anabolia nervosa	6	17	0.029	0.029	
Anacaena bipustulata	3	19	0.014	0.033	
Anacaena globulus	39	135	0.188	0.234	
Anacaena limbata	68	259	0.329	0.449	
Anacaena lutescens	28	119	0.135	0.206	
Anax imperator	9	9	0.043	0.016	
Ancylus fluviatilis	1	3	0.005	0.005	
Anisosticta 19 punctata	11	72	0.053	0.125	
Anisus leucostoma	27	41	0.130	0.071	
Anisus vortex	27	107	0.130	0.185	
Anodonta anatina	0	1	0.000	0.002	
Anodonta cygnea	1	10	0.005	0.017	
Apatamia muliebris	1	0	0.005	0.000	
Aphelocheirus aestivalis	0	1	0.000	0.002	
Aphthona nonstriata	0	8	0.000	0.014	
Aplexa hypnorum	5	9	0.024	0.016	
Aquarius paludum	0	1	0.000	0.002	
Arctocorisa germari	1	4	0.005	0.007	
Argyroneta aquatica	2	41	0.010	0.071	
Armiger crista	42	110	0.203	0.191	
Asellus aquaticus	130	294	0.628	0.510	
Asellus meridianus	16	111	0.077	0.192	

Athripsodes aterrimus	14	47	0.068	0.081
Athripsodes bilineatus	0	1	0.000	0.002
Athripsodes cinereus	1	4	0.005	0.007
Austropotamobius pallipes	0	1	0.000	0.002
Baetis rhodani	0	1	0.000	0.002
Baetis vernus	0	1	0.000	0.002
Bathyomphalus contortus	6	53	0.029	0.092
Batracobdella paludosa	1	0	0.005	0.000
Beraea pullata	2	2	0.010	0.003
Beraeodes minutus	0	1	0.000	0.002
Berosus affinis	0	1	0.000	0.002
Berosus luridus	2	4	0.010	0.007
Berosus signaticollis	1	10	0.005	0.017
Bithynia leachi	2	11	0.010	0.019
Bithynia tentaculata	22	35	0.106	0.061
Brachycentrus subnubilus	0	1	0.000	0.002
Caenis horaria	24	26	0.116	0.045
Caenis luctuosa	9	18	0.043	0.031
Caenis macrura	0	1	0.000	0.002
Caenis rivulorum	5	3	0.024	0.005
Caenis robusta	5	19	0.024	0.033
Callicorixa praeusta	29	46	0.140	0.080
Callicorixa wollastoni	4	3	0.019	0.005
Cataclysta lemnata	20	39	0.097	0.068
Centroptilum pennulatum	1	2	0.005	0.003
Ceraclea fulva	1	0	0.005	0.000
Ceraclea nigronervosa	0	1	0.000	0.002
Cercyon convexiusculus	18	74	0.087	0.128
Cercyon granarius	1	2	0.005	0.003
Cercyon impressus	0	7	0.000	0.012
Cercyon marinus	2	5	0.010	0.009
Cercyon obsoletus	0	1	0.000	0.002
Cercyon sternalis	0	4	0.000	0.007
Cercyon tristis	0	9	0.000	0.016
Cercyon ustulatus	4	24	0.019	0.042
Ceriagrion tenellum	0	4	0.000	0.007
Chaetarthria seminulum	0	2	0.000	0.003
Chaetocnema concinna	1	2	0.005	0.003
Chalcoides aurea	0	2	0.000	0.003
Cheumatopsyche lepida	0	1	0.000	0.002
Chloroperla torrentium	0	1	0.000	0.002
Chrysolina polita	0	6	0.000	0.010
Cloeon dipterum	110	283	0.531	0.490
Cloeon simile	9	38	0.043	0.066
Coccidula rufa	11	48	0.053	0.083
Coelambus confluens	3	14	0.014	0.024
Coelambus impressopunctatus	17	76	0.082	0.132
Coelambus paralellogrammus	0	1	0.000	0.002
Coelostoma orbiculare	12	58	0.058	0.101
Coenagrion puella pulchellum	57	207	0.275	0.359
Colymbetes fuscus	38	207	0.184	0.359
Copelatus haemorrhoidalis	12	76	0.058	0.132
Corixa affinis	0	1	0.000	0.002
Corixa dentipes	7	10	0.034	0.017

