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1 **The effects of water chemistry and lock-mediated connectivity on**
2 **macroinvertebrate diversity and community structure in a canal in**
3 **northern England**

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13 fieldwork for this study and for providing information on the Leeds-Liverpool Canal.

14 **ABSTRACT**

15 Freshwater ecosystems are under threat from habitat loss, partly due to urban expansion. However,
16 some elements of urban freshwaters are already integral parts of the urban landscape and so are
17 more resilient to loss, representing opportunities for the enhancement of freshwater resources within
18 cities. This study investigated the biodiversity value of the Leeds-Liverpool Canal in Leeds, UK, in
19 relation to its landscape context. Specifically, we tested the hypotheses that (i) biodiversity value is
20 lowest nearest to the urban core, and (ii) the pattern of canal locks structured ecological communities.
21 Nutrients, metals and dissolved carbon all existed at relatively low concentrations, contrary to what is
22 often seen in urban water bodies, although concentrations were higher in the urban core. This
23 gradient of chemical stress was associated with a decline in macroinvertebrate diversity towards the
24 city centre, which manifested as pollution-sensitive taxa being excluded from this area. Community
25 structures were found to vary between groups of sampling sites separated by locks, suggesting that
26 locks may act as barriers for aquatic invertebrates by restricting dispersal. The results in this study
27 indicate that canals in urban areas can be high-quality habitats, despite the associated anthropogenic
28 stressors, and locks may represent a unique model for researching relationships between connectivity
29 and community structure.

30

31 Keywords: canal, macroinvertebrate, pollution, connectivity, biodiversity, community structure

32

33 **Introduction**

34 Anthropogenic activities are continuing to alter the environment, resulting in the loss of biodiversity
35 and changes in the structure and functionality of natural ecosystems. Anthropogenic stressors include
36 habitat loss, pollution, the introduction of invasive species and the over-exploitation of natural
37 resources, among others (Sala *et al.* 2000). These changes are a substantial threat to the ecosystem
38 services necessary for human society, such as food and water production, carbon storage, climate
39 regulation and nutrient cycling (Hooper *et al.* 2005). Urbanisation is one of the primary causes of
40 habitat loss and is a major threat to biodiversity as it impairs ecosystems more severely than
41 conversion to other types of land use (McKinney 2006). Urbanisation leads to habitat fragmentation

42 (York *et al.* 2011), improves the fitness of invasive species (McKinney 2006) and impairs gene flow by
43 restricting the dispersal of many species (Riley *et al.* 2005). Urbanisation also causes global habitat
44 homogenisation to the extent that cities on different continents can support more similar biodiversity
45 than a city and its surrounding countryside (McKinney 2006). The majority of people in developed
46 countries live in cities and urban populations are growing in many developing countries (Cohen 2006).
47 As the global human population continues to grow, urban areas are expected to expand to
48 accommodate them (Seto *et al.* 2012). As a result, the total land area covered by urban environments
49 globally is expected to increase by 85% by 2030 (Seto *et al.* 2012). With current rates of biodiversity
50 loss and the expansion of urban areas, it is important to incorporate biodiversity into urban planning to
51 create more sustainable cities, buffering the damage caused by inevitable urbanisation in the future
52 (Kowarik 2011). In addition to reducing biodiversity loss, ecosystems in urban environments are
53 beneficial for human health and well-being (Dallimer *et al.* 2012). Urban ecology has largely been
54 overlooked in comparison to the study of 'natural' ecosystems. However, for the reasons described
55 above, urban ecosystems have received more attention from ecologists in recent years (Kowarik
56 2011).

57

58 Freshwater ecosystems are among the many affected by urbanisation. Indeed, freshwater
59 ecosystems are experiencing a greater rate of biodiversity loss than many of their terrestrial
60 counterparts, due to freshwaters often exhibiting higher initial levels of biodiversity (Sala *et al.* 2000).
61 Vermonden *et al.* (2009) showed that urban drainage systems can support comparable levels of
62 biodiversity to rural drainage systems and natural watercourses, and that they can support IUCN Red
63 List species. Despite the high levels of biodiversity that freshwater ecosystems can support (Lundberg
64 *et al.* 2000; Finlay 2002; Dudgeon *et al.* 2006), they cover only 0.8% of the Earth's surface and
65 contain 0.01% of the Earth's water (Dudgeon *et al.* 2006). This relatively scarce resource is estimated
66 to be of enormous value in terms of ecosystem services, such as food production, drinking water,
67 waste assimilation and recreation. In their estimation of the value of ecosystem services, Costanza *et al.*
68 (1997) valued those associated with freshwater at US\$6,579 x 10⁹ per year, exceeding the
69 estimated value of all others except marine ecosystems.

70

71 Biodiversity in urban freshwaters, in particular, is not well understood. Besides natural waterbodies
72 such as rivers and streams, artificial freshwaters are common in urban areas and are created for a
73 wide range of functions, including transport and flood management, as well as aesthetic and
74 recreational purposes (Hassall 2014). The potential that these waterbodies have for providing islands
75 of habitat in an urban setting and thus increasing biodiversity has long been overlooked and
76 potentially underestimated (Hill *et al.* 2017). For example, networks of urban ponds increase
77 biodiversity in urban areas by acting as 'stepping stones', thereby allowing species to move through
78 urban landscapes (Fortuna *et al.* 2006; Garden *et al.* 2010), and urban ponds can support high levels
79 of biodiversity, despite the increase in anthropogenic stressors (Brand *et al.* 2010). Previous studies
80 have shown that urban ponds contain significantly greater macroinvertebrate family diversity ($15.1 \pm$
81 0.6 SE) families than non-urban ponds (13.6 ± 0.3), although species richness did not differ (Hill *et al.*
82 2018).

83

84 The ecology of canals is under-represented in the urban and freshwater ecology literature despite
85 their prevalence in urbanised landscapes. There are 3,500km of canals in the UK alone, representing
86 at least 7.4km² of freshwater (assuming an average width of 2.1m). While some research has focused
87 on the relationship between canals and the dispersal of invasive species (Pimentel 2005; Leuven *et*
88 *al.* 2009), few have investigated the ecological and physical parameters of canals, such as their
89 hydrology, biodiversity, community structures or ecosystem functions, such as nutrient cycling
90 (Vermonden *et al.* 2009). As a result, their value as urban habitats and as refuges for freshwater
91 organisms is unclear (Chester & Robson 2013). As part of the European Union Water Framework
92 Directive (WFD), European rivers are expected to meet targets of good ecological quality, and are
93 therefore monitored for relevant ecological variables (e.g. biodiversity and physio-chemical
94 parameters; European Environment Agency 2017). Canals are rarely included in the WFD and so little
95 of their ecological quality is known. Even with the WFD in place, many rivers do not meet the targets
96 (Haase *et al.* 2013). With the lack of environmental targets set for canals in the UK and elsewhere, it
97 is likely that their potential for providing a good-quality habitat is not being utilised effectively. Canals
98 have a contrasting morphology to natural waterbodies such as rivers and streams; canals are
99 generally straighter and often have vertical banks, resulting in reduced morphological complexity.
100 Canals also have a unique hydrology and connectivity due to the action of locks. By opening and

101 closing locks, sections of a canal alternate between a chain of standing, isolated waterbodies and a
102 connected, running waterbody. Additionally, while the flow of water is generally downstream,
103 adhesion on canal boats and the action of bow waves result in some upstream movement (Liddle &
104 Scorgie 1980). The action of locks may facilitate the transport of nutrients, pollutants and organisms
105 between sections of a canal and there is evidence that hydrological connectivity across canal
106 networks drives changes in algal community structure (Kelly & Hassall 2018). Furthermore, when a
107 lock opens, the increased turbidity of the water elevates the concentration of dissolved oxygen, which
108 has the potential to increase productivity and ecosystem function (Boets *et al.* 2010). These features
109 make canals unique habitats, which, due to a lack of previous research, have uncertain effects on
110 canal biodiversity. Like other urban ecosystems, canals are often subject to higher levels of pollution
111 when compared with rural freshwaters (Paul & Meyer 2001). While rural freshwaters can be polluted
112 by agricultural run-off, canals are often polluted due to their proximity to roads and industrial sites, and
113 from sewage outlets that feed into the water (Paul & Meyer 2001). However, due to the lack of
114 research on canal ecology, the effects of pollution on biodiversity and community structure are
115 unclear (Gessner *et al.* 2004).

