

1 **Distribution of macroinvertebrate communities across surface and groundwater**
2 **habitats in response to hydrological variability**

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14
15 **Abstract**

16 Macroinvertebrate communities are strongly influenced by hydrological variability in
17 surface waters. However, the response of these communities in corresponding
18 groundwater-dependent habitats is not well understood. This study characterised the
19 macroinvertebrate fauna and physicochemical characteristics of a chalk aquifer and
20 its river in southern England. Samples were collected over one year from three
21 perennial and two temporary paired benthic-hyporheic sites and seven phreatic sites
22 in the surrounding aquifer. The study was preceded by a period of below average
23 rainfall, providing an opportunity to assess the response of macroinvertebrate
24 communities to unseasonal declines in river discharge and groundwater levels.
25 Benthic, hyporheic and phreatic habitats each supported a distinct macroinvertebrate
26 community, with the hyporheic habitat supporting both epigeal taxa and stygofauna.
27 As discharge declined, the composition of these communities changed. In particular,
28 the abundance of the epigeal amphipod *Gammarus pulex* was higher in hyporheic
29 than benthic habitats during periods of low surface discharge, suggesting potential
30 refuge-seeking behaviour. Similarly, fluctuations in the abundance and distribution of
31 two stygofauna, *Crangonyx subterraneus* and *Niphargus fontanus*, coincided with
32 marked changes in groundwater levels, suggesting that the contraction of available
33 habitat and changes in connectivity also influenced the phreatic community. The
34 variable distribution of macroinvertebrates between these habitats, especially in
35 response to hydrological variability, suggests a dynamic connection between the river
36 and its aquifer. This connection is an important consideration for the assessment and
37 conservation management of both surface and groundwater communities and may
38 help to underpin integrated, catchment-based environmental management, especially
39 in river systems with temporary reaches.

40
41 **Keywords:** *Groundwater Ecology, Benthic, Hyporheic, Macroinvertebrate, Flow*
42 *Permanence, Drought*

43
44 **Introduction**

45 Hydrological variability is a primary influence on the structure of lotic habitats
46 and communities (Dole-Olivier & Marmonier 1992, Monk et al. 2008). Variability occurs
47 as a result of disturbance events, such as floods and droughts, or predictably in
48 temporary rivers, which periodically cease to flow and may dry (Lake 2003, Datry et
49 al. 2016). While periods of high discharge amplify hydrological connectivity, periods of

50 low discharge and drying cause aquatic habitats to contract, reducing connectivity and
51 leading to fragmentation (Lake 2003).

52 The relationship between the benthic community, which comprises epigean
53 species occupying the upper layer of the substratum, and hydrological variability has
54 been widely investigated (Extence et al. 1999, Dunbar et al. 2010, Datry et al. 2014,
55 White et al. 2017). The response of the benthic taxa to periods of low discharge and
56 streambed drying varies. While some species can be lost if they become trapped or
57 stranded in conditions exceeding their tolerance, others may persist using species-
58 specific physiological, morphological, life strategy or behavioural adaptations (Lytle &
59 Poff 2004). One behavioural adaptation is the vertical migration of normally benthic
60 taxa into deeper sediments during periods of adverse conditions (the Hyporheic
61 Refuge Hypothesis; Palmer et al. 1992; Dole-Olivier 2011, Stubbington 2012).
62 However, our understanding of the responses of communities occupying groundwater-
63 dependent (hyporheic and phreatic) habitats to hydrological variability is limited (but
64 see Wood et al. 2010, Stubbington & Wood 2013, Stubbington et al. 2015, Reiss et al.
65 2019).

66 The hyporheic habitat comprises the interstitial spaces in the sediments
67 beneath the streambed where exchange occurs between surface water and
68 groundwater (Orghidan 1959). Connectivity between the river and its aquifer shapes
69 hyporheic communities which comprise temporary and permanent residents with
70 varying degrees of surface and groundwater affinity, including generalist epigean
71 species, hyporheic specialists and taxa which spend all or part of their life cycle in
72 groundwater-dominated habitats (stygo fauna; Gibert et al. 1994, Hancock et al. 2005).

73 The phreatic habitat occurs in the aqueous voids and interstices within an
74 aquifer, with biological communities occurring most commonly in porous or fractured
75 aquifers (such as limestone or chalk) that provide hydrogeological connectivity
76 (Robertson et al. 2009, Johns et al. 2015). The phreatic community comprises
77 stygo fauna, many members of which occur nowhere else and display convergent
78 morphological, physiological and behavioural adaptations such as the loss of
79 pigmentation, ocular regression and vermiform body shape, which facilitate the
80 exploitation of this dark, resource and nutrient-poor environment (Gibert et al. 1994,
81 Robertson et al. 2009). Internationally, stygo fauna comprise a diverse range of
82 nematodes, beetles, crustaceans and snails; however, in Great Britain they are limited
83 to less than 30 recorded species, seven of which are macroinvertebrates (not including
84 *Antrobathynella stammeri*; Robertson et al. 2009, Knight & Gledhill 2010). The relative
85 contribution of surface and groundwater to hyporheic and phreatic habitats can vary
86 spatially and temporally, while hydrological connectivity is considered to be the primary
87 factor shaping these communities, they are also influenced by geology,
88 hydrometeorological fluctuations and anthropogenic pressures (Poole et al. 2006,
89 Thulin & Hahn 2008).

90 The assessment of benthic, hyporheic and phreatic communities and their
91 responses to hydrological variability requires an integrated approach which recognises
92 the connectivity between them. However, most previous work has focussed on the
93 assessment of a single habitat (or by exception, paired benthic and hyporheic studies,
94 such as Datry et al. (2007), Wood et al. (2010), Peralta-Maraver et al. (2018)) and has
95 often been limited to a single sampling occasion or season. Our aim was to address
96 this research gap by exploring the spatial and temporal distribution of
97 macroinvertebrates across all three habitats during a one-year period of hydrological
98 variability. We hypothesized that: (1) distinct macroinvertebrate communities would
99 occur within each habitat, but (2) that these communities would increasingly converge

100 during periods of low river discharge and declining groundwater levels as connectivity
101 decreased and available habitat contracted.

102

103 **Study area**

104 The study area, a temperate groundwater-dominated system in the North
105 Downs (Kent, south-east England), included five riverine sites on the Little Stour River
106 (and its tributary, the Nailbourne, 51°15'53.0"N, 1°09'11.7"E) and River Dour
107 (51°08'45.6"N, 1°15'35.1"E) and seven phreatic sites across their associated aquifer
108 (Fig.1). The study area is underlain by Carboniferous Chalk, a geology which forms
109 semi-karstic aquifers characterised by high rates of transmissivity and hydraulic
110 conductivity (Allen et al. 1997). The rivers display features typical of lowland chalk
111 streams, such as tufa deposits and characteristic flora (*Hildenbrandia* and *Ranunculus*
112 *penicillatus* [*pseudofluidans*]). The surrounding land use is predominantly agricultural
113 with some residential areas.