Coving panzovi	7	13	0.034	0.023
Corixa panzeri	50	238	0.034	0.023
Corixa punctata Corixidae nymph	41	238	0.242	0.412
Considue nymph Crangonyx pseudogracilis	123	190	0.198	0.002
Cymatia bonsdorffi	4	5	0.019	0.009
Cymatia coleoptrata	6	13	0.019	0.003
, i	18	13	0.029	0.023
Cymbiodyta marginella	18 0	3	0.087	0.220
Cyphon coarctatus Cyphon hilaria	11	25	0.000	0.003
	1	4	0.005	0.043
Cyphon padi Cyphon variabilis	0	4	0.003	0.007
	0 7	6	0.000	0.002
Cyrnus flavidus	15	4	0.034	0.010
Cyrnus trimaculatus Dendrocoelum lacteum	13	4 8		
	2	8 14	0.068	0.014
Deroceras laeve		2	0.010	0.024
Donacia marginata	0		0.000	0.003
Donacia simplex	5	28	0.024	0.049
Donacia versicolorea	1	1	0.005	0.002
Donacia vulgaris	1	13	0.005	0.023
Dryops ernesti	0	1	0.000	0.002
Dryops luridus	3	20	0.014	0.035
Dryops similaris	0	7	0.000	0.012
Dryops striatellus	0	3	0.000	0.005
Dugesia lugubris	7	24	0.034	0.042
Dugesia polychroa	20	11	0.097	0.019
Dugesia tigrina	17	16	0.082	0.028
Dytiscus circumcinctus	0	2	0.000	0.003
Dytiscus circumflexus	0	13	0.000	0.023
Dytiscus marginalis	23	53	0.111	0.092
Dytiscus semisulcatus	2	10	0.010	0.017
Ecdyonurus dispar	0	1	0.000	0.002
Ecnomus tenellus	0	2	0.000	0.003
Elmis aenea	1	4	0.005	0.007
Elophila nymphaeata	13	62	0.063	0.107
Enallagma cyathigerum	23	91	0.111	0.158
Enochrus affinis	2	11	0.010	0.019
Enochrus bicolor	0	1	0.000	0.002
Enochrus coarctatus	28	114	0.135	0.198
Enochrus fuscipennis	0	6	0.000	0.010
Enochrus halophilus	0	2	0.000	0.003
Enochrus isotae	0	5	0.000	0.009
Enochrus melanocephalus	1	13	0.005	0.023
Enochrus nigritus	0	1	0.000	0.002
Enochrus ochropterus	0	18	0.000	0.031
Enochrus testaceus	26	78	0.126	0.135
Ephemera danica	1	1	0.005	0.002
Ephemera vulgata	1	3	0.005	0.005
Ephemerella ignita	0	1	0.000	0.002
Erpobdella octoculata	45	136	0.217	0.236
Erpobdella testacea	35	52	0.169	0.090
Erythromma najas	12	33	0.058	0.057
Euconulus alderi	1	5	0.005	0.009
Ferrissia wautieri	2	5	0.010	0.009
Galerucella cf grisescens	0	2	0.000	0.003