116

117 Research into the biodiversity and biotic community structure of canals, as well as the effects of
118 pollution and lock-generated connectivity, could contribute to a greater understanding of the value of
119 canals as urban ecosystems and their ecological functionality. Improving understanding of the
120 ecology of canals is imperative to improving management practices to ensure that canals are
121 managed in a way that increases urban biodiversity without negative effects, such as transferring
122 invasive species (Pimentel 2005) or the development of algal blooms (Vos & Roos 2005). Such
123 improvements would contribute to alleviating the biodiversity loss associated with urban expansion
124 and improving the quality of urban ecosystems for the benefit of human health and well-being. The
125 aim of this study was to investigate the biodiversity value of canals in cities, focusing on the roles of
126 water quality and lock-mediated connectivity. To do this, macroinvertebrate and water samples were
127 collected from a 2km stretch of the Leeds-Liverpool Canal in northern England. Two hypotheses were
128 tested: (1) macroinvertebrate diversity decreases with increasing nutrient and heavy-metal pollution
129 through the exclusion of pollution-sensitive taxa at more polluted sites and (2) locks structure
130 macroinvertebrate community assemblages by creating barriers between sites.

131

132 **Methods**

133 Fieldwork took place in Leeds, West Yorkshire, UK, on the Leeds-Liverpool Canal, which runs
134 between Hull and Liverpool, connecting the North and Irish Seas. The canal sits within the 1,100km²
135 catchment of the River Aire. This catchment has a population density of 1,000 people per km²
136 concentrated in major conurbations of Leeds and Bradford in the lower catchment where our study is
137 situated. The upper catchment is dominated by grassland and pastoral landscape with low population
138 densities. Our site sits within the most highly urbanised area of the Aire catchment and so might
139 represent one of the more impacted areas of freshwater in the region. A 2km stretch of the canal from
140 Leeds city centre (53.793°N, -1.550°E) towards Armley (53.802°N, -1.576°E) (Fig. 1) was used in this
141 study, in which 15 sites were selected. This stretch of canal is publicly accessible via a towpath that
142 runs along its northern bank. The southern bank varies in accessibility. The canal has no strict
143 dredging regime and is dredged on an ad-hoc basis. The section of canal used in this study has not
144 been dredged since at least 2010 and high levels of silt on the canal bed have been reported,
145 although no data regarding silt composition or concentrations are available. A stretch of canal that is
146 designated as a site of special scientific interest (SSSI; sites allocated legal protection under UK
147 legislation for their wildlife and/or geological interest, which are maintained to preserve or enhance
148 their habitats or features; Natural England 2013) is located 750m west of the most westward site. All
149 sites were at least 100m away from locks and each other. All fieldwork was conducted with
150 permission from the Canal and River Trust and took place in June and July 2019.

151

152 Due to a lack of information regarding the water quality and pollution levels of the site, a wide range of
153 potential pollutants, including nutrients, metals and dissolved carbon, were tested for: ammonia (NH₄),
154 nitrite (NO₂), nitrate (NO₃), phosphate (PO₄), calcium (Ca), magnesium (Mg), sodium (Na), potassium
155 (K), arsenic (As), aluminium (Al), lead (Pb), zinc (Zn), copper (Cu), cobalt (Co), manganese (Mn),
156 chromium (Cr), cadmium (Cd), iron (Fe), dissolved organic carbon (OC) and dissolved inorganic
157 carbon (IC). Two water samples were collected at each site between 24th June and 8th July 2019. A
158 bucket, fixed to a rope, was lowered into the canal to collect a mixed water sample that included the
159 lower and middle depths of the canal. The water was mixed and two water samples were then taken

160 from the bucket in 50ml sampling pots. Water samples were then filtered using 0.45µm nylon syringe
161 filters that had been washed using deionised water. All samples were filtered on the same day as
162 collection. For each water sample, 10ml of filtered water was pipetted into 15ml test tubes in
163 preparation for analysis for nutrients and metals. For samples to be analysed for metals, 15µl of
164 concentrated nitric acid (HNO₃; ARISTAR grade) was then pipetted into each sample to reduce the
165 loss of metals between filtration and analysis. For dissolved carbon analysis, 2ml glass auto-sampler
166 vials were filled with filtered water from each sample. Nutrients, dissolved carbon and metals were
167 analysed using an Auto Analyser (Skalar San++), Combustion Analyser (Analytik Jena Multi N/C
168 2100) and Inductively Coupled Plasma – Optical Emission Spectrophotometer (ICP-OES; Thermo
169 Scientific iCAP7600), respectively. Analyses were performed on individual tubes, rather than in
170 replicates.

171

172 Invertebrate samples were taken via sweep sampling, using a pond net fixed to an extendable metal
173 pole. The net was used to disturb the canal bed before being moved in a figure-of-eight motion five
174 times. The contents of the net were then emptied into a plastic tray containing a small amount of
175 canal water. Any large pieces of vegetation were checked for invertebrates before being discarded.
176 The contents of the tray were then emptied into a 4l sampling pot and taken to the laboratory for
177 identification. Invertebrates were identified to family level (Dobson *et al.* 2012) and the number of
178 individuals of each family was recorded. The identified invertebrates were then stored in ethanol, so
179 that they could be reviewed later if required. All invertebrate samples were identified and preserved
180 on the same day as collection to reduce the likelihood of predation within the sample. All sampling
181 equipment was soaked in hot water for at least 30 minutes after use to ensure that successive
182 samples were not contaminated. Two samples were collected at each site. The first samples were
183 taken between 24th June and 10th July 2019 and the second samples were taken between 12th July
184 and 22nd July 2019. Sites were sampled in the same order both times so that the length of time
185 between sample collection was roughly consistent across all 15 sites.

186

187 Statistical analysis of the data was carried out in R v3.5.1 (R Core Team 2018). To test the effects of
188 water chemistry variables on macroinvertebrate diversity and community structure, water chemistry

189 variables were combined into principal components (PCs) and Shannon's Diversity Index (H' ;
190 Shannon 1948) was calculated for each macroinvertebrate sample using the "vegan" R package
191 (Oksanen *et al.* 2019). Shannon's Diversity Index was chosen over other indices because it accounts
192 for evenness of species, rather than being skewed by the presence of rare species, of which some
193 were found. Shannon's Diversity Index has also been commonly used in previous studies
194 investigating freshwater invertebrate diversity (Hirst *et al.* 2002; Moore & Palmer 2005), so the use of
195 this index allows for direct comparisons to be made with existing literature. Mean diversity was
196 calculated for each site and relationships between the first three PCs and macroinvertebrate diversity
197 were tested. A redundancy analysis (RDA) was then calculated using water chemistry PCs, sites and
198 taxa, and relationships between PCs and community structure were tested.

199

200 To test the effects of lock-mediated connectivity on community structure, sampling sites 2-7 were
201 grouped into "Lock A", 8-9 into "Lock B", 10-11 into "Lock C" and 12-15 into "Lock D" based on groups
202 of sites separated by locks (Fig. 1). An RDA was carried out using lock groups, sites and taxa, and
203 differences in community structure between lock groups was tested using a permutational ANOVA.

204

205 **Results**

206 Of the water chemistry variables tested, all were found to be present in the water samples except Cd,
207 Co and Cr. K and Mg were excluded from statistical analysis due to them showing little variation
208 between sites. Cu, Al, As and Pb were also excluded due to concentrations at or below the lower
209 detection limits of the instruments used ($Cu \leq 0.004 \text{ mg/L}$, $Al \leq 0.03 \text{ mg/L}$, $As \leq 0.2 \text{ mg/L}$, $Pb \leq 0.1 \text{ mg/L}$) and
210 showing little variation between sites. The mean concentrations of water chemistry variables at each
211 site are shown in Table S1.

