

1 **Benthic and interstitial habitats of a lentic spring as invertebrate refuges during**
2 **supra-seasonal drought**

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11

12 **Abstract**

13 Instream refuges are places where invertebrates persist during disturbances due to
14 reduced adverse impacts. During droughts, low flows may be accompanied by
15 elevated temperatures, and potential refuges including subsurface sediments and
16 spring-fed headwaters are therefore characterized by hydrological and thermal
17 stability. This study examined invertebrate use of benthic and interstitial habitats
18 (analogous to the hyporheic zone) in a groundwater-dominated, perennial limnocrone
19 spring during a supra-seasonal drought. Although exceptionally high air temperatures
20 occurred as flow declined, environmental conditions in the spring were relatively
21 stable, and refuge-seeking vertical migrations into interstitial habitats did not coincide
22 with peak temperatures. However, maximum benthic abundance of two amphipods
23 (*Gammarus pulex* and *Crangonyx pseudogracilis*) occurred shortly after the period of
24 elevated temperatures. It is suggested that this temporary increase in the abundance of
25 these mobile taxa reflected upstream migrations triggered by a combination of refuge-
26 seeking behaviour and thermally-stimulated activity. In addition, the spring provided
27 a passive refuge for many lotic invertebrate taxa. A conceptual model is presented,
28 which illustrates the potential contribution of multiple refuges to invertebrate
29 persistence in drought-impacted ecosystems.

30

31 **Keywords:** hyporheic, headwaters, refugium, macroinvertebrate, migration,
32 temperature

33

34

35 **Introduction**

36 Hydrological droughts include seasonal and supra-seasonal events, the latter being
37 unpredictable, infrequent and sometimes prolonged disturbances that increase
38 gradually in intensity (Lake, 2000, 2003). In freshwater ecosystems, supra-seasonal
39 droughts manifest as unusually long and/or severe reductions in surface flow
40 (Humphries & Baldwin, 2003, Fleig et al., 2006), and while surface water is retained
41 in perennial systems, instream habitats may experience significant changes.
42 Submerged habitat availability, habitat heterogeneity and flow velocities may
43 decrease, accompanied by reductions in water quality and changes to water
44 temperature (Caruso, 2002, Dewson et al., 2007, Wood et al., 2010). Consequent
45 impacts on invertebrate communities depend on the extent and nature of changes in
46 the availability of suitable habitat. Taxonomic richness typically declines due to
47 reduced habitat heterogeneity, while the abundance of individual taxa may either
48 decrease or increase (Wood & Armitage, 2004, Dewson et al., 2007, Stubbington et
49 al., 2011).

50
51 Behavioural adaptations that promote persistence of benthic invertebrates during
52 supra-seasonal droughts include active migrations into refugial habitats, in which
53 environmental conditions remain favourable (Boulton, 1989, Lancaster & Belyea,
54 1997, Boulton, 2003). The interstitial habitat of the hyporheic zone has been identified
55 as a patchy refuge (Dole-Olivier et al., 1997), its utility depending on the fulfilment of
56 various environmental criteria (see Dole-Olivier, 2011 and Stubbington, 2012 for
57 recent reviews). While several studies have indicated that benthic invertebrates use
58 the hyporheic zone as a refuge during floods (Williams & Hynes, 1974, Dole-Olivier
59 & Marmonier, 1992, Holomuzki & Biggs, 2000) and streambed drying (Delucchi,
60 1989, Clinton et al., 1996, Fenoglio et al., 2006), evidence of refuge use is equivocal
61 during other hydrological disturbances (Dole-Olivier, 2011, Stubbington, 2012). In
62 particular, there is little evidence that interstitial sediments act as a refuge during low
63 flows in perennial streams (James et al., 2008, James & Suren, 2009), although Wood
64 et al. (2010) reported active migrations of the amphipod *Gammarus pulex* into the
65 hyporheic zone, these migrations being associated with elevated water temperatures
66 during a supra-seasonal drought.