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Galerucella sagittariae	0	15	0.000	0.026
Gammarus lacustris	4	0	0.019	0.000
Gammarus pulex	58	69	0.280	0.120
Garrmarus zaddachi	0	1	0.000	0.002
Gastrophysa polygoni	0	3	0.000	0.005
Gerris argentatus	0	5	0.000	0.009
Gerris costai	0	1	0.000	0.002
Gerris gibbifer	5	3	0.024	0.005
Gerris lacustris	71	191	0.343	0.331
Gerris lateralis	0	4	0.000	0.007
Gerris odontogaster	12	54	0.058	0.094
Gerris thoracicus	9	47	0.043	0.081
Glossiphonia complanata	37	92	0.179	0.159
Glossiphonia heteroclita	15	70	0.072	0.121
Glyphotaelius pellucidus	22	44	0.106	0.076
Grammotaulius nigropunctatus	3	6	0.014	0.010
Graptodytes flavipes	0	3	0.000	0.005
Graptodytes granularis	1	9	0.005	0.016
Graptodytes pictus	1	17	0.005	0.029
Gyraulus albus	66	150	0.319	0.260
Gyraulus laevis	4	2	0.019	0.003
Gyrinus caspius	0	3	0.000	0.005
Gyrinus distinctus	1	0	0.005	0.000
Gyrinus marinus	2	33	0.010	0.057
Gyrinus substriatus	20	84	0.097	0.146
Gyrinus urinator	1	1	0.005	0.002
Haemopis sanguisuga	11	46	0.053	0.080
Halesus digitatus	0	1	0.000	0.002
Halesus radiatus	2	6	0.010	0.010
Haliplus confinis	16	19	0.077	0.033
Haliplus flavicollis	2	30	0.010	0.052
Haliplus fluviatilis	1	11	0.005	0.019
Haliplus fulvus	6	26	0.029	0.045
Haliplus heydeni	1	7	0.005	0.012
Haliplus immaculatus	9	40	0.043	0.069
Haliplus laminatus	2	4	0.010	0.007
Haliplus lineatocollis	16	71	0.077	0.123
Haliplus lineolatus	2	3	0.010	0.005
Haliplus obliquus	6	20	0.029	0.035
Haliplus ruficollis	63	178	0.304	0.308
Haliplus variegatus	0	1	0.000	0.002
Haliplus wehnckei	3	28	0.014	0.049
Hebrus pusillus	0	2	0.000	0.003
Hebrus ruficeps	0	6	0.000	0.010
Helobdella stagnalis	69	118	0.333	0.205
Helochares lividus	24	76	0.116	0.132
Helochares punctatus	8	53	0.039	0.092
Helophorus aequalis	12	52	0.058	0.090
Helophorus alternans	0	1	0.000	0.002
Helophorus avernicus	0	1	0.000	0.002
Helophorus brevipalpis	68	340	0.329	0.589
Helophorus dorsalis	0	1	0.000	0.002
Helophorus flavipes	5	28	0.024	0.049
Helophorus fulgidicollis	0	1	0.000	0.002