114 The hydrological regime follows a characteristic, seasonal pattern, with peak
115 discharge between December and February and baseflow occurring between August
116 and September (Wood and Armitage 2004). Mean annual catchment precipitation is
117 593 mm (Met Office, Manston Weather Station, 51° 35' N, 1° 34'E; 49 m.a.s.l.; 1981-
118 2010). However, this study was conducted between November 2011 and September
119 2012, coinciding with an extended period of below average rainfall which resulted in
120 limited winter recharge and drought conditions both locally and nationally (Fig. 2;
121 Marsh et al. 2013). Within the study area, below average rainfall extended baseflow
122 conditions into November 2011. Riverine discharge remained below the long-term
123 average at perennial sites but flow did not cease, suggesting that this drought was not
124 as severe locally as previous events (1949, 1991-92 and 1996-97) which caused parts
125 of the river to dry completely (Wood et al. 2004, Wood et al. 2010). Groundwater levels
126 were also below average during the study period, ranging from 0.27 to -9.72 m.a.s.l.
127 below site-specific, long-term averages recorded by the Environment Agency. The
128 drought broke in April 2012 following a period of high rainfall. River discharge returned
129 to typical seasonal magnitudes in May 2012 and groundwater levels returned to usual
130 seasonal levels at most sites in July 2012.

131 Flow permanence differed between riverine sites. The two headwater sites (1
132 and 5) are located downstream of near-perennial springheads (which cease to flow
133 only during extended periods of low rainfall) and are temporary, drying predictably
134 during summer months both historically (Holmes 2006) and during this study. Site 1
135 dried in November 2011, with no water recorded in either the benthic or hyporheic
136 habitats, prohibiting sample collection. Discharge from the springhead upstream of
137 Site 1 resumed in January 2012, but connectivity between this site and the
138 downstream sections of the river was not restored for the duration of the study. Flow
139 ceased at site 5, although water remained in both benthic and hyporheic habitats in
140 isolated pools. Sites further downstream (2, 3 and 4) were characterised by perennial
141 discharge during the study period, although some have historically dried during supra-
142 seasonal droughts (Wood et al. 2010).

143

144 **Material and Methods**

145 *Invertebrate sampling and processing*

146 Samples were collected bimonthly: paired benthic and hyporheic samples from
147 the five riverine sites, and phreatic samples from seven unscreened boreholes and
148 wells. The boreholes and wells were between 3 and 30 m deep with a diameter of 25

149 to 85 cm and were situated between 5 and 6000 m from the river channel. All of the
150 boreholes and wells, with the exception of Sites A and C, were covered.

151 At each riverine site, replicate benthic and hyporheic samples were collected at
152 four points distributed equidistantly across the channel. Benthic samples were
153 collected using a Surber sampler (0.1 m² frame, 63-µm mesh net) in which the
154 substratum was manually disturbed to a depth of 5 cm for 30 seconds. Hyporheic
155 samples were collected using a Bou-Rouch pump (Duncan and Associates, England;
156 unmodified) in which a perforated steel pipe was driven 30-50 cm into the substratum
157 using a sledgehammer before priming the pump with filtered river water and extracting
158 6 L of water. Phreatic samples were collected using a weighted net (Institut Für
159 Grundwasser Ökologie, Germany; unmodified, 63-µm mesh) which was lowered to the
160 bottom of the borehole or well and then raised and lowered 10 times (Malard et al.
161 2002).

162 All invertebrate samples were sieved to a fraction 63-µm for consistency
163 between habitats. As this study focussed on the comparison of macroinvertebrate
164 assemblages between habitats, meiofauna (Acari, Copepoda, Nematoda, Ostracoda
165 and Cladocera) and Oligochaeta were excluded from the analyses.
166 Macroinvertebrates were live sorted under a stereomicroscope within 48 hours of
167 sample collection. Stygofauna were preserved in >90% ethanol and all other fauna in
168 70% Industrial Methylated Spirits, before identification to species level (with the
169 exception of some early instar larvae, damaged specimens, and Diptera) using
170 standard keys.

171

172 *Characterisation of abiotic variables*

173 Temperature (°C), pH, conductivity (µS cm⁻¹), dissolved oxygen (mg L⁻¹) and total
174 alkalinity (mg L⁻¹) were measured in the field using calibrated standard portable meters
175 (Hach HQ Series) and test kits (Hach Alkalinity Titration). Dissolved oxygen was not
176 measured in hyporheic samples due to the bias produced by the sampling method.
177 Water samples from all three habitats were filtered using Whatman GF/C Glass
178 Microfibre Filters and returned to the laboratory for nutrient (PO₄³⁻ and NO₃⁻) and
179 geochemical (calcium, strontium, magnesium, sodium and potassium) analyses using
180 Hach Lange DR2800 spectrophotometer and Varian 720-ES Inductively Coupled
181 Plasma Optical Emission Spectrometer.

182 Water width, depth and mean flow velocity (0.6 × depth) were recorded at each
183 riverine site. Flow velocity was measured using a Valeport impeller flow meter
184 mounted on a wading rod (BFM 002 S-N 1855). These measurements were used to
185 calculate discharge after Fetter (2001) and contextualised using long-term continuous
186 hydrological data provided by the Environment Agency from the Littlebourne
187 Ultrasonic Gauge, the Crabble Mill v-notch weir (Fig. 1) and meteorological data from
188 the Met Office (Manston weather station). Groundwater levels over the study period
189 were assessed using water level data collected by the Environment Agency at each
190 phreatic site.

191

192 *Data analysis*

193 Spatiotemporal variability between sites, sampling occasions and habitats in
194 environmental parameters and biological metrics (abundance and richness) was
195 assessed using one-way analysis of variance (ANOVA) tests supplemented as
196 necessary by post-hoc Tukey (HSD) tests. All biological samples were ordinated using
197 non-metric multidimensional scaling (NMDS; Bray-Curtis distance matrix) to examine
198 broad-scale differences in composition by habitat. An iterative process was used to

199 determine the best fit for the ordination and tested using a Shepard plot to assess the
200 scatter around the fitted line. Due to the differences in collection methods between
201 habitats, NMDS was performed on untransformed proportional abundance rather than
202 abundance data (preliminary analyses indicated that presence / absence data
203 obscured variability between habitat types). The first hypothesis, that distinct
204 communities would occur within each habitat, was tested using Analysis of Similarities
205 (ANOSIM) to assess compositional differences between the invertebrates recorded in
206 these habitats. The second hypothesis, that communities would converge during
207 periods of low discharge and groundwater levels, was also tested using ANOSIM to
208 assess differences in community composition between the three habitats during
209 drought conditions (November to March). The test was repeated using samples
210 collected during post-drought conditions (May to September). Further ANOSIM testing
211 was undertaken to assess convergence between the benthic and hyporheic habitats
212 at temporary (1 and 5) and perennial (2, 3 and 4) sites during drought and post-drought
213 conditions. Similarity percentages (SIMPER) were used to identify influential taxa
214 between groups. The relationship between the abundance of influential taxa, habitat
215 and sampling occasion was analysed with two-way (interactive) ANOVA. The
216 distribution of influential taxa, and specifically their use of the hyporheic habitat, was
217 assessed by dividing the abundance of a taxon in the hyporheic habitat by its total
218 (benthic + hyporehic) abundance to calculate the hyporheic proportion (after
219 Stubbington et al. 2011). Caution should be exercised in interpreting the results of the
220 second hypothesis testing due to limited replication, particularly within the temporary
221 sites group. All analyses were performed in the R software platform version 3.4.2 (R
222 Core Team, 2016).