67
68 Other research has indicated that habitats in the surface stream provide preferable
69 refuges to subsurface sediments. Boulton (1989), for example, noted invertebrate
70 persistence in nearby perennial waters rather than the hyporheic zone following
71 streambed drying, while James et al. (2008) reported that benthic sediments remained
72 preferable to interstitial habitats during periods of reduced flow. Depending on
73 longitudinal patterns of flow recession, surface refuges may occur in headwaters
74 (Lake, 2003), particularly in streams fed by hydrologically stable groundwater springs
75 (Erman & Erman, 1995, Smith & Wood, 2002). The additional thermal stability of
76 such upwelling springs may simultaneously provide a buffer against elevated water
77 temperatures (Berrie, 1992, Smith et al., 2003, Barquín & Death, 2011). Headwater
78 springs are therefore potential drought refuges for taxa capable of active upstream
79 migration as well as offering passive protection to resident fauna (Hughes, 1970,
80 Williams & Williams, 1993, Erman & Erman, 1995).

81
82 Few studies have concurrently considered the effects of hydrological variability on
83 benthic and interstitial invertebrates (but see Belaidi et al., 2004, Wood et al., 2010,
84 Stubbington et al., 2011, Datry, 2012), and the present study is the first to compare the
85 responses of these contiguous communities in a limnocrone spring. The study was
86 conducted during the latter stages of a supra-seasonal drought (Marsh, 2007) in which

87 extremely high surface water temperatures were linked to invertebrate migrations into
88 the hyporheic zone in lotic reaches downstream of the spring (Wood et al., 2010). It
89 was predicted that: i) benthic invertebrates would not migrate into interstitial
90 sediments in the spring unless adverse conditions developed in the surface water; and
91 ii) densities of mobile taxa would fluctuate in response to migrations between the
92 spring and downstream reaches, if unfavourable conditions affected the latter.

93 94 **Materials and methods**

95 96 *Study site*

97 The Little Stour River is a lowland chalk stream which rises 4 km east of Canterbury
98 (Kent, U.K.; 51° 15.9'N 1° 09.4'E) and flows for 11.5 km, draining an area of ~213
99 km²; characteristics of the catchment are described by Wood et al. (2000, 2010). The
100 present study was conducted in the headwater spring source of the Little Stour: a
101 limnocrene spring comprising a permanent area of lentic water of approximately 100
102 m by 20 m (Fig. 1) and with a maximum depth of <2 m. The spring is shaded by
103 riparian trees and macrophyte growth is therefore limited, while filamentous algae
104 (*Cladophora* spp.) are abundant. Surface sediments are gravel dominated, with some
105 sand and finer sediments. The interstitial sediments are fed by upwelling groundwater
106 and are analogous in character to hyporheic sediments of downstream lotic reaches.

107
108 Mean annual precipitation in the catchment is c.650 mm (Wood & Petts, 1999) and
109 the mean annual air temperature is 10.2 °C (Met Office, 2008). However, this study
110 was conducted during the latter stages of a supra-seasonal drought which affected
111 southern England between 2004 and 2006 (Marsh, 2007). Discharge in the Little
112 Stour River declined from mid-June to mid-August 2006 then remained low until
113 early September; the hydrological conditions experienced in lotic reaches downstream
114 of the spring are described by Wood et al. (2010). The limnocrene spring is
115 characterized by relatively stable hydrology, and reductions in water depth during the
116 drought exposed only gently-sloping marginal areas. Air temperatures increased to a
117 maximum of 26.3 °C in July, these temperatures being extreme and the highest
118 observed in the 348-year regional record (Prior & Beswick, 2007). Temperatures then
119 declined for the rest of the study period, except for a brief increase in September,
120 when mean daily maxima reached 17.7 °C (Fig. 2).

121 122 *Field sampling*

123 Paired samples of benthic and interstitial invertebrate assemblages were collected at
124 monthly intervals between April and November 2006 inclusive. Five sampling points
125 were located in shallow (<0.5 m) areas of the spring, this number of replicates being
126 selected to minimize impacts on the sensitive ecosystem (Cantonati et al., 2006).
127 Benthic invertebrates were collected using a Surber sampler (0.1 m² frame, 250 µm
128 mesh net) by manually disturbing the sediments within the frame to a depth of ~5 cm
129 for 30 seconds. A current was generated manually to carry disturbed invertebrates into
130 the net. Large clasts located within the sample area were inspected individually and
131 attached invertebrates included in the sample.