212

213 Pearson correlation tests were used to test for collinearity between all chemical variables except Zn,
214 for which a Spearman's rank test was used. The correlation tests were carried out in a matrix, using
215 the "Hmisc" R package (Harrell *et al.* 2019). Chemical concentrations were scaled so that the varying
216 magnitudes did not cause the data to skew. A principal component analysis (PCA) was then used to

217 visualise the correlations between variables (Fig. 2A) and the resulting PCs were used to explain
218 general patterns in the correlated water chemistry variables. PC1 has positive loadings ($R>0.6$) of
219 NH_4 , NO_2/NO_3 , PO_4 , ZN, and MN and negative loadings of Na. PC2 has positive loadings of NO_2 , Ca,
220 and inorganic C. Pearson correlations showed that there was a significant decline in PC1 across the
221 sites ($R=-0.812$, $P<0.001$; Fig. 2B), but not in PC2 ($R=-0.325$, $P=0.238$) or PC3 ($R=-0.041$, $P=0.886$).

222

223 A total of 6636 macroinvertebrate specimens were collected across the 15 sampling sites, belonging
224 to 19 orders and 35 families (Table S2). Site 1 was the least diverse (Shannon's diversity 0.490),
225 while site 12 was the most diverse (2.044). There was a significant increase in diversity from sites 1 to
226 15 ($R=0.575$, $P=0.025$; Fig. 3). Correlation tests showed that there was a significant correlation
227 between diversity and water chemistry PC1 ($R=-0.575$, $P=0.025$) but not between diversity and PC2
228 ($R=-0.036$, $P=0.899$) or PC3 ($R=0.229$, $P=0.411$).

229

230 Macroinvertebrate community data were transformed using Hellinger transformation in order to give
231 low weight to instances where no or small numbers of animals were found. A redundancy analysis
232 (RDA) was then calculated using water chemistry PCs, sites and taxa, and a subsequent ANOVA-like
233 permutation test indicated that there were relationships between the first three PCs and community
234 structure (PC1: $F_{1,11}=2.465$, $P=0.030$; PC2: $F_{1,11}=3.203$, $P=0.013$; PC3: $F_{1,11}=2.358$, $P=0.036$).

235 Lumbriculidae was found to be marginally positively associated with PC1 ($R=0.514$, $P=0.050$) and
236 Asellidae was found to be positively associated with PC2 ($R=0.544$, $P=0.036$).

237

238 An RDA was calculated using lock groups, sites and taxa, and a subsequent one-way ANOVA
239 showed that there was a significant difference in community structure among lock groups
240 ($F_{3,10}=2.852$, $P=0.002$; Fig. 4). One-way ANOVAs showed that there was a significant difference in
241 Asellidae ($F_{3,10}=9.415$, $P=0.003$), Chydoridae ($F_{3,10}=4.372$, $P=0.033$) and Gammaridae ($F_{3,10}=4.302$,
242 $P=0.034$) abundance between lock groups. Tukey post-hoc tests showed that the greatest differences
243 were between groups A and D for Asellidae ($P=0.002$) and Chydoridae ($P=0.021$), and between
244 groups A and C for Gammaridae ($P=0.034$).

245

246 **Discussion**

247 Both water chemistry and locks were found to have a significant association with macroinvertebrate
248 community structure. Macroinvertebrate diversity significantly increased with increasing distance from
249 the city centre – a pattern associated with lower concentrations of certain pollutants. Invertebrate
250 communities also showed strong structuring by locks, which may indicate a role for lock-mediated
251 connectivity in driving community organisation.

252

253 Overall, the stretch of canal that was used in this study exhibited low concentrations of pollutants for
254 an urban waterbody (Smolders 2003; Santoro *et al.* 2009). We find a mean concentration of
255 0.051mg/L (range 0.019-0.101) for NH₄, 0.038 (0-0.077) for NO₃ and 0.022 (0.009-0.045) for PO₄.
256 These concentrations are lower than those found in an urban river catchment in northern England
257 (NH₄: 0.500 [0.400-0.600]; NO₃: 2.88 [0.900-4.300]; PO₄: 0.360 [0.100-0.600]) (Medupin 2019).
258 Concentrations of NO₃ and PO₄ have also been shown increase with urbanisation (Rothwell *et al.*
259 2010). Most of the water chemistry variables were also low in comparison to a rural upland headwater
260 (NH₄: 0.060 [<0.004-2.160]; NO₃: 2.000 [<0.500-6.000]) and a rural lowland catchment with intensive
261 cattle farming (NH₄: 1.080 [0.020-13.800]; NO₃: 9.000 [<0.500-52.000]) (Jarvie *et al.* 2008), both in
262 south west England. However, we did find a higher mean concentration of Ca (38.967mg/L [38.700-
263 41.900]) than the aforementioned rural waterbodies (2.300 [0.9-11.6] and 24.900 [10.200-103.000],
264 respectively), although the maximum Ca concentration found in the lowland catchment was higher
265 than that of our study. Ca has been shown to vary considerably across catchments (0.002-
266 6636.000mg/L), with less variability in NH₄ (0.100-30.000) and NO₃ (0.001-1.600) (Rothwell *et al.*
267 2010). The low concentrations of heavy-metals found in water samples, and the absence of some
268 such as Cd and Pb, is particularly surprising given the well-documented association between these
269 pollutants and run-off from industries in urban environments (Davis *et al.* 2001). This is especially true
270 for this study site, as there is a lack of riparian vegetation at the site, which would be expected to
271 coincide with higher concentrations of pollutants associated with road run-off and other typical urban
272 pollution sources due to a lack of filtration and phytoremediation by riparian plants (Schlosser & Karr
273 1981; Valera *et al.* 2019). While the site is close to a stretch of canal designated as a SSSI,

274 suggesting the pollution levels may be low, the apparent high water quality shown in this study
275 indicates that this is a high-quality habitat despite its location in the centre of a large city. That being
276 said, there are still variations in the water chemistry variables found that correlate with the biological
277 community of the Leeds-Liverpool Canal.

278

279 The results from this study show that canal habitats can contain comparable macroinvertebrate
280 diversity to other, more natural habitat types. We find a mean family richness of 10.6 (range 4-20)
281 which is similar to reports of agricultural UK ditch habitats (mean 9.0 [2-15] and 9.8 [1-18]), while
282 being lower than agricultural ponds (16.3 [2-32] and 14.5 [3-27]), rivers (29.4 [16-36]), or streams
283 (13.9 [3-31] and 12.5 [6-16]) (Davies *et al.* 2008), as well as lower than UK urban ponds (15.1 [2-46])
284 (Hill *et al.* 2018) and a nearby UK urban river (19.8 [13-23]) (Medupin 2020). Macroinvertebrate
285 diversity significantly increased from sites 1-15, moving out from Leeds city centre. The results also
286 show a significant decrease in diversity as PC1 increases, indicating a shift in water chemistry
287 variables from higher concentrations of Na, NO₂ and IC towards higher concentrations of NH₄, Mn,
288 PO₄, NO₃ and Zn. One contributing factor in this shift in water chemistry along the stretch of canal is
289 possibly the sewage outlet approximately 90m east of site 1 (Fig. 1), as water chemistry variables
290 associated with sewage, such as NH₄, NO₃ and PO₄ (Neal *et al.* 2005), are in higher concentrations
291 closer to site 1. This may cause the exclusion of more pollution-sensitive taxa, such as
292 Ephemeroptera and Coleoptera, close to the sewage outlet, and may explain the dominance of highly
293 tolerant taxa, such as Lumbriculidae, at site 1 (Lumbriculidae accounted for 32% of the mean number
294 of organisms found at site 1 across the two samples). Lumbriculidae are highly tolerant to pollution
295 and are low-scoring on water quality measuring procedures such as the biological monitoring working
296 party (BMWP; Paisley & Walley 2014). Santoro *et al.* (2009) found that, of the macroinvertebrates that
297 they studied, Lumbriculidae was the only taxon to be resistant to polluted sites in their study of a
298 heavily polluted river in Italy. They found that all organisms had large quantities of heavy-metals in
299 their tissues, though this apparently did not affect the fitness of the Lumbriculidae. In our study,
300 Ephemeroptera, Coleoptera and some Trichoptera were more abundant and diverse in sites furthest
301 from the city centre. Buss *et al.* (2002) found that chironomids (Diptera), a pollution-tolerant taxon,
302 increased in abundance with higher levels of pollution. While chironomids were found to be almost
303 ubiquitous in this study (present at sites 2-15), they were absent from the site with seemingly the