235

240 **Results**

241 **Physical and chemical variables**

242 Environmental conditions varied by site, sampling occasion and between
243 habitats (see Appendix 1 for mean \pm SE values and ANOVA results). Water
244 temperatures were cooler in the hyporheic (8.6-15.9°C) than the benthic (7.0-17.1°C)
245 and phreatic habitats (11-17.9°C), and varied by sampling occasion, with the lowest
246 values recorded during the winter (January) and highest values during the summer
247 (July and September), but were comparable within habitats and between temporary
248 and perennial sites. Dissolved oxygen did not vary statistically between sites or
249 sampling occasions in the benthic (7.4-14.3 mg L⁻¹) or phreatic (1.54–9.86 mg L⁻¹)
250 habitats and did not fall below documented thresholds of 1.0 mg L⁻¹ for stygofauna
251 persistence (Hahn 2006). Conductivity ranged from 458 to 747 μ S cm⁻¹ and was
252 spatially variable only between sites in the hyporheic habitat, with the highest values
253 recorded at temporary sites. Total alkalinity ranged from 89 to 336 mg L⁻¹ and was
254 spatially variable between sites in the benthic habitat, with the highest values recorded
255 at temporary sites. pH was typically circumneutral (6.7 to 8.6) but varied between sites
256 in the phreatic habitat, with the lowest values recorded at sites on the western edge of
257 the catchment. Nitrate (0.50 to 5.70 mg L⁻¹) and Phosphate (0.01 to 1.53 mg L⁻¹) did
258 not vary statistically by site or sampling occasion. Geochemical parameters varied
259 spatially and temporally, particularly for parameters related to the dissolution and
260 diagenesis of Chalk geologies. Calcium (66.38 to 141.19 mg L⁻¹) and Strontium (0.16
261 to 0.39 mg L⁻¹) both varied spatially between hyporheic sites, with higher values
262 recorded on the western edge of the catchment. Magnesium (1.99 to 4.77 mg L⁻¹) was
263 comparable between the three habitats and sampling locations but varied between
264 phreatic sites with higher values recorded at downstream locations on the Little Stour.

265 Sodium (0.41 to 1.36 mg L⁻¹) varied spatially between sites in the benthic and well as
266 phreatic habitats, with higher values recorded on the eastern side of the catchment,
267 and between sampling occasions in the hyporheic habitat. Potassium (0.09 to 1.59 mg
268 L⁻¹) was spatially and temporally comparable in benthic and hyporheic habitats but
269 varied between sampling occasions in the phreatic habitat, with the highest values
270 recorded during the summer (July).

271

272 **Benthic Community Composition and Distribution**

273 A total of 2,493 macroinvertebrate individuals representing 54 taxa were
274 recorded from 29 samples (Table 1). *Gammarus pulex* dominated the benthic
275 community, accounting for 31% of total macroinvertebrate abundance with *Agapetus*
276 *fuscipes* (17%), Chironomidae (9%), *Asellus aquaticus* (5%) and *Baetis rhodani* (5%)
277 comprising the next greatest proportions. No stygofauna were recorded within the
278 benthic habitat during the study period. Macroinvertebrates were recorded on all
279 sampling occasions except at Site 1 when it was dry. Three taxa were recorded at
280 Site 1 in January, following the resumption of discharge: *A. aquaticus* (n=1),
281 Chironomidae (n=1) and *G. pulex* (n=12), with *A. fuscipes* (n=1) additionally recorded
282 in March. Macroinvertebrate abundance did not vary significantly among sites, in
283 relation to flow permanence or between sampling occasions; but was highest in
284 November and lowest in January and March (Figs. 3 and 4). Richness did not vary by
285 site or sampling occasion but was lower at temporary sites (12.18 ± 1.63) than at
286 perennial sites (20.00 ± 1.07, ANOVA, p<0.001, F=18.3).

287

288 **Hyporheic Community Composition and Distribution**

289 A total of 402 macroinvertebrate individuals representing 10 taxa were recorded
290 from 29 hyporheic samples (Table 1). The community was dominated by the epigeal
291 amphipod *G. pulex*, which accounted for 84% of total macroinvertebrate abundance
292 with Chironomidae (8%), *A. fuscipes* (2%), *Elmis aenea* (2%) and *A. aquaticus* (2%)
293 comprising the next greatest proportions. The hyporheic community primarily
294 comprised epigeal taxa but also included stygofauna *Niphargus aquilex* (1%) and
295 *Crangonyx subterraneus* (<1%). Macroinvertebrates were recorded on all sampling
296 occasions except, as with the benthic community, at Site 1 when it was dry. Following
297 the resumption of discharge at Site 1, the same taxa were recorded in the hyporheic
298 habitat as the benthic habitat in January (*A. aquaticus* (n=1), Chironomidae (n=2) and
299 *G. pulex* (n=11)), with *E. aenea* (n=1) additionally recorded in March.
300 Macroinvertebrate abundance varied between sampling occasions (ANOVA, p=0.008;
301 F=8.37), with the highest values recorded in January and March (21.00 ± 3.77), but
302 did not differ between sites. Faunal richness varied by site (ANOVA, p=0.04 F=4.54)
303 and was markedly higher at Site 1 (3.67 ± 0.96), but did not differ between sampling
304 occasions. No significant differences in either abundance or richness were recorded
305 between temporary and perennial sites.