132
133 Prior to the study, a stainless steel T-bar was used to insert one open-ended PVC tube
134 (internal diameter 19 mm) 20 cm into the sediments at each sampling point, to act as a
135 semi-permanent interstitial habitat sampling well (Boulton & Stanley, 1995,
136 Stubbington et al., 2009). Wells were sealed with bungs between sampling occasions
137 to prevent colonisation by benthic fauna. Each month, 6 L (three 2 L aliquots) of
138 interstitial water were extracted from the base of each well by manually operating a

139 bilge pump, as detailed in Boulton & Stanley (1995). The extracted water was filtered
140 through a 90 µm-mesh sieve to retain invertebrates. All invertebrate samples were
141 preserved in the field using a 4 % formaldehyde solution.

142

143 Water temperature (°C), dissolved oxygen (DO; mg l⁻¹), pH and conductivity (µS
144 cm⁻¹) were measured in situ for both surface and interstitial water using standard
145 instrumentation (Hanna Instruments, Leighton Buzzard). Interstitial water
146 measurements were recorded from the second 2 L aliquot.

147

148 *Laboratory analysis*

149 Invertebrates were identified to the lowest taxonomic resolution possible, in many
150 cases species level. Some taxonomically demanding groups, including all Diptera
151 families, Baetidae (Ephemeroptera), Planariidae (Tricladida), Oligochaeta, and all
152 meiofauna (Ostracoda, Copepoda, Cladocera and Hydracarina), were left at the
153 taxonomic resolutions stated. Adults and larvae of an individual taxon were
154 considered as separate taxa due to recognized differences in environmental
155 requirements (Elliott, 2008).

156

157 *Data analysis*

158 Temporal change in surface and interstitial water quality variables (temperature, DO,
159 pH and conductivity) was analysed using one-way analysis of variance (ANOVA) in
160 IBM SPSS Statistics 19.0 (IBM Corporation, New York). Levene's tests were used to
161 verify the ANOVA assumption of homoscedasticity. Where ANOVA indicated
162 significant temporal variability, differences between individual months were
163 examined using Tukey's *post-hoc* multiple comparison tests.

164

165 Benthic and interstitial communities were analysed separately, to facilitate
166 observation of contrasting patterns of temporal change in community composition.
167 Total invertebrate abundance (TIA), taxon richness (number of taxa), and the
168 abundance of common taxa were calculated for each month and expressed as the
169 mean ± 1 standard error. TIA was calculated for all taxa and also for all non-insect
170 taxa, to acknowledge the influence of seasonal insect emergence on abundance
171 patterns. 'Common taxa' were defined as those comprising >1 % of all individuals
172 recorded in benthic or interstitial samples. Abundance data were square-root
173 transformed prior to further analysis. All metrics were then used as dependent
174 variables in one-way ANOVA tests to determine the significance of temporal
175 changes, as described for environmental variables.

176

177 Detrended correspondence analysis (DCA) was conducted by segments in the
178 program CANOCO 4 (ter Braak & Šmilauer, 2006) to examine temporal variability in
179 invertebrate community composition. Rare species were downweighted and data log
180 transformed ($y' = \log(y + 1)$) to reduce the influence of very abundant taxa. The
181 significance of temporal change in sample scores on axis 1 and 2 was examined using
182 one-way ANOVA tests. Relationships between axis scores and TIA, taxon richness
183 and the abundance of individual taxa were examined using Pearson's correlation
184 coefficients (r), to determine those parameters having a significant effect on patterns
185 of community change.