304 worst water quality and were not found to associate significantly with water chemistry PCs. Similarly to
305 this study, Godfrey (1978) found that Asellidae were ubiquitous, likely due to their tolerance to
306 pollution. They also found that not all Trichoptera were affected by pollution. In this study,
307 Leptoceridae (Trichoptera) were found at all sites except site 1, despite them apparently being
308 pollution-sensitive indicators of good water quality, according to the BWMP (Paisley & Walley 2014). It
309 is unclear why this taxon would be so widespread when other Trichoptera, such as Odontoceridae
310 (found only at sites 12 and 13), appeared to be more restricted, but it is interesting that another study
311 found a similar inconsistency in the distribution of Trichoptera (Godfrey 1978). Hirst *et al.* (2002) found
312 that macroinvertebrate diversity decreased with increasing metal pollution. However, in contrast to our
313 study, they found that a rise in pollution increased evenness rather than excluding sensitive taxa. It is
314 clear that there is still a lack of consensus on the exact effects of water chemistry variations on
315 freshwater ecosystems, exacerbated by the fact that different studies test for different variables,
316 making comparisons problematic.

317

318 In other habitat types, it might have been tempting to suggest that other factors could have influenced
319 macroinvertebrate diversity or the variations in community structure. Heino (2000) found that physical
320 habitat variables such as size of waterbody, depth and vegetation cover accounted for more variation
321 in macroinvertebrate community structure than water chemistry variables. For example, crustaceans
322 were ubiquitous throughout the stretch of canal; isopods and amphipods were found at all sites and
323 water fleas were found at all sites except site 4. These taxa are less effective colonisers than semi-
324 aquatic taxa such as Diptera and may favour the stability of canals and potentially other man-made,
325 highly maintained habitats (Gasith & Resh 1999). However, much of the difficulty in making
326 comparisons between the findings of this study and others is that there is a lack of studies
327 investigating canal ecology, so comparisons can only be drawn from studies researching other types
328 of waterbody. The hydromorphological structure of canals is relatively uniform and so this variable is
329 unlikely to be a key factor. Some studies have found that the influence of water chemistry variables is
330 secondary to that of hydrological connectivity (Heino 2000; Gallardo *et al.* 2008), especially as
331 variations in water chemistry are often related to connectivity (Zimmer *et al.* 2000). It is, therefore,
332 difficult to say whether variations in water chemistry are truly driving differences in diversity and
333 community structure in this study, or whether all three variables are similarly influenced by

334 connectivity. However, again the aforementioned studies have investigated natural waterbodies with
335 different connectivity mechanisms to canals. Connectivity within the canal network is present at a low
336 level, with a steady but slow flow from summit to mouth and long retention times during periods of low
337 lockage. Further research into the interactions between variations in water chemistry and the unique
338 connectivity system of canals is needed. For example, do pollutants accumulate downstream as a
339 result of the direction of flow? Do locks act as barriers, reducing the speed of movement of pollutants
340 through canal systems? Where water quality is low due to point source pollution, how far from the
341 source are the effects felt? Answering questions such as these would provide a greater understanding
342 of how (or even *if*) pollutants are transported through canal networks.

343

344 Another potential factor that could contribute to the observed variations in diversity and community
345 assemblage is the proximity of the tested sites to surrounding waterbodies. Ponds and ditches in the
346 surrounding area could provide a source of taxa that can disperse to the canal. If this is the case, it
347 may explain the increase in macroinvertebrate diversity from sites 1-15, as more built-up urban areas
348 make it more difficult for invertebrates to disperse (Riley *et al.* 2005). As a result, sites further from the
349 city centre may be more likely to be colonised by nearby habitats. For example, some studies have
350 found that invertebrate diversity increases along an urban-rural gradient, with diversity and species
351 richness in agricultural areas being double that of urban areas (Moore & Palmer 2005). However, the
352 densely urbanised land surrounding the stretch of canal that was studied here lacks almost any
353 surrounding water bodies within 1km of the canal apart from the River Aire which flows in parallel
354 along the whole stretch.

355

356 Groups of sites separated by locks were found to have significantly different community structures.
357 This would suggest that, despite the fact that lock gates open frequently, allowing water to flow
358 between sections, locks act as barriers for aquatic organisms and limit dispersal, therefore creating
359 differences in community structure. Taking the overall low concentrations of water chemistry variables
360 in this particular stretch of the canal into account, the effects of connectivity could be considered to be
361 more important than the effects of variations in water chemistry. In the RDA (Figure 4), the majority of
362 taxa are aggregated in the centre of the plot, indicating a lack of variation in the abundance of these

363 taxa between lock groups. It is interesting that none of the outlying taxa away from the central cluster
364 are semi-aquatic, with the exception of Leptoceridae (Trichoptera). Many semi-aquatic taxa, such as
365 flying insects, are effective dispersers due to the adults' ability to fly between potential breeding sites
366 without having to rely as heavily on connectivity as fully aquatic taxa (Bilton *et al.* 2001). It may be
367 that, for this reason, locks are relatively ineffective as barriers to semi-aquatic taxa compared to
368 others such as the crustaceans Asellidae, Gammaridae and Chydoridae, although dispersal rates of
369 aquatic invertebrates that rely on vectors other than flight have not been well documented
370 (Vanschoenwinkel *et al.* 2008). However, it is difficult to confirm whether macroinvertebrate
371 assemblages are truly structured by locks or simply as a function of distance between sites and the
372 dispersal abilities of the taxa in question. Figure 4 shows that lock groups A-C are arranged in a
373 somewhat linear orientation, with group D removed from the trend. It may be that the differences in
374 community structure between groups A-C are due to the distances between them and the inability of
375 taxa to disperse over those distances, rather than because of a lack of connectivity. However, Figure
376 4 does not suggest a gradual trend through ordination space that might be indicative of isolation by
377 distance, but rather a clustering of sites within the lock groups. Interestingly, our data for canals
378 resemble findings from semi-natural systems of linked ponds: spatial structuring of community
379 structure at a larger scale with influence of local hydrogeochemical factors (Cottenie *et al.* 2001).
380 Future studies investigating the differing representation of semi-aquatic and fully aquatic taxa in
381 canals, over a larger spatial scale than this study, could allow for a better understanding of the effects
382 of locks on the dispersal of different taxa.

383

384 Other studies have shown that hydrological connectivity is a crucial factor in predicting
385 macroinvertebrate community structures (Gallardo *et al.* 2008) and this hydrological connectivity has
386 been shown to influence algal community structure in canals (Kelly & Hassall 2018). Taken together,
387 these findings suggest that canal lock systems could be analogous to other hydrological networks and
388 may provide an interesting testbed for ecological hypotheses about connectivity. For example, some
389 studies have investigated the effects of connectivity between rivers and floodplain wetlands, but,
390 unlike sections of canals, these systems are often connected for relatively short periods of time and
391 over a greater distance (Bornette *et al.* 2002; Sheldon *et al.* 2002). It is clear that more research into
392 the effects of connectivity on the biology of canals is needed.