306

307 **Phreatic Community Composition and Distribution**

308 A total of 39 macroinvertebrate individuals representing four species of
309 stygofauna were recorded from 42 samples. *Niphargus kochianus* (46%) and *C.*
310 *subterraneus* (39%) were the most abundant while *N. fontanus* (13%) was the most
311 widespread (occurring at four sites). In addition, a single, eyeless individual of
312 Gammaridae (*Gammarus* sp.) was recorded at Site A, for which morphological
313 analysis was indeterminate (further molecular analyses were also indeterminate for
314 these specimen, please see Blackman et al. 2017 for methods), while polymorphism

315 among Gammaridae is well documented (Karaman and Pinkster, 1977), this record is
316 notable due to its location (6000 m from the river in a well 30 m deep). Two species of
317 Collembola, *Folsomia candida* (a cosmopolitan, unpigmented, blind springtail) and
318 *Heteromurus nitidus* (a cosmopolitan springtail which is not blind or unpigmented
319 despite its troglobite affiliation) were also recorded at four phreatic sites but were
320 omitted from further analyses as they are not representative of the aquatic
321 environment (Wilson 1975, Fountain & Hopkin 2005). Macroinvertebrates were
322 recorded at six out of the seven phreatic sites over the study period, but not at site B.
323 The number of positive samples varied spatially and temporally, with
324 macroinvertebrates recorded on every sampling occasion at Site A but only once at
325 sites C and D. The greatest number of positive samples occurred in July (n=6), when
326 *N. fontanus* was recorded at four sites close to the Little Stour River (<100 m; C-F)
327 having not previously been recorded in this habitat. Abundance did not vary
328 significantly between sites or sampling occasions, although there was a notable peak
329 of *C. subterraneus* individuals at Site E in March (n=10). Richness varied among sites
330 (ANOVA, p=0.001; F=4.72) and was greatest at Sites A and E (1.20 ± 0.13), but did
331 not differ between sampling occasions. No association was found between the number
332 of positive samples, abundance or richness and the depth, diameter, proximity of the
333 site to the river or if site was fitted with a cover.

334

335 **Community Composition and Distribution Across Three Habitats**

336 Community composition differed between the three habitats, with limited
337 overlap between the benthic and hyporheic habitats (ANOSIM, R=0.80; p=0.001; Fig.
338 5). The overlap between benthic and hyporheic habitats reflected the common
339 occurrence of influential taxa such *G. pulex* (SIMPER, 24% contribution) and *Agapetus*
340 *fuscipes* (15% contribution). Community composition did not converge between the
341 three habitats during (ANOSIM R=0.73; p=0.001) or following (R=0.74; p=0.001) the
342 drought. Benthic and hyporheic community composition did not converge at perennial
343 (ANOSIM, R=0.85; p=0.001) or temporary (R=0.33; p=0.002) sites. An interaction
344 between habitat and sampling occasion was identified for *G. pulex* abundance (two-
345 way ANOVA, p=0.02, F=2.99), which peaked in the hyporheic habitat in March,
346 coinciding with the lowest surface water discharge (Fig. 6). The calculated hyporheic
347 proportion of *G. pulex* reflected the pattern in Figure 6, in which proportion gradually
348 increased in the hyporheic habitat from November (0.41 ± 0.03) to January ($0.42 \pm$
349 0.02), peaking in March (0.56 ± 0.02) before declining through May (0.20 ± 0.02), July
350 (0.17 ± 0.03) and September (0.14 ± 0.03).

351

352

353 **Discussion**

354 *Community composition across the benthic, hyporheic and phreatic habitats*

355 The three habitats supported distinct macroinvertebrate communities, providing
356 support for the first hypothesis. The benthic community comprised epigeal taxa typical
357 of chalk streams in England (such as *A. fuscipes* and *Drusus annulatus*, Wood & Petts
358 1999). The distribution of the benthic community varied between temporary and
359 perennial sites, with the former supporting a greater number of specialist taxa
360 associated with drying events (such as *Anacaena limbata* and *Helophorus*
361 *brevipalpis*), reflecting the influence of discharge permanence on community
362 composition as highlighted in previous research (Wood & Petts 1999, Williams, 2006,
363 Stubbington et al. 2009, Datry et al. 2014).

364 The hyporheic community included both surface and groundwater fauna. With
365 the exception of *N. aquilex*, taxa were also recorded in benthic (*A. fuscipes*) and
366 phreatic (*C. subterraneus*) habitats. Compared to the other habitats, hyporheic
367 communities were characterised by greater variability. Temporal changes in
368 abundance, which was highest at the drought peak, and spatial differences in richness,
369 which was highest at temporary sites, suggests that the composition of this community
370 is more dynamic, especially during periods of low discharge. These results support
371 previous findings in which the abundance (and proportion) of benthic taxa, specifically
372 *G. pulex*, increased in the hyporheic habitat during periods of flow recession
373 (Stubbington et al. 2010, Stubbington et al. 2011). However, contrary to the findings
374 of Datry et al. (2007), hyporheic abundance did not vary significantly between
375 temporary and perennial sites. This difference is attributed to the similarities in
376 community composition between temporary and perennial sites across the study area
377 (Datry 2012).

378 The phreatic community was distinct, comprised exclusively of stygofauna
379 typical of carbonate aquifers in England (Johns et al. 2015, Maurice et al. 2016). The
380 abundance, richness and number of positive samples was consistent with similar
381 studies where stygofauna were found to be absent from up to 30% of sampled
382 boreholes (Hahn 2006; Johns et al. 2015). Although richness varied by site, this
383 community was the least dynamic of the three habitats, with little variation in
384 abundance or richness over the study period.

385 386 *Community response to changes in river discharge*

387 We did not find support for our second hypothesis, that communities between
388 habitats would converge during periods of low river discharge and declining
389 groundwater levels; however, the results do suggest species-specific responses to
390 these changes. For example, the hyporheic proportion of *G. pulex* was highest at the
391 drought peak when surface water discharge was lowest. Despite its benthic affiliations,
392 this species has been reported to migrate into hyporheic habitats in response to
393 adverse conditions including increased water temperatures, declining water levels and
394 biotic interactions (Dole-Olivier et al. 1997, Stubbington & Wood 2013, Vadher et al.
395 2018). The dominance of *G. pulex* within benthic and hyporheic habitats and its rapid
396 recovery following the drying of these habitats suggests that vertical migration enables
397 this species to enter, persist within and recolonise from hyporheic sediments in these
398 catchments. Similar patterns were observed for other predominantly epigeal species,
399 such as *A. aquaticus* and *E. aenea*, which were recorded in the hyporheic habitat at
400 Site 1 following rewetting of the dry channel. As surface water connectivity between
401 this site and the downstream reach was not re-established during the study, the
402 presence of these species suggests that hydrological connectivity was maintained
403 within the hyporheic habitat, supporting the Hyporheic Refuge Hypothesis.

404 Previous work has suggested that the hyporheic refuge may also be used by
405 stygofauna (specifically *N. aquilex* and *Proasellus cavaticus*), in response to adverse
406 groundwater conditions (Wood et al. 2010). Although we did not record this use of the
407 hyporheic habitat by stygofauna, our results do suggest their movement within the
408 wider aquifer in response to environmental variability. Specifically, the high number of
409 *C. subterraneus* observed in March and wider distribution of *N. fontanus* in July
410 corresponded to the lowest and highest groundwater levels (respectively) recorded
411 during the study period. Although the flow paths in this catchment are not well
412 understood, these results suggest that, as in surface water systems, hydrodynamic
413 factors such as habitat availability and connectivity may influence the distribution of

414 phreatic communities. This influence may be particularly pronounced in the study area
415 as the variable phreatic temperatures may indicate hydrological exchange with the
416 river. The Hyporheic Corridor Concept, describing the hyporheic habitat as a
417 subsurface corridor connecting laterally and longitudinally along the river (Stanford &
418 Ward 1993), is useful to characterise this connectivity. However, these results suggest
419 that the movement of individual species between habitats, especially in response to
420 hydrological variability, should be viewed as evidence of a spatiotemporally dynamic
421 connection between the river and its aquifer.