186

187 To examine use of subsurface sediments by benthic invertebrates, the proportion of a
188 population present in interstitial habitat (i.e. the *interstitial proportion* of the
189 population, analogous to the hyporheic proportion *sensu* Stubbington et al., 2011) was
190 determined for each month. The interstitial proportion was calculated by dividing a

191 taxon's interstitial abundance by its total (benthic + interstitial) abundance at a
192 sampling point, facilitating comparison of assemblages sampled using contrasting
193 methods. The interstitial proportion was calculated for TIA and for individual taxa
194 which: i) were found predominantly in benthic samples; ii) comprised >1 % of all
195 interstitial invertebrates, and; iii) were not insects subject to development-related
196 seasonal changes in vertical distribution. The calculated proportions were arcsine
197 square-root transformed prior to inclusion in ANOVA tests to analyse temporal
198 change in the use of interstitial habitat by benthic taxa.

199

200 **Results**

201

202 *Environmental conditions*

203 Surface water temperatures increased from 8.1 °C in April to 13.4 °C in July, while
204 mean interstitial water temperatures were less variable, increasing from 8.5 °C in
205 April to 12.8 °C in July (Fig. 2). Considering both environments, temporal change was
206 significant (ANOVA, $p < 0.001$) due to successive increases between April, May and
207 June (Tukey's tests, $p < 0.05$), but remained within 3.2 °C of the mean annual air
208 temperature. Surface water DO concentrations peaked in April, at 12 mg l⁻¹, decreased
209 to 5 mg l⁻¹ in August and were particularly low (2.6 mg l⁻¹) in September. Interstitial
210 DO concentrations also peaked in April at 8 mg l⁻¹ and mean values varied between 4-
211 6 mg l⁻¹ in later months. Considering both environments, temporal variability was
212 significant (ANOVA, $p < 0.001$) due to the reduction in DO concentrations between
213 April and June (Tukey's test, $p < 0.001$). Conductivity peaked at 730 μS cm⁻¹ in April
214 then declined and remained between 550-600 μS cm⁻¹ between May and October in
215 both surface and interstitial waters, before increasing in November (ANOVA, p
216 < 0.001). The pH was stable and circumneutral (7.2-7.6) in both surface and interstitial
217 waters.

218

219 *Invertebrate communities*

220 A total of 17771 individuals from 55 taxa were recorded from the benthic sediments
221 (Table 1). The family Chironomidae dominated this community, accounting for 36.3
222 % of all individuals. The Oligochaeta, Ostracoda, Hydracarina, Planariidae, and two
223 Amphipoda (*Crangonyx pseudogracilis* and *G. pulex*) each comprised between 4.0-
224 18.5 % of the community. Lentic water specialists were present at low abundance,
225 including *Berosus affinis* (Coleoptera) and *Plea leachi* (Hemiptera). In addition, three
226 stygobiotic amphipods were recorded: *Niphargus aquilex*, *N. fontanus* and *Crangonyx*
227 *subterraneus* the former accounting for 2.2 % of TIA (Table 1). No crenobiotic taxa
228 (spring specialists) were identified.

229

230 In total, 2747 individuals from 29 taxa were collected from interstitial habitats (Table
231 1). Of these, 25 were also found in the benthic zone and four were restricted to the
232 subsurface: Cladocera, *Valvata cristata* (Gastropoda), an unidentified Diptera larva,
233 and *Proasellus cavaticus* (Isopoda), a nationally notable stygobiont (Robertson et al.,
234 2009). Ostracoda were numerically dominant (59.1 %), and Copepoda, Chironomidae,
235 *G. pulex* and Oligochaeta each accounted for 2.2-13.6 % of the community. *N.*
236 *aquilex*, *N. fontanus*, *C. subterraneus* and *P. cavaticus* comprised the stygobiotic
237 component of the interstitial community, while a coarse taxonomic resolution
238 prevented assignment of habitat preferences to other taxa and no crenobionts were
239 recorded.