393

394 **Conclusion**

395 This study presents evidence that canals can have high water quality and support a diverse range of
396 macroinvertebrates, even in urban areas. With urban areas expected to continue to expand in the
397 next decade (Seto *et al.* 2012), it is important that urban habitats such as canals are managed in a
398 way that allows them to support as much biodiversity as possible, for the benefit of both wildlife and
399 humans (Pimentel *et al.* 1997). Additionally, this study may be the first to attempt to evaluate the
400 effects of locks on biodiversity and community structures. If locks are in fact the cause of the
401 observed differences in macroinvertebrate community structure, as the results suggest, this study
402 presents evidence that community assemblages in canals are structured in a way that is unique to
403 this type of waterbody. Therefore, canals may represent not only potential ecosystems for increasing
404 biodiversity in urban spaces, but also a unique model system for researching the effects of
405 connectivity on biotic community structures.

406

407 Moving forward, it is clear that more research on canal ecology is needed in order to put the results of
408 this study into context and to assess the ecological quality of other canals. One issue in interpreting
409 the results of this study is that the vast majority of studies investigating the effects of water chemistry
410 and connectivity on biodiversity were researching natural waterbodies, making comparisons difficult.
411 More research into this topic on other canals, particularly in more polluted areas and over larger
412 spatial scales, would help to improve understanding of ecological patterns in canals. Results of such
413 research would help to inform protection legislation and management decisions that could improve
414 biodiversity in canals and the wider urban landscape. Currently, some canal management decisions
415 are made purely with the needs of humans in mind, rather than biodiversity. For example, the stretch
416 of canal used in this study is only dredged when sediment accumulation becomes an issue for boats,
417 and lock gates are only opened for passing boats. If, for example, locks are consistently found to limit
418 the dispersal of certain species, opening lock gates more regularly may help to promote dispersal.
419 Alternatively, if locks are found to limit the dispersal of invasive species like *Dikerogammarus villosus*,
420 locks may be a useful tool in slowing their spread. However, further research into specific
421 management decisions such as this would be required to assess their effectiveness.

422

423 **References**

424 Bilton DT, Freeland JR, Okamura B (2001) Dispersal in freshwater invertebrates. *Annu Rev Ecol Syst*
425 32:159-181.

426 Boets P, Lock K, Goethals PL (2011) Using long-term monitoring to investigate the changes in
427 species composition in the harbour of Ghent (Belgium). *Hydrobiologia* 6631:55-166.

428 Bornette G, Amoros C, Lamouroux N (1998) Aquatic plant diversity in riverine wetlands: the role of
429 connectivity. *Freshwater Biol* 39:267-283.

430 Brand AB, Snodgrass JW (2010) Value of artificial habitats for amphibian reproduction in altered
431 landscapes. *Conserv Biol* 24:295-301

432 Buss DF, Baptista DF, Silveira MP, Nessimian JL, Dorvillé LF (2002) Influence of water chemistry and
433 environmental degradation on macroinvertebrate assemblages in a river basin in south-east
434 Brazil. *Hydrobiologia* 481:125-136.

435 Chester ET, Robson BJ (2013) Anthropogenic refuges for freshwater biodiversity: their ecological
436 characteristics and management. *Biol Conserv* 166:64-75.

437 Cohen B (2006) Urbanization in developing countries: Current trends, future projections, and key
438 challenges for sustainability. *Technol Soc* 28:63-80.

439 Costanza R, d'Arge R, De Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill RV,
440 Paruelo J, Raskin RG (1997) The value of the world's ecosystem services and natural
441 capital. *Nature* 387:253-260.

442 Cottenie K, Nuytten N, Michels E, De Meester L (2001) Zooplankton community structure and
443 environmental conditions in a set of interconnected ponds. *Hydrobiologia* 442:339-350.

444 Dallimer M, Irvine KN, Skinner AM, Davies ZG, Rouquette JR, Maltby LL, Warren PH, Armsworth PR,
445 Gaston KJ (2012) Biodiversity and the feel-good factor: understanding associations between self-
446 reported human well-being and species richness. *BioScience* 62:47-55.

447 Davies B, Biggs J, Williams P, Whitfield M, Nicolet P, Sear D, Bray S, Maund S (2008) Comparative
448 biodiversity of aquatic habitats in the European agricultural landscape. *Agr Ecosyst Environ* 125:1-8

449 Davis AP, Shokouhian M, Ni S (2001) Loading estimates of lead, copper, cadmium, and zinc in urban
450 runoff from specific sources. *Chemosphere* 44:997-1009.

451 Dobson M, Pawley S, Fletcher M, Powell A (2012). *Guide to freshwater invertebrates. The Freshwater*
452 *Biological Association. Ambleside, UK.*

453 Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Lévêque C, Naiman RJ, Prieur-
454 Richard AH, Soto D, Stiassny ML, Sullivan CA (2006). *Freshwater biodiversity: importance, threats,*
455 *status and conservation challenges. Biol Rev* 81:163-182.

456 European Environment Agency (2017) <https://www.eea.europa.eu/themes/water/intro>. Accessed
457 August 2019.

458 Esri (2017) *ArcGIS Desktop Release 10.1*. Redlands, CA.

459 Finlay BJ (2002) Global dispersal of free-living microbial eukaryote species. *Science* 296:1061-1063.

460 Fortuna MA, Gómez-Rodríguez C, Bascompte J (2006) Spatial network structure and amphibian
461 persistence in stochastic environments. *P Roy Soc B-Biol Sci* 273:1429-1434.

462 Gallardo B, García M, Cabezas Á, González E, González M, Ciancarelli C, Comín FA (2008)
463 Macroinvertebrate patterns along environmental gradients and hydrological connectivity within a
464 regulated river-floodplain. *Aquat Sci* 70:248-258.

465 Garden JG, McAlpine CA, Possingham HP (2010) Multi-scaled habitat considerations for conserving
466 urban biodiversity: native reptiles and small mammals in Brisbane, Australia. *Landscape*
467 *Ecol* 25:1013-1028.

468 Gasith A, Resh VH (1999) Streams in Mediterranean climate regions: abiotic influences and biotic
469 responses to predictable seasonal events. *Annu Rev Ecol Syst*, 30:51-81.

470 Gessner MO, Inchausti P, Persson L, Raffaelli DG, Giller PS (2004) Biodiversity effects on ecosystem
471 functioning: insights from aquatic systems. *Oikos* 104:419-422.

472 Godfrey PJ (1978) Diversity as a measure of benthic macroinvertebrate community response to water
473 pollution. *Hydrobiologia* 57:111-122.

474 Haase P, Hering D, Jähnig SC, Lorenz AW, Sundermann A (2013) The impact of hydromorphological
475 restoration on river ecological status: a comparison of fish, benthic invertebrates, and
476 macrophytes. *Hydrobiologia* 704:475-488.

477 Harrell FE Jr, with contributions from Charles Dupont and many others (2019) Hmisc: Harrell Miscella
478 neous. R package version 4.2-0. <https://CRAN.R-project.org/package=Hmisc>

479 Hassall C (2014) The ecology and biodiversity of urban ponds. *WIREs Water*, 1:187-206.

480 Heino J (2000) Lentic macroinvertebrate assemblage structure along gradients in spatial
481 heterogeneity, habitat size and water chemistry. *Hydrobiologia* 418:229-242.

482 Hill, MJ, Biggs J, Thornhill I, Briers RA, Gledhill DG, White JC, Wood PJ, Hassall C (2017) Urban
483 ponds as an aquatic biodiversity resource in modified landscapes. *Glob Change Bio* 23:986-999.

484 Hirst H, Jüttner I, Ormerod SJ (2002) Comparing the responses of diatoms and macro-invertebrates
485 to metals in upland streams of Wales and Cornwall. *Freshwater Biol* 47:1752-1765.

486 Hooper DU, Chapin FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M,
487 Naeem S, Schmid B (2005) Effects of biodiversity on ecosystem functioning: a consensus of current
488 knowledge. *Ecol Monogr* 75:3-35.