Helophorus grandis	54	286	0.261	0.496
Helophorus granularis	4	11	0.019	0.019
Helophorus griseus	2	6	0.010	0.010
Helophorus longitarsis	0	1	0.000	0.002
Helophorus minutus	36	122	0.174	0.211
Helophorus nanus	3	11	0.014	0.019
Helophorus obscurus	13	73	0.063	0.127
Helophorus strigifrons	2	4	0.010	0.007
Helophorus terrestrial	3	0	0.014	0.000
Helophorus tuberculatus	0	1	0.000	0.002
Hemiclepsis marginata	9	10	0.043	0.017
Heptagenea sulphurea	0	1	0.000	0.002
Hesperocorixa castanea	6	36	0.029	0.062
Hesperocorixa linnei	24	80	0.116	0.139
Hesperocorixa moesta	11	10	0.053	0.017
Hesperocorixa sahlbergi	72	250	0.348	0.433
Heterocerus fenestratus	0	3	0.000	0.005
Hippeutis complanatus	44	106	0.213	0.184
Hippuriphila modeeri	0	8	0.000	0.014
Holocentropus dubius	5	16	0.024	0.028
Holocentropus picicornis	8	17	0.039	0.029
Holocentropus stagnalis	2	7	0.010	0.012
Hydaticus seminiger	5	19	0.024	0.033
Hydraena britteni	0	1	0.000	0.002
Hydraena riparia	6	27	0.029	0.047
Hydraena testacea	4	14	0.019	0.024
Hydrobius fuscipes	83	296	0.401	0.513
Hydrochara caraboides	2	3	0.010	0.005
Hydrochus angustatus	1	17	0.005	0.029
Hydrochus brevis	0	1	0.000	0.002
<i>Hydrochus carinatus</i>	0	3	0.000	0.005
<i>Hydrochus elongatus</i>	1	4	0.005	0.007
Hydroglyphus geminus	2	15	0.010	0.026
Hydroglyphus pusillus	2	0	0.010	0.000
Hydrometra gracilenta	0	1	0.000	0.002
Hydrometra stagnorum	42	71	0.203	0.123
Hydroporus angustatus	38	147	0.184	0.255
Hydroporus discretus	1	9	0.005	0.016
Hydroporus erythrocephalus	12	72	0.058	0.125
Hydroporus glabriusculus	0	2	0.000	0.003
Hydroporus gyllenhalii	10	58	0.048	0.101
<i>Hydroporus incognitus</i>	6	37	0.029	0.064
Hydroporus longicornis	0	1	0.000	0.002
Hydroporus longulus	0	1	0.000	0.002
Hydroporus marginatus	0	1	0.000	0.002
<i>Hydroporus melanarius</i>	0	1	0.000	0.002
Hydroporus memnonius	15	72	0.072	0.125
Hydroporus neglectus	8	14	0.039	0.024
Hydroporus nigrita	10	61	0.048	0.106
Hydroporus obscurus	3	17	0.014	0.029
Hydroporus obsoletus	2	0	0.014	0.000
Hydroporus palustris	57	251	0.275	0.435
Hydroporus planus	46	270	0.273	0.468
Hydroporus pubescens	12	270	0.058	0.133
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Hydroporus rufifrons	0	1	0.000	0.002
Hydroporus striola	12	50	0.058	0.087
Hydroporus tesselatus	6	71	0.029	0.123
Hydroporus tristis	1	22	0.005	0.038
Hydroporus umbrosus	2	32	0.010	0.055
Hydropsyche angustipennis	2	0	0.010	0.000
Hydrothassa marginella	0	8	0.000	0.014
Hydrovatus clypealis	1	2	0.005	0.003
Hygrobia hermanni	8	53	0.039	0.092
Hygrotus decoratus	2	8	0.010	0.014
Hygrotus inaequalis	41	209	0.198	0.362
Hygrotus versicolor	5	3	0.024	0.005
Hyphydrus ovatus	24	136	0.116	0.236
Hyrdochus ignicollis	0	1	0.000	0.002
Ilybius ater	23	144	0.111	0.250
Ilybius fenestratus	2	7	0.010	0.012
Ilybius fuliginosus	30	173	0.145	0.300
Ilybius guttiger	5	19	0.024	0.033
Ilybius quadriguttatus	6	47	0.029	0.081
Ilybius subaeneus	0	11	0.000	0.019
Ilyocoris cimicoides	26	93	0.126	0.161
Ischnura elegans	72	176	0.348	0.305
Ischnura pumilio	0	1	0.000	0.002
Laccobius atratus	0	1	0.000	0.002
Laccobius biguttatus	25	91	0.121	0.158
Laccobius bipunctatus	10	39	0.048	0.068