240

241 *Temporal change in the benthic invertebrate community*

242 Mean benthic TIA peaked at 846 ± 358 individuals (ind.) m^{-2} in July, before
243 decreasing in each subsequent month to 130 ± 10 ind. m^{-2} in November; these
244 temporal changes were not significant (ANOVA, $p = 0.110$). TIA was influenced by
245 seasonal development and emergence of insect taxa, particularly the Chironomidae,
246 and exclusion of insects from this analysis shifted peak abundance to August
247 (ANOVA, $p < 0.05$). Abundance peaks varied between common non-insect taxa,
248 occurring in April for Ostracoda, June for Hydracarina and Planariidae, and August
249 for Oligochaeta, *G. pulex* (Fig. 3a) and *C. pseudogracilis* (Fig. 3b); these changes
250 were not significant (ANOVA, $p > 0.05$). Mean taxon richness was lower in
251 September (13.6 ± 1.0 taxa $0.1 m^{-2}$) and October (13.2 ± 1.0 taxa $0.1 m^{-2}$) than in
252 other months ($\geq 16.6 \pm 0.5$ taxa $0.1 m^{-2}$); temporal change was not significant
253 (ANOVA, $p = 0.492$).

254

255 Axis 1 of the benthic community DCA explained 21.1 % of the variation in the
256 species data (Fig. 4a). Sample scores on axis 1 changed significantly over time,
257 increasing between April and September then decreasing moderately in later months
258 (ANOVA, $p < 0.001$). Moderate ($r = -0.41$ – -0.63), significant ($p < 0.01$) negative
259 correlations with axis 1 scores were recorded for TIA, taxon richness, and the
260 abundance of *Limnophilus lunatus*, *Valvata piscinalis*, Hydracarina, *Asellus* and
261 *Drusus annulatus*. Positive correlations with this axis were highly significant for
262 *Haliplus lineatocollis* adults and *Haliplus* spp. larvae (both $r = 0.68$, $p < 0.001$). Axis 2
263 explained an additional 8.6 % of community variation. Sample scores did not vary
264 significantly between months on axis 2 (ANOVA, $p = 0.182$) but were negatively
265 correlated with *G. pulex* abundance ($r = -0.66$, $p < 0.001$).

266

267 *Temporal change in interstitial invertebrate community*

268 Mean interstitial TIA peaked in November (251 ± 208 ind. $6 L^{-1}$), due to exceptionally
269 high ostracod abundance, was also high in June (109 ± 29 ind. $6 L^{-1}$) and was lowest
270 in September (12 ± 3 ind. $6 L^{-1}$); these changes were not significant (ANOVA, $p =$
271 0.120) and exclusion of insect taxa had no significant effects on the observed patterns
272 (ANOVA, $p = 0.430$). Considering predominantly benthic non-insect taxa, *G. pulex*
273 abundance peaked in October, was also high in July and was low in all other months
274 ($p = 0.140$; Fig. 3a). The other common benthic amphipod, *C. pseudogracilis*,
275 occurred at very low abundance in interstitial habitats (Fig. 3b). The abundance of all
276 stygobionts was low and temporally variable, for example a single *P. cavaticus* was
277 recorded in July; seven *C. subterraneus* were found in May; and *N. aquilex* abundance
278 peaked at nine individuals in August. Taxon richness was particularly high in
279 November (10 ± 0.5 taxa $6 L^{-1}$), partly due to the occurrence of new Diptera families
280 at low abundance. Richness in November was significantly higher than in April,
281 September, October and May (Tukey's tests, $p < 0.05$), the latter month having lowest
282 mean richness (4 ± 0.5 taxa $6 L^{-1}$).

283

284 Axis 1 of the interstitial DCA ordination (Fig. 4b) explained 15.5 % of the variance in
285 the species data. Sample scores changed significantly over time on this axis (p
286 < 0.001) but followed no clear trajectory, being highest in April and August and
287 particularly low in July. Axis 2 explained a further 9.2 % of the community variance,
288 and temporal change was again significant ($p < 0.01$) due to low sample scores in May
289 (Fig. 4b). The abundance of *Niphargus aquilex* was positively correlated with axis 1
290 scores ($r = 0.46$, $p < 0.01$), while the chironomid abundance was negatively correlated
291 with axis 2 ($r = -0.49$, $p = 0.001$).