489 Kelly LA, Hassall C (2018) The spatial ecology of phytoplankton blooms in UK canals. *Inland Waters*
490 8:422-433.

491 Kowarik I (2011) Novel urban ecosystems, biodiversity, and conservation. *Environ Pollut* 159:1974-
492 1983.

493 Leuven RS, van der Velde G, Baijens I, Snijders J, van der Zwart C, Lenders HR, bij de Vaate A
494 (2009) The river Rhine: a global highway for dispersal of aquatic invasive species. *Biol*
495 *Invasions* 11:1989.

496 Liddle MJ, Scorgie HRA (1980) The effects of recreation on freshwater plants and animals: a
497 review. *Biol Conserv* 17:183-206.

498 Lundberg JG, Kottelat M, Smith GR, Stiassny ML, Gill AC (2000) So many fishes, so little time: an
499 overview of recent ichthyological discovery in continental waters. *Ann Mo Bot Gard* 87:26-62.

500 McKinney ML (2006) Urbanization as a major cause of biotic homogenization. *Biol Conserv* 127:247-
501 260.

502 Medupin C (2019) Distribution of benthic macroinvertebrate communities and assessment of water
503 quality in a small UK river catchment. *SN Applied Sciences* 1:544.

504 Medupin C (2020) Spatial and temporal variation of benthic macroinvertebrate communities along an
505 urban river in Greater Manchester, UK. *Environ Monit Assess* 192:84.

506 Moore AA, Palmer MA (2005) Invertebrate biodiversity in agricultural and urban headwater streams:
507 implications for conservation and management. *Ecol Appl* 15:1169-1177.

508 Muñoz I, Prat N (1996) Effects of water abstraction and pollution on macroinvertebrate community in
509 a Mediterranean river. *Limnetica* 12:9-16.

510 Natural England (2013) Sites of special scientific interest: managing your land.
511 <http://gov.uk/guidance/protected-areas-sites-of-special-scientific-interest>. Accessed 24th May 2020

512 Neal C, Jarvie HP, Neal M, Love AJ, Hill L, Wickham H (2005) Water quality of treated sewage effluent
513 in a rural area of the upper Thames Basin, southern England, and the impacts of such effluents on
514 riverine phosphorus concentrations. *J Hydrol* 304:103-1177.

515 Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlenn D, Minchin PR, O'Hara RB, Simp
516 son GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2019) *Vegan: Community Ecology Packag*
517 *e*. R package version 2.5-5. <https://CRAN.R-project.org/package=vegan>.

518 Paisley MF, Trigg DJ, Walley WJ (2014) Revision of the biological monitoring working party (bmwp) sc
519 ore system: derivation of present-only and abundance-related scores from field data. *River Res App*
520 *l* 30:887-904.

521 Paul MJ, Meyer JL (2001) Streams in the urban landscape. *Annu Rev Ecol Syst* 32:333-365.

522 Pimentel D, Wilson C, McCullum C, Huang R, Dwen P, Flack J, Tran Q, Saltman T, Cliff B (1997)
523 Economic and Environmental Benefits of Biodiversity. *BioScience* 47:747-757.

524 Pimentel D (2005) Aquatic nuisance species in the New York State Canal and Hudson River systems
525 and the Great Lakes Basin: an economic and environmental assessment. *Environ Manage* 35:692-
526 702.

527 R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statis
528 tical Computing, Vienna, Austria. URL: <https://www.R-project.org/>.

529 Riley SP, Busted GT, Kats LB, Vandergon TL, Lee LF, Dagit RG, Kerby JL, Fisher RN, Sauvajot RM
530 (2005) Effects of urbanization on the distribution and abundance of amphibians and invasive species
531 in southern California streams. *Conserv Biol* 19:1894-1907.

532 Rothwell JJ, Dise NB, Taylor KG, Allott TEH, Scholefield P, Davies H, Neal C (2010) A spatial and
533 seasonal assessment of river water chemistry across North West England. *Sci Total Environ* 408:841-
534 855.

535 Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF,
536 Jackson RB, Kinzig A, Leemans R (2000) Global biodiversity scenarios for the year 2100. *Science*
537 287:1770-1774.

538 Santoro A, Blo G, Mastrolitti S, Fagioli F (2009) Bioaccumulation of heavy metals by aquatic
539 macroinvertebrates along the Basento River in the South of Italy. *Water Air Soil Poll* 201:19-31.

540 Schlosser IJ, Karr JR (1981) Riparian vegetation and channel morphology impact on spatial patterns
541 of water quality in agricultural watersheds. *Environ Manage* 5:233-243.

542 Seto KC, Güneralp B, Hutyra LR (2012) Global forecasts of urban expansion to 2030 and direct
543 impacts on biodiversity and carbon pools. *P Natl Acad Sci USA* 109:16083-16088.

544 Shannon CE (1948) A mathematical theory of communication. *Bell Syst Tech J* 27:379-423.

545 Sheldon F, Boulton AJ, Puckridge JT (2002) Conservation value of variable connectivity: aquatic
546 invertebrate assemblages of channel and floodplain habitats of a central Australian arid-zone river,
547 Cooper Creek. *Biol Conserv* 103:13-31.

548 Smolders AJP, Lock RAC, Van der Velde G, Hoyos RM, Roelofs JGM (2003). Effects of mining
549 activities on heavy metal concentrations in water, sediment, and macroinvertebrates in different
550 reaches of the Pilcomayo River, South America. *Arch Environ Con Tox* 44:314-323.

551 Tiegs SD, Clapcott JE, Griffiths NA, Boulton AJ (2013) A standardized cotton-strip assay for
552 measuring organic-matter decomposition in streams. *Ecological indicators* 32:131-139.

553 Valera CA, Pissarra TCT, Valle Júnior RFD, Oliveira CF, Moura JP, Sanches Fernandes LF, Pacheco
554 FAL (2019) The buffer capacity of riparian vegetation to control water quality in anthropogenic
555 catchments from a legally protected area: a critical view over the Brazilian new forest
556 code. *Water* 11:549.

557 Vanschoenwinkel B, Gielen S, Vandewaerde H, Seaman M, Brendonck L (2008) Relative importance
558 of different dispersal vectors for small aquatic invertebrates in a rock pool metacommunity. *Ecography*
559 31:567-577.

560 Vermonden K, Leuven RS, van der Velde G, van Katwijk MM, Roelofs JG, Hendriks AJ (2009) Urban
561 drainage systems: An undervalued habitat for aquatic macroinvertebrates. *Biol Conserv* 142:1105-
562 1115.

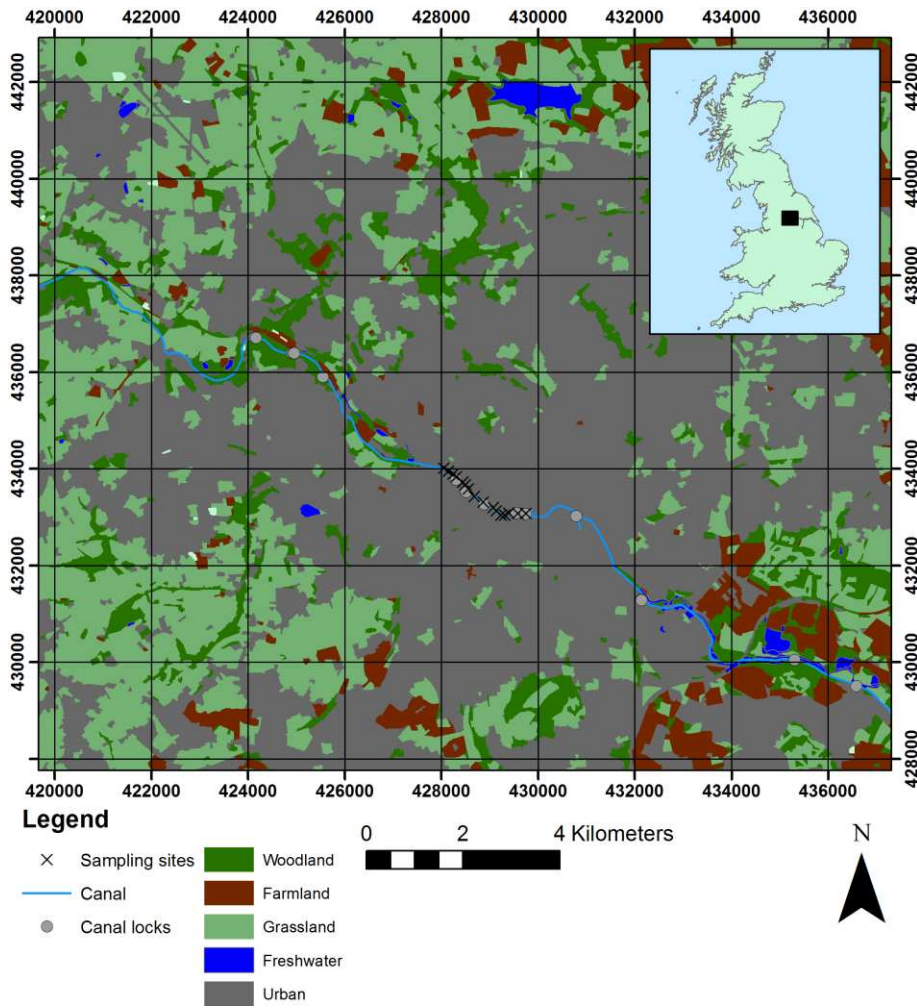
563 Vos AT, Roos JC (2005) Causes and consequences of algal blooms in Loch Logan, an urban
564 impoundment. *Water SA* 31:385-392.