Laccobius colon	0	1	0.000	0.002
Laccobius minutus	9	30	0.043	0.052
Laccobius sinuatus	1	1	0.005	0.002
Laccobius striatulus	0	1	0.000	0.002
Laccobius ytenensis	0	1	0.000	0.002
Laccophilus hyalinus	1	2	0.005	0.003
Laccophilus minutus	36	224	0.174	0.388
Laccornis oblongus	0	1	0.000	0.002
Lasiocephala basalis	1	0	0.005	0.000
Lepidostoma hirtum	0	2	0.000	0.003
Leptocerus tineiformis	5	5	0.024	0.009
Leptophlebia marginata	7	7	0.034	0.012
Leptophlebia vespertina	1	3	0.005	0.005
Lestes sponsa	3	37	0.014	0.064
Leuctra fusca	0	2	0.000	0.003
Libellula depressa	13	8	0.063	0.014
Libellula quadrimaculata	11	36	0.053	0.062
Limnebius nitidus	1	6	0.005	0.010
Limnebius papposus	1	1	0.005	0.002
Limnebius truncatellus	7	28	0.034	0.049
Limnephilus affinis incisus	9	21	0.043	0.036
Limnephilus auricula	13	42	0.063	0.073
Limnephilus binotatus	4	2	0.019	0.003
Limnephilus bipunctatus	1	3	0.005	0.005
Limnephilus centralis	6	21	0.029	0.036
Limnephilus decipiens	7	2	0.034	0.003
Limnephilus extricatus	0	2	0.000	0.003
Limnephilus flavicornis	48	84	0.232	0.146
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Limnephilus griseus	1	1	0.005	0.002
Limnephilus hirsutus	0	2	0.000	0.003
Limnephilus ignavus	0	1	0.000	0.002
Limnephilus lunatus	47	109	0.227	0.189
Limnephilus marmoratus	20	51	0.097	0.088
Limnephilus nigriceps	1	0	0.005	0.000
Limnephilus politus	1	0	0.005	0.000
Limnephilus rhombicus	6	3	0.029	0.005
Limnephilus sparsus	1	3	0.005	0.005
Limnephilus stigma	5	17	0.024	0.029
Limnephilus vittatus	24	154	0.116	0.267
Limnius volckmari	0	3	0.000	0.005
Limnoxenus niger	0	1	0.000	0.002
Lymnaea auricularia	1	18	0.005	0.031
Lymnaea glabra	4	8	0.019	0.014
Lymnaea palustris	31	97	0.150	0.168
Lymnaea peregra	89	253	0.430	0.438
Lymnaea stagnalis	59	100	0.285	0.173
Lymnaea truncatula	7	61	0.034	0.106
Lype reducta	2	2	0.010	0.003
Megasternum obscurum	1	3	0.005	0.005
Mesovelia furcata	1	0	0.005	0.000
Microcara testacea	0	13	0.000	0.023
Micronecta poweri	10	0	0.048	0.000
Micronecta scholtzi	3	1	0.014	0.002
Micropterna lateralis	0	4	0.000	0.007
Microvelia buenoi	0	2	0.000	0.003
Microvelia pygmaea	0	2	0.000	0.003
Microvelia reticulata	7	61	0.034	0.106
Molanna angustata	6	3	0.029	0.005
Musculium lacustre	15	64	0.072	0.111
Mystacides azurea	7	8	0.034	0.014
<i>Mystacides longicornis</i>	15	8	0.072	0.014
Mystacides nigra	1	3	0.005	0.005
Myxas glutinosa	0	1	0.000	0.002
Nebrioporus depressus	1	13	0.005	0.023
Nebrioporus elegans	1	0	0.005	0.000
Nemoura cambrica	0	1	0.000	0.002
Nemoura cinerea	7	50	0.034	0.087
Nemurella picteti	5	9	0.024	0.016
Nepa cinerea	32	91	0.155	0.158
Niphargus aquilex	0	1	0.000	0.002
Noterus clavicornis	62	169	0.300	0.293
Noterus crassicornis	0	16	0.000	0.028
Notonecta glauca	91	329	0.440	0.570
Notonecta maculata	13	0	0.063	0.000
Notonecta marmorea	5	17	0.024	0.029
Notonecta obliqua	3 7	7	0.034	0.012
Nymphula stagnata	0	4	0.000	0.007
Ochthebius dilatatus	1	1	0.005	0.007
Ochthebius marinus	0	3	0.000	0.002
Ochthebius minimus	16	107	0.000	0.185
Ochthebius nanus	0	107	0.000	0.183
Ochthebius punctatus	0	1	0.000	0.002
Senineorus punciulus	0	1	0.000	0.002