422

423 *Implications for ecological assessment and environmental management*

424 We used an integrated approach to assess the benthic, hyporheic and phreatic
425 habitats and the macroinvertebrate communities they support. While each of these
426 habitats supported distinctive communities, the results suggest a dynamic connection
427 between the rivers and their aquifer. This connection is particularly notable during
428 periods of hydrological variability and suggests responses from both surface and
429 groundwater communities. Recognition of this connection is important for the
430 assessment of ecological responses to, and recovery from, environmental change and
431 is especially relevant to temporary rivers, in which biological communities are more
432 likely to have adapted to regular drying events (Leigh et al. 2015). Further research is
433 required to determine the nature and extent of this connection and if it can be viewed
434 as a continuum.

435 Despite increasing support from environmental managers to take a catchment-
436 based approach (Defra 2013) and further integrate the management of surface and
437 groundwater systems (Environment Agency 2009), the ecological status of lotic
438 systems is conventionally based on the assessment of surface water habitats and
439 communities. Although groundwater community assessment frameworks have been
440 proposed (Hahn 2006, Griebler et al. 2010) and the requirement for an improved
441 understanding of the distribution, sensitivity and ecology of groundwater fauna has
442 been recognised (Robertson et al. 2008), these frameworks have also largely
443 focussed on a single habitat. This study contributes to the growing body of evidence
444 which suggests that our understanding of the status and functioning of lotic
445 ecosystems can be enhanced through the integration of surface and groundwater
446 assessments to the benefit of both sustainable management and the conservation of
447 groundwater communities (Gibert et al. 2009, Robertson et al. 2009, Boulton et al.
448 2010).

449

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461 not necessarily represent the views or policies of the Environment Agency.

462

463

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668 **Figure captions**

669 **Fig. 1.** The Nailbourne, Little Stour and Dour study area showing the location of paired
670 benthic-hyporheic riverine sampling sites (1-5) and their flow permanence, phreatic
671 sampling sites (A-G), and gauging stations (GS).

672
673 **Fig. 2.** Hydrograph of mean daily discharge ($\text{m}^3 \text{s}^{-1}$) recorded between January 2011
674 and October 2012 at the Environment Agency gauging stations at Littlebourne on the
675 Little Stour (solid line) and at Crabble Mill on the Dour (dotted line).

676
677 **Fig. 3.** Mean ± 1 SE macroinvertebrate abundance and richness by site in benthic (a-
678 b), hyporheic (c-d) and phreatic (e-f) habitats (respectively) from November 2011 to
679 September 2012 using habitat-specific sampling methods.

680
681 **Fig. 4.** Mean ± 1 SE macroinvertebrate abundance and richness by sampling occasion
682 in benthic (a-b), hyporheic (c-d) and phreatic (e-f) habitats, from November 2011 to
683 September 2012 using habitat-specific sampling methods.

684
685 **Fig. 5** Non-metric multidimensional scaling (NMDS) ordination plot of
686 macroinvertebrate taxa in benthic, hyporheic and phreatic habitats. Polygons enclose
687 samples in groups by habitat. Analysis performed on Bray-Curtis dissimilarities for
688 proportional abundance data over the study period.

689
690 **Fig. 6.** Mean ± 1 SE *Gammarus pulex* abundance in the benthic and hyporheic
691 samples from November 2011 to September 2012 using habitat-specific sampling
692 methods.

693
694 **Table captions**

695 **Table 1.** Presence (n samples) and occurrence (n sites) of macroinvertebrate taxa
696 with presence >1 , (except *Gammarus* sp.) in benthic, hyporheic and phreatic samples
697

698 **Table 1.** created in Excel

Taxon		Benthic		Hyporheic		Phreatic		
		n Sample	n Site	n Sample	n Site	n Sample	n Site	
Crustacea	<i>Asellus aquaticus</i>	26	5	5	1			
	<i>Crangonyx subterraneus</i>			1	1	6	3	
	<i>Gammarus pulex</i>	29	5	29	5			
	<i>Gammarus</i> sp.					1	1	
	<i>Niphargus aquilex</i>			3	2			
	<i>Niphargus fontanus</i>					4	4	
	<i>Niphargus kochianus</i>					9	3	
Ephemeroptera	<i>Proasellus meridianus</i>	11	3					
	<i>Baetis rhodani</i>	20	4					
	<i>Serratella ignita</i>	12	4					
Plecoptera	<i>Nemoura</i> sp.	2	1	1	1			
Trichoptera	<i>Agapetus fuscipes</i>	28	5	5	3			
	<i>Athripsodes</i> spp.	6	3					
	<i>Drusus annulatus</i>	2	2					
	<i>Hydropsyche siltalai</i>	3	2					
	<i>Hydroptila</i> sp.	6	3					
	<i>Limnephilus lunatus</i>	5	4					
	<i>Limnephilus marmoratus</i>	2	2					
	<i>Lype reducta</i>	2	1					
	<i>Polycentropus flavomaculatus</i>	2	2					
	<i>Rhyacophila dorsalis</i>	11	4					
	<i>Sericostoma personatum</i>	14	4					
	<i>Silo nigricornis</i>	9	4					
	Megaloptera	<i>Sialis lutaria</i>	7	3				
	Coleoptera	<i>Anacaena limbata</i>	2	1				
		<i>Elmis aenea</i>	21	4	5	2		
<i>Haliplus lineatocollis</i>		6	3					
<i>Helodes</i> sp.		5	2	2	1			
<i>Helophorus brevipalpis</i>		2	1					
<i>Hydrobius fuscipes</i>		2	1					
<i>Limnius volckmari</i>		21	3	2	2			
<i>Nebrioporus elegans</i>		7	2					
Mollusca	<i>Bithynia tentaculata</i>	14	3					
	<i>Lymnaea palustris</i>	3	2					
	<i>Lymnaea peregra</i>	4	2					
	<i>Pisidium</i> sp.	8	2					
	<i>Planorbis planorbis</i>	10	3					
	<i>Potamopyrgus antipodarum</i>	24	4					
Tricladida	<i>Dendrocoelum lacteum</i>	7	3					
	<i>Polycelis</i> spp.	18	5					
Rhynchobdellida	<i>Erpobdella octoculata</i>	21	4					
	<i>Glossiphonia complanata</i>	17	4					
	<i>Helobdella stagnalis</i>	17	4					
	<i>Piscicola geometra</i>	4	2					
Diptera	Ceratopogonidae	21	5					
	Chironomidae	29	5	15	5			
	<i>Dicranota</i> sp.	3	2					
	Simuliidae	7	4					
	<i>Tipula</i> sp.	14	5					