565 York AM, Shrestha M, Boone CG, Zhang S, Harrington JA, Prebyl TJ, Swann A, Agar M, Antolin MF,
566 Nolen B, Wright JB (2011) Land fragmentation under rapid urbanization: A cross-site analysis of
567 Southwestern cities. *Urban Ecosyst* 14:429-455.

568 Zimmer KD, Hanson MA, Butler MG (2000) Factors influencing invertebrate communities in prairie
569 wetlands: a multivariate approach. *Can J Fish Aquat Sci* 57:76-85.

570

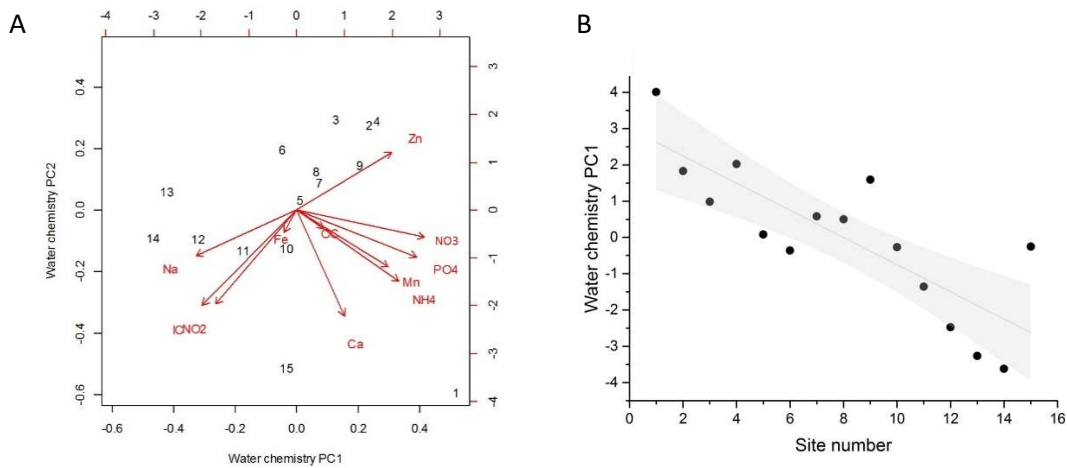
571 **Figures**



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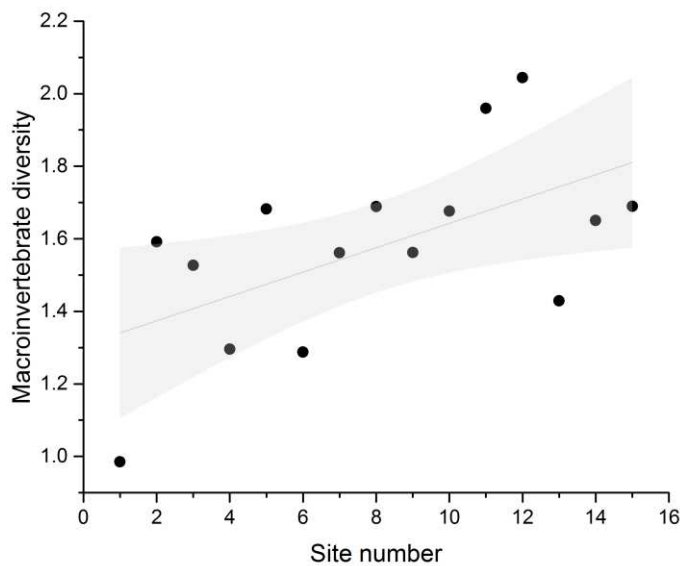
573 **Figure 1** Map showing the location of sampling sites and locks on the Leeds-Liverpool Canal,
 574 England. Sites are numbered 1-15 from East to West. Locations of the sewage outlet and water
 575 abstraction point were provided by the Canal and River Trust.

576

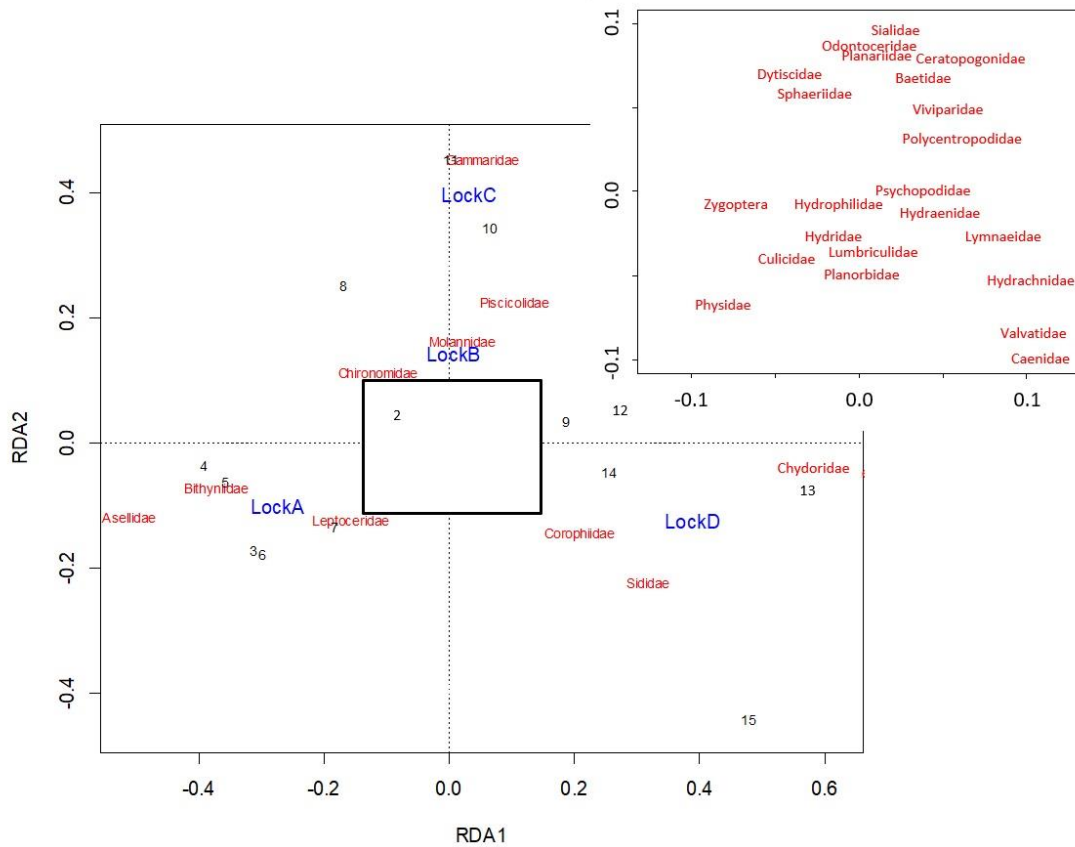


577

578 **Figure 2 A)** Principal components analysis of water chemistry variables for the 15 sampling sites.
 579 Numbers represent sites, which are consecutive along the canal from east (closest to urban core) to
 580 west (furthest from urban core). B) PC1 represents a shift from higher concentrations of Na, NO₂ and
 581 inorganic carbon (IC) to higher concentrations of NH₄, Mn, PO₄, NO₃ and Zn, and was found to have a
 582 significant negative relationship with site. The trendline represents a significant correlation (R=-5.013,
 583 P<0.001) and the shaded area represents the 95% CI of the regression line. PC2 represents a shift
 584 from higher concentrations of Zn to higher concentrations of IC, NO₂ and Ca, and did not have a
 585 significant relationship with site (R=1.238, P=0.238).