Ochthebius viridis	0	4	0.000	0.007
Oecetis lacustris	2	3	0.010	0.005
Oecetis ochracea	2	4	0.010	0.007
Oligotricha striata	0	2	0.000	0.003
Oreodytes sanmarkii	0	1	0.000	0.002
Orthetrum cancellatum	2	2	0.010	0.003
Orthetrum coerulescens	0	2	0.000	0.003
Oulimnius tuberculatus	2	7	0.010	0.012
Oxyloma pfeifferi	4	24	0.019	0.042
Paracorixa concinna	4	0	0.019	0.000
Paracymus scutellaris	0	8	0.000	0.014
Paraleptophlebia submarginata	0	1	0.000	0.002
Paraponyx stratiotata	1	3	0.005	0.005
Peltodytes caesus	1	3	0.005	0.005
Phaedon armoraciae	9	46	0.043	0.080
Phryganea bipunctata	18	10	0.087	0.017
Phryganea grandis	0	1	0.000	0.002
Physa acuta	24	20	0.116	0.035
Physa fontinalis	23	22	0.111	0.038
Physa heterostropha	1	0	0.005	0.000
Piscicola geometra	8	9	0.039	0.016
Pisidium casertanum	1	2	0.005	0.003
Pisidium hybernicum	3	1	0.014	0.002
Pisidium nitidum	1	2	0.005	0.003
Pisidium subtruncatum	3	3	0.014	0.005
Pisidium supinum	1	0	0.005	0.000
Planaria torva	1	0	0.005	0.000
Planorbarius corneus	44	51	0.213	0.088
Planorbis carinatus	22	56	0.106	0.097
Planorbis planorbis	18	29	0.087	0.050
Platambus maculatus	1	2	0.005	0.003
Plateumaris discolor	0	1	0.000	0.002
Plateumaris sericea	0	2	0.000	0.003
Plea leachi	9	48	0.043	0.083
Plectrocnemia conspersa	1	3	0.005	0.005
Polycelis felina	0	3	0.000	0.005
Polycelis nigra	11	28	0.053	0.049
Polycelis tenuis	33	159	0.159	0.276
Polycentropus flavomaculatus	1	1	0.005	0.002
Porhydrus lineatus	0	34	0.000	0.059
Potamanthus luteus	0	1	0.000	0.002
Potamonectes assimilis	0	1	0.000	0.002
Potamophylax latipennis	1	1	0.005	0.002
Potamopyrgus antipodarum	44	55	0.213	0.095
Prasocuris phellandrii	1	19	0.005	0.033
Prasocurus junci	0	6	0.000	0.010
Procloeon bifidum	0	1	0.000	0.002
Psylliodes affinis	0	4	0.000	0.007
Pyrrhosoma nymphula	37	88	0.179	0.153
Radix auricularia	2	0	0.010	0.000
Radix balthica	4	0	0.019	0.000
Ranatra linearis	7	5	0.034	0.009
Rhantus exsoletus	1	15	0.005	0.026
Rhantus frontalis	0	1	0.000	0.002
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Rhantus grapii	0	3	0.000	0.005
Rhantus suturalis	4	4	0.019	0.007
Rhantus suturellus	1	4	0.005	0.007
Scirtes hemisphaericus	6	32	0.029	0.055
Sericostoma personatum	1	4	0.005	0.007
Sialis fuliginosa	1	0	0.005	0.000
Sialis lutaria	55	141	0.266	0.244
Sigara concinna	6	43	0.029	0.075
Sigara distincta	31	117	0.150	0.203
Sigara dorsalis	57	132	0.275	0.229
Sigara falleni	28	63	0.135	0.109
Sigara fossarum	8	53	0.039	0.092
Sigara lateralis	24	53	0.116	0.092
Sigara limitata	10	27	0.048	0.047
Sigara nigrolineata	13	56	0.063	0.097
Sigara scotti	1	14	0.005	0.024
Sigara semistriata	1	17	0.005	0.029
Sigara stagnalis	1	0	0.005	0.000
Sigara venusta	0	2	0.000	0.003
Siphlonurus lacustris	0	2	0.000	0.003
Sisyra fuscata	1	0	0.005	0.000
Sphaerium corneum	19	112	0.092	0.194
Sphaerium rivicola	1	0	0.005	0.000
Stagnicola palustris	1	0	0.005	0.000
Stenophylax permistus	0	1	0.000	0.002
Stictonectes lepidus	1	4	0.005	0.007
Stictotarsus duodecimpustulatus	0	8	0.000	0.014
Succinea putris	2	5	0.010	0.009
Suphrodytes dorsalis	11	59	0.053	0.102
Sympetrum danae	1	2	0.005	0.003
Sympetrum flaviolum	0	1	0.000	0.002
Sympetrum fonscolombii	1	0	0.005	0.000
Sympetrum sanguineum	8	34	0.039	0.059
Sympetrum striolatum	27	89	0.130	0.154
Tanysphyrus lemnae	2	17	0.010	0.029
Theromyzon tessulatum	34	89	0.164	0.154
Tinodes waeneri	5	2	0.024	0.003
Triaenodes bicolor	14	38	0.068	0.066
Tricholeiochiton fagesii	0	1	0.000	0.002
Trichostegia minor	3	18	0.014	0.031
Trocheta bykowskii	0	1	0.000	0.002
Valvata cristata	0	15	0.000	0.026
Valvata macrostoma	0	2	0.000	0.003
Valvata piscinalis	9	10	0.043	0.017
Velia caprai	4	8	0.019	0.014
Viviparus contectus	0	2	0.000	0.003
Viviparus viviparus	1	2	0.005	0.003
Zonitoides nitidus	7	4	0.034	0.007