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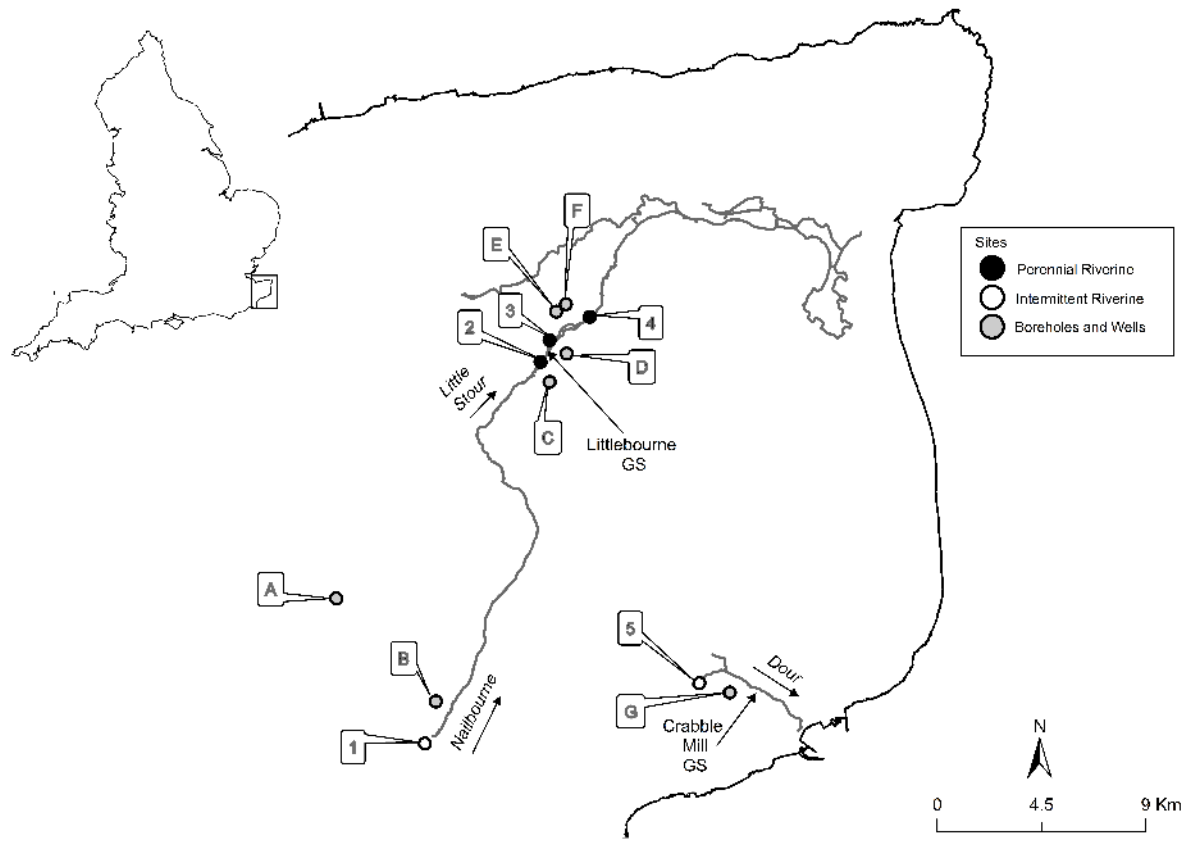
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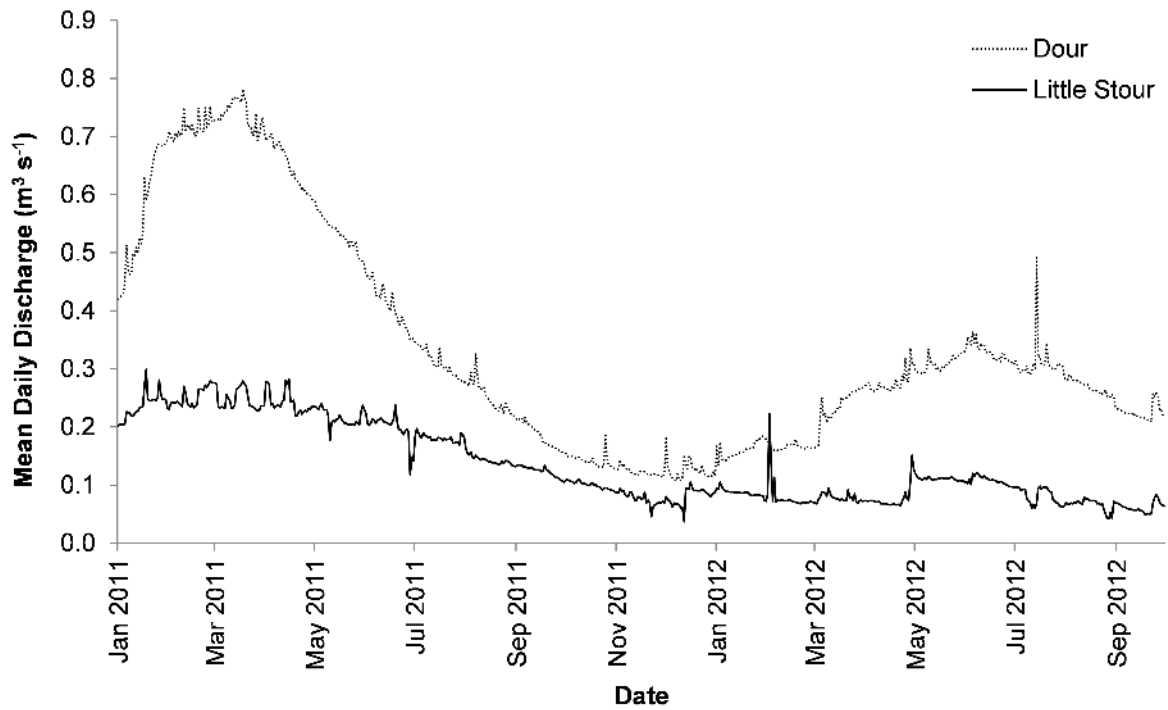
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704 **Figure 1.** Created in ArcMap 10.2.2