586
 587 **Figure 3** Shannon's Diversity Index significantly increased across sampling sites. The trendline
 588 represents a significant correlation (R=0.575, P=0.025) and the shaded area represents the 95% CI of
 589 the regression line.
 590



591

592 **Figure 4** Redundancy analysis (RDA) showing the effects of lock groups (A, B, C, and D) on
 593 macroinvertebrate community structure. Numbers represent sites, which are consecutive along the
 594 canal from east to west. The central region indicated by the black square is expanded in the upper
 595 right to show species more clearly.

596

597 **Appendix**

598 **Table S1** Mean concentration (mg/L) of water chemistry variables found in water samples from each
 599 site. OC and IC refer to organic carbon and inorganic carbon, respectively.

Site	NH4	NO2	NO2+NO3	PO4	Ca	Na	Zn	Mn	Fe	OC	IC
1	0.101	0.003	0.08	0.043	40.7	12.894	0.007	0.043	0.050	0.81	34.74
2	0.047	0.001	0.05	0.020	38.7	12.346	0.007	0.035	0.009	1.90	32.49
3	0.021	0.001	0.04	0.022	39.0	12.473	0.007	0.032	0.038	1.29	32.91
4	0.061	0.003	0.06	0.031	37.8	12.689	0.013	0.027	0.055	0.71	32.70
5	0.050	0.002	0.05	0.024	39.5	13.905	0.006	0.015	0.067	1.55	33.49
6	0.045	0.002	0.04	0.024	38.2	14.078	0.006	0.023	0.099	1.03	32.92
7	0.058	0.002	0.06	0.023	38.7	13.398	0.007	0.021	0.042	1.58	34.02
8	0.061	0.002	0.04	0.023	38.7	13.331	0.008	0.024	0.053	1.06	33.67

9	0.071	0.002	0.04	0.024	39.2	12.736	0.013	0.024	0.023	1.14	33.69
10	0.061	0.002	0.04	0.018	39.6	14.353	0.008	0.025	0.069	1.67	34.83
11	0.034	0.005	0.04	0.015	39.6	13.987	0.009	0.024	0.017	1.62	35.26
12	0.036	0.004	0.04	0.010	38.9	14.109	0.001	0.020	0.072	1.65	35.25
13	0.036	0.004	0.02	0.016	38.0	13.950	0.000	0.021	0.011	0.00	35.90
14	0.020	0.005	0.01	0.013	38.9	13.701	0.000	0.023	0.060	0.63	36.06
15	0.073	0.006	0.04	0.029	39.4	13.608	0.000	0.032	0.057	1.93	35.24

600

601 **Table S2** Abundance of macroinvertebrate families. ST refers to site number and SA refers to sample

602 number (i.e. ST1SA1 is the first sample taken from site 1).

Halipidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
Baetidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	1	
Hydrachnida	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	2	0	2	0	10	0	33
Sidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	25	300	20
Hydridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Phryganeida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Corophiidae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	0	3	1	1	3	2	
Hydrophilida	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

Hydraenidae	Planariidae	Polycentropo	Odontocerid	Sialidae	Viviparidae	Molannidae	Caenidae	Valvatidae	Lymnaeidae	Psychodidae	Chaoboridae
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	2	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	4	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	4	1	0	0
0	3	0	0	0	0	0	0	0	0	0	0
0	0	2	0	0	0	0	0	0	2	0	0
0	0	2	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0
0	0	0	1	1	1	4	0	0	0	0	0
0	0	0	0	2	0	0	0	0	0	0	0
0	0	0	0	1	1	0	6	3	0	0	0
0	0	0	0	0	1	0	2	0	0	0	0
0	0	0	0	0	0	0	2	1	2	1	0
0	0	2	0	0	0	1	0	1	1	0	0
0	0	0	0	0	0	0	0	2	0	0	2
10	0	4	0	1	0	0	0	2	2	0	1
0	0	9	0	0	0	0	3	3	6	0	0
0	1	0	0	0	1	0	0	1	2	0	0

Chydoridae	Gammaridae	Zygoptera	Leptoceridae	Bithyniidae	Planorbidae	Piscicolidae	Chironomida	Sphaeriidae	Ceratopogon	Physidae	Dysticidae
2	0	0	0	0	0	0	0	0	0	0	0
3	18	0	0	0	0	0	0	0	0	0	0
1	3	0	0	0	0	0	0	0	0	0	0
4	5	0	1	1	0	0	1	0	0	2	0
8	4	4	8	45	1	0	0	0	0	0	0
3	19	0	6	7	1	0	1	9	1	0	0
0	6	0	4	11	0	1	50	0	0	0	0
0	11	0	3	3	1	6	6	4	0	0	0
6	22	6	10	62	0	8	43	8	0	0	0
1	14	0	0	4	0	1	3	3	1	0	0
40	24	1	20	57	1	5	18	2	4	1	0
1	22	0	2	34	3	3	6	20	0	1	0
6	7	1	5	0	0	0	4	0	0	0	1
0	2	0	5	0	0	0	3	0	0	1	0
21	133	0	6	55	3	104	13	16	0	0	0
8	135	0	4	27	2	48	2	15	2	8	0
300	38	0	4	7	1	2	23	10	0	0	0
0	56	0	4	13	0	6	4	1	0	0	0
11	15	0	0	0	0	15	4	0	0	0	0
9	19	0	1	0	0	1	3	0	1	0	0
6	23	1	0	2	0	0	10	4	1	0	1
2	8	0	1	0	0	0	0	1	0	0	0
5	6	0	2	0	0	0	7	1	0	0	0
15	15	0	1	0	0	10	0	0	0	0	0
400	30	0	0	0	0	3	17	2	3	0	0
85	15	0	5	0	4	9	2	2	3	0	0
300	55	1	2	0	1	3	6	8	2	0	0
5	100	0	1	0	1	4	3	2	0	1	0
200	13	1	3	2	0	0	9	1	2	1	0
100	31	0	3	0	0	0	1	4	3	0	0

Family	Lumbriculida	Asellidae	Culicidae
ST1SA1	20	1	1
ST1SA2	0	15	0
ST2SA1	1	6	0
ST2SA2	1	11	0
ST3SA1	0	78	0
ST3SA2	0	38	1
ST4SA1	0	104	1
ST4SA2	7	90	0
ST5SA1	7	274	0
ST5SA2	7	12	0
ST6SA1	1	405	1
ST6SA2	0	174	0
ST7SA1	0	41	0
ST7SA2	0	17	0
ST8SA1	12	288	0
ST8SA2	1	152	0
ST9SA1	21	123	0
ST9SA2	7	55	0
ST10SA1	0	42	0
ST10SA2	0	9	0
ST11SA1	1	16	0
ST11SA2	0	4	0
ST12SA1	0	3	0
ST12SA2	0	23	0
ST13SA1	0	23	0
ST13SA2	7	15	0
ST14SA1	12	231	0
ST14SA2	25	91	0
ST15SA1	4	59	1
ST15SA2	1	69	0