Habitat	Taxon	statistic	p-value
Non-urban ponds	Nemouridae	0.341	0.007
	Heptageniidae	0.196	0.021
Urban ponds	Chironomidae	0.719	0.001
	Oligochaeta	0.690	0.001
	Crangonyctidae	0.632	0.001
	Sphaeriidae	0.511	0.001
	Ceratopogonidae	0.477	0.001
	Dixidae	0.463	0.001
	Hydrobiidae	0.458	0.001
	Culicidae	0.449	0.001
	Physidae	0.447	0.001
	Psychodidae	0.426	0.001
	Hydrometridae	0.412	0.001
	Nepidae	0.377	0.001
	Dugesidae	0.362	0.001
	Stratiomyidae	0.302	0.001
	Hydroptilidae	0.278	0.003
	Dendrocoelidae	0.275	0.001
	Hydrachnidae	0.213	0.001
	Chaoboridae	0.161	0.01
	Ptychopteridae	0.161	0.017
	Simuliidae	0.161	0.014

Table S10 - Aquatic macroinvertebrate families identified as statistically significant indicator species for urban or non-urban ponds.

Habitat	Taxon	statistic	p-value
Non-urban ponds	Hydroporus planus	0.573	0.001
	Hydroporus pubescens	0.390	0.001
	Helochares punctatus	0.382	0.001
	Hydroporus erythrocephalus	0.373	0.003
	Cymbiodyta marginella	0.365	0.005
	Lymnaea truncatula	0.362	0.001
	Copelatus haemorrhoidalis	0.346	0.004
	Hydroporus gyllenhalii	0.339	0.001
	Hydroporus tesselatus	0.327	0.003
	Bathyomphalus contortus	0.318	0.009
	Hesperocorixa castanea	0.298	0.023
	Argyroneta aquatica	0.298	0.004
	Hydroporus memnonius	0.283	0.011
	Hydroporus umbrosus	0.262	0.037
	Coelostoma orbiculare	0.248	0.04
	Hydroporus tristis	0.246	0.007
	Enochrus ochropterus	0.246	0.004
	Hydroporus nigrita	0.238	0.018
	Ilybius quadriguttatus	0.234	0.05
	Haliplus flavicollis	0.231	0.035
	Aeshna juncea	0.223	0.015
	Hydroporus obscurus	0.215	0.026
	Valvata cristata	0.215	0.029
	Sigara scotti	0.198	0.035
Jrban ponds	Crangonyx pseudogracilis	0.688	0.001
•	Lymnaea stagnalis	0.499	0.001
	Gammarus pulex	0.480	0.001
	Planorbarius corneus	0.468	0.001
	Potamopyrgus antipodarum	0.442	0.001
	Hydrometra stagnorum	0.409	0.003
	Erpobdella testacea	0.406	0.001
	Physa fontinalis	0.368	0.001
	Dugesia polychroa	0.354	0.001
	Aeshna grandis	0.347	0.002
	Dugesia tigrina	0.338	0.001
	Phryganea bipunctata	0.328	0.001
	Caenis horaria	0.306	0.035
	Haliplus confinis	0.295	0.003
	Dendrocoelum lacteum	0.294	0.001
	Mystacides longicornis	0.290	0.001
	Cataclysta lemnata	0.285	0.001
	Physa acuta	0.284	0.009
	Agraylea multipunctata	0.281	0.001

Table S11 - Aquatic macroinvertebrate species identified as statistically significant indicator species for urban or non-urban ponds.

Micronecta poweri	0.280	0.001
Notonecta maculata	0.265	0.001
Cyrnus trimaculatus	0.253	0.001
Hesperocorixa moesta	0.250	0.018
Ilyocoris cimicoides	0.250	0.002
Libellula depressa	0.247	0.004
Hemiclepsis marginata	0.237	0.011
Anax imperator	0.228	0.028
Limnephilus decipiens	0.220	0.004
Aeshna mixta	0.217	0.001
Zonitoides nitidus	0.217	0.002
Piscicola geometra	0.214	0.013
Caenis rivulorum	0.189	0.015
Agraylea sexmaculata	0.189	0.02
Molanna angustata	0.189	0.012
Hygrotus versicolor	0.182	0.039
Paracorixa concinna	0.177	0.008
Gammarus lacustris	0.177	0.011
Radix balthica	0.177	0.008
Limnephilus binotatus	0.168	0.041
Agrypnia pagetana	0.168	0.034

References

Oskanen J, Kindt R, Legendre P, O'Hara B, Stevens MHM (2007) *vegan: Community ecology package*, R package version 1.8-8. <u>http://cran.r-project.org/, http://r-forge.r-project.org/projects/vegan/</u>.

R Core Team (2015) *R: A language and environment for statistical computing,* R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/.