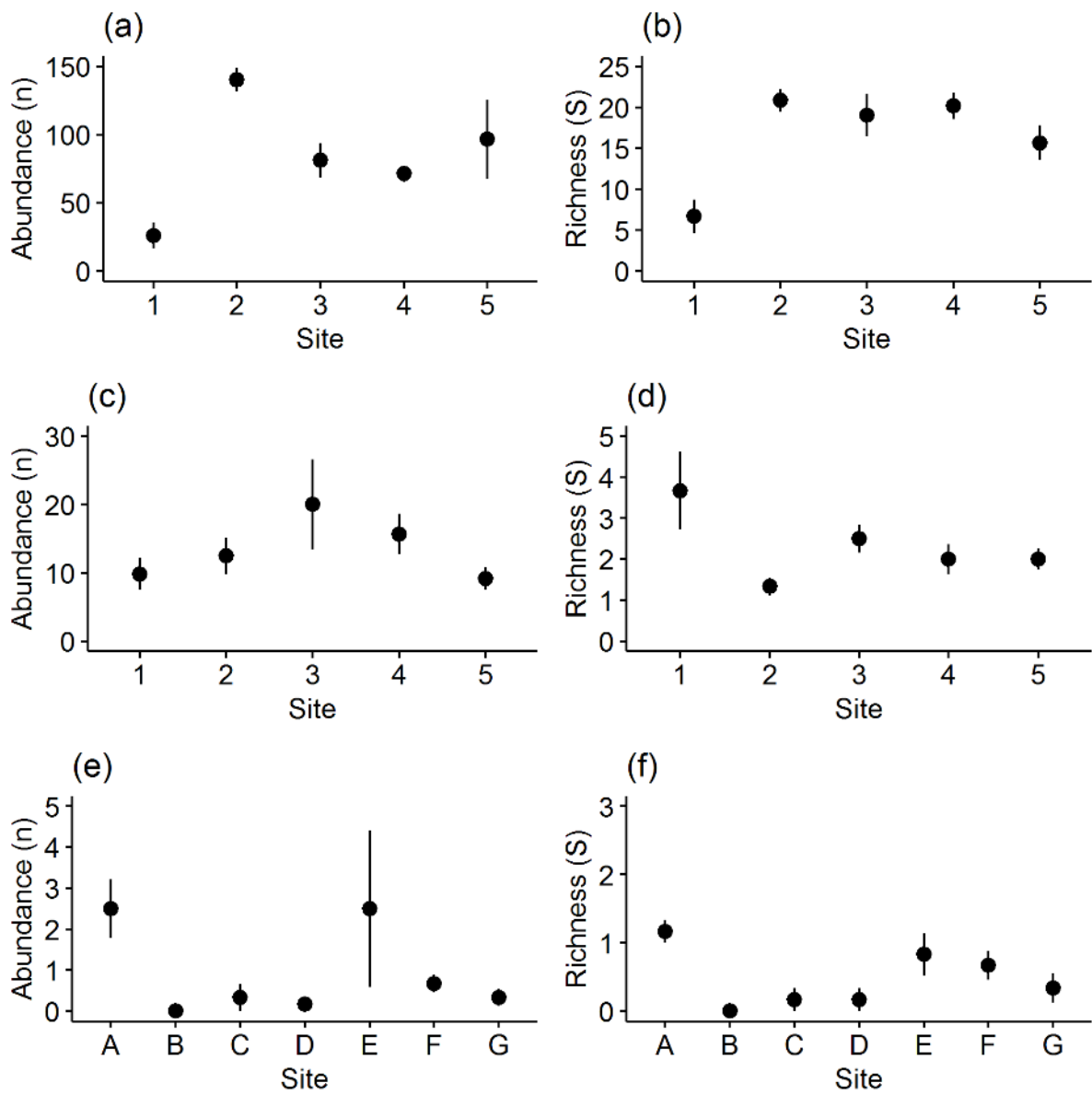
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Figure 2. Created in Excel



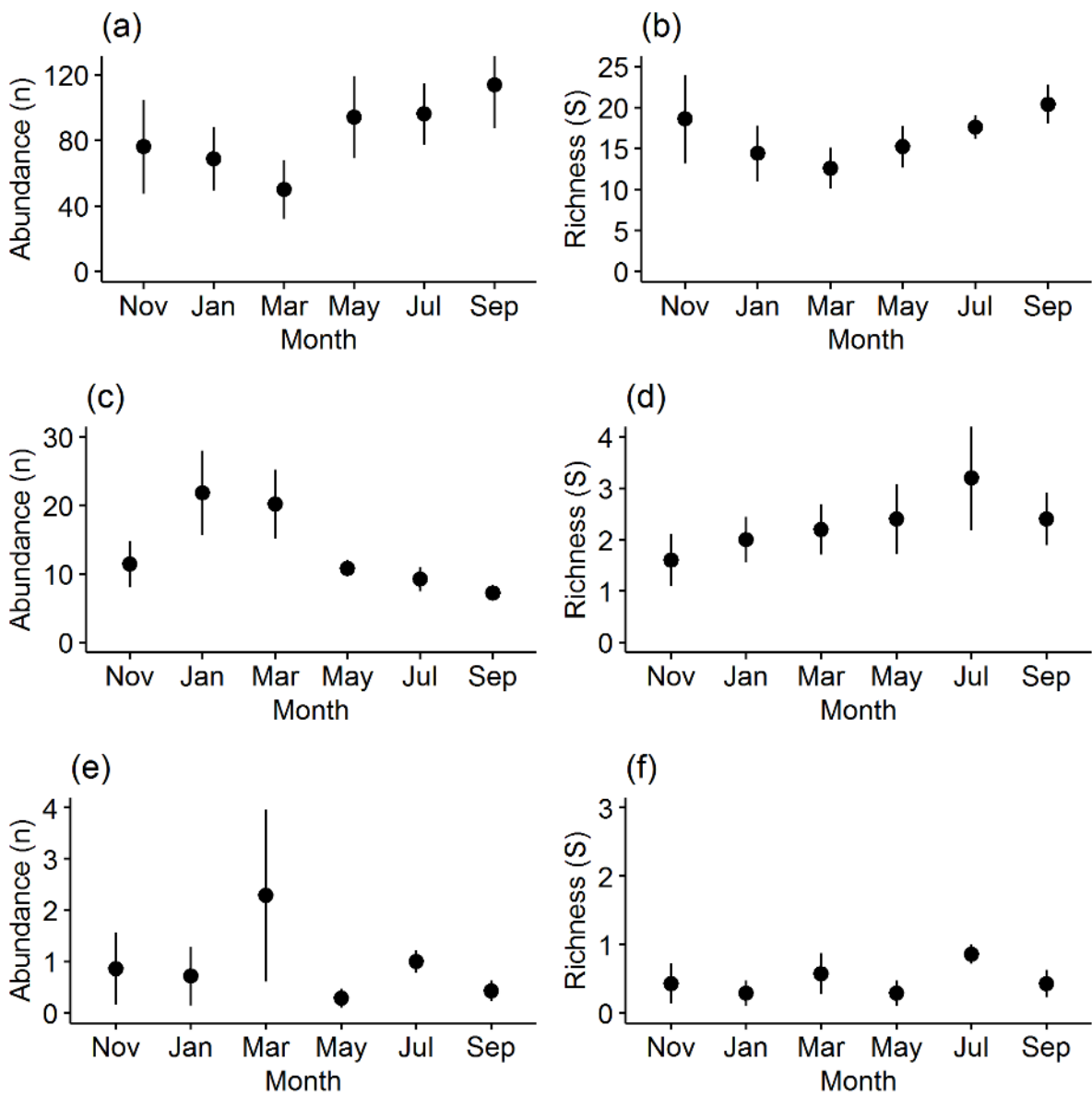
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713 **Figure 3.** Created in R



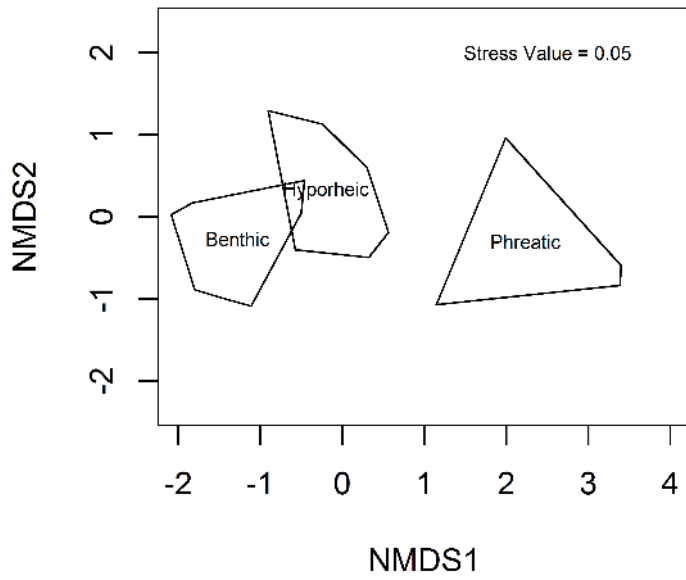
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731 **Figure 4.** Created in R

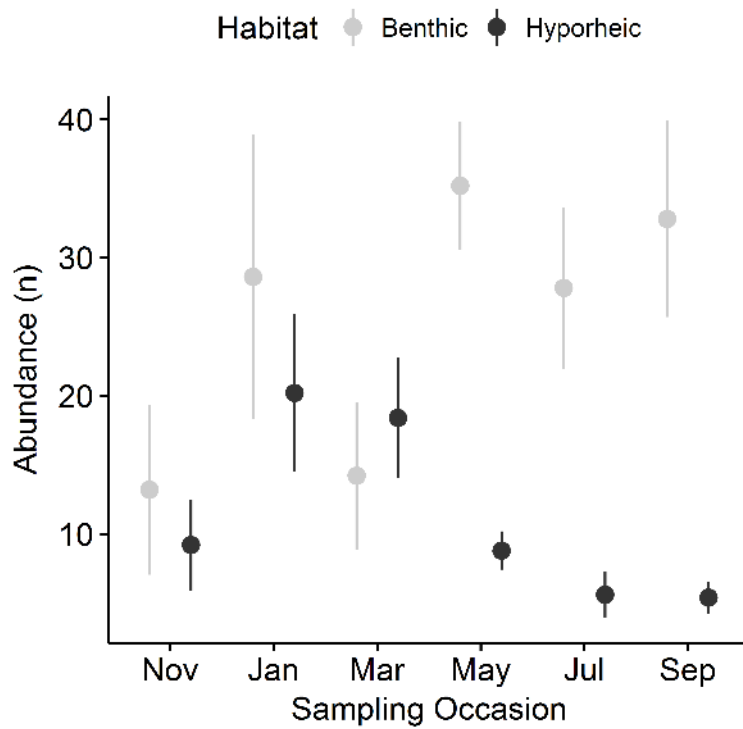


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749 **Figure 5.** Created in R



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752 **Figure 6.** Created in R



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Appendix 1. Supplementary Information.

Mean \pm 1 SE for environmental variables measured in benthic, hyporheic and phreatic habitats and assessed spatially (between sites) and temporally (between sampling occasions) using ANOVA tests; ** indicates $p < 0.001$, * $p < 0.05$ and ns $p > 0.05$.

Habitat Scale	Benthic		Hyporheic		Phreatic	
	Spatial	Temporal	Spatial	Temporal	Spatial	Temporal
Temperature (°C)	12.47 \pm 0.44 ns	12.47 \pm 0.44 **	11.85 \pm 0.30 ns	11.85 \pm 0.30 **	13.72 \pm 0.29 ns	13.72 \pm 0.29 *
Dissolved Oxygen (mg L ⁻¹)	9.89 \pm 0.33 ns	9.89 \pm 0.33 ns	NA		6.68 \pm 0.33 ns	6.68 \pm 0.33 ns
Conductivity (μ S cm ⁻¹)	630.07 \pm 9.08 ns	630.07 \pm 9.08 ns	628.00 \pm 6.76 **	628.00 \pm 6.76 ns	632.07 \pm 8.45 ns	632.07 \pm 8.45 ns
pH	7.57 \pm 0.06 ns	7.57 \pm 0.06 ns	7.41 \pm 0.04 ns	7.41 \pm 0.04 ns	7.29 \pm 0.06 *	7.29 \pm 0.06 ns
Total Alkalinity (mg L ⁻¹)	239.28 \pm 8.51 *	239.28 \pm 8.51 ns	248.86 \pm 7.89 ns	248.86 \pm 7.89 ns	203.41 \pm 9.78 ns	203.41 \pm 9.78 ns
N (NO ₃ ⁻) (mg L ⁻¹)	3.34 \pm 0.16 ns	3.34 \pm 0.16 ns	3.02 \pm 0.20 ns	3.02 \pm 0.20 ns	2.80 \pm 0.17 ns	2.80 \pm 0.17 ns
P (PO ₄ ³⁻) (mg L ⁻¹)	0.16 \pm 0.06 ns	0.16 \pm 0.06 ns	0.13 \pm 0.04 ns	0.13 \pm 0.04 ns	0.30 \pm 0.07 ns	0.30 \pm 0.07 ns
Ca (mg L ⁻¹)	107.91 \pm 2.09 ns	107.91 \pm 2.09 ns	108.77 \pm 2.41 **	108.77 \pm 2.41 ns	106.23 \pm 2.53 ns	106.23 \pm 2.53 ns
Mg (mg L ⁻¹)	2.52 \pm 0.07 ns	2.52 \pm 0.07 ns	2.62 \pm 0.11 ns	2.62 \pm 0.11 ns	2.86 \pm 0.11 *	2.86 \pm 0.11 ns
Na (mg L ⁻¹)	0.68 \pm 0.02 **	0.68 \pm 0.02 ns	0.73 \pm 0.03 ns	0.73 \pm 0.03 *	0.76 \pm 0.03 *	0.76 \pm 0.03 ns
K (mg L ⁻¹)	0.14 \pm 0.01 ns	0.14 \pm 0.01 ns	0.17 \pm 0.02 ns	0.17 \pm 0.02 ns	0.43 \pm 0.06 ns	0.43 \pm 0.06 *
Sr (mg L ⁻¹)	0.25 \pm 0.01 ns	0.25 \pm 0.01 ns	0.25 \pm 0.01 *	0.25 \pm 0.01 ns	0.25 \pm 0.01 ns	0.25 \pm 0.01 ns