292

293 *Proportion of benthic invertebrates in interstitial habitats*

294 The interstitial proportion of the total (benthic + interstitial) population was calculated
295 for TIA and two taxa which fulfilled the stated criteria: *G. pulex* and Oligochaeta. The
296 interstitial proportion of TIA varied considerably between months, declining
297 gradually between June (0.22 ± 0.12) and September (0.03 ± 0.01) then peaking at
298 0.37 ± 0.14 in November due to high ostracod abundance (ANOVA, $p < 0.05$). The
299 interstitial proportion of the *G. pulex* population increased gradually from April (0.11
300 ± 0.10) to July (0.60 ± 0.23), fell in September (0.10 ± 0.09), then increased to 1 in
301 October, when all 53 individuals occurred in interstitial habitat (ANOVA, $p < 0.01$;
302 Fig. 3a); all significant differences related to comparisons between this October value
303 and earlier months (Tukey's tests, $p < 0.05$). The interstitial proportion of the
304 Oligochaeta was low (< 0.1) and comparable in all months (ANOVA, $p = 0.790$).

305

306 Discussion

307

308 *Environmental conditions*

309 Water temperatures in the spring peaked at 13.4 °C and 12.8 °C in surface and
310 interstitial water respectively, considerably lower than equivalent values (19.5 °C and
311 20.4 °C) at the river site 1.5 km downstream (Fig. 1; Wood et al., 2010). The
312 temperature range was also low in the spring: 5.3 °C and 4.3 °C in surface and
313 interstitial water, respectively, compared to and 9.5 °C and 11.4 °C at the river site.
314 Both surface and interstitial water were therefore characterized by relative thermal
315 stability at the spring, as is typical of groundwater-fed headwaters (Mackey & Berrie,
316 1991, Barquín & Death, 2011). DO concentrations were reduced in August and
317 September, despite decreases in temperature and potential increases in upwelling
318 water velocities (see Wood et al., 2010); the absence of a clear pattern may reflect
319 complex pathways of interstitial flow and/or biological activity in the chalk aquifer
320 (Malard and Hervant 1999).

321

322 *The spring as an invertebrate refuge*

323 Few studies have compared the invertebrate communities of springs and their
324 associated streams (Smith & Wood, 2002, von Fumetti et al., 2007, Wood et al.,
325 2005). While the present study examined only a limnocrene spring, comparisons with
326 lotic reaches of the Little Stour are possible using previously published data
327 (Stubbington et al., 2009, Wood et al., 2010). In this system, longitudinal linkages are
328 rarely severed by streambed drying, and never by drying of the spring. This
329 connectivity increases the potential of the spring to act as a refuge for benthic taxa
330 that predominantly inhabit lotic waters.

331

332 Mobile taxa capable of upstream migrations may exploit nearby refuges during
333 droughts (Henry & Danielopol, 1998, Wood et al., 2005). Such migrations are largely
334 restricted to strong swimmers, exemplified in the Little Stour by *G. pulex* and *C.*
335 *pseudogracilis*. Amphipod metabolic and behavioural activities are positively related
336 to temperature (Williams, 1980, Lagerspetz & Vainio, 2006), and upstream
337 migrations may be promoted by moderate temperature increases (Hultin, 1971). The
338 benthic abundance of both *G. pulex* and *C. pseudogracilis* was particularly high in
339 August (Fig. 3) and these sudden, temporary, abundance peaks may be lagged
340 evidence of migrations from downstream lotic reaches exposed to elevated
341 temperatures in July; further research employing multidirectional cage traps (e.g.
342 Elser, 2001) would be required to test this hypothesis.

343

344 Wood et al. (2010) attributed peak hyporheic abundance of *G. pulex* to vertical
345 migrations triggered by high water temperatures. The maximum surface water

346 temperature recorded by Wood et al. (2010) was 22.7 °C, which promotes high
347 metabolism and activity in *G. pulex* (Wijnhoven et al., 2003, Maazouzi et al., 2011).
348 Equally, survival may decline at only 20 °C (Maazouzi et al., 2011); Wijnhoven et al.
349 (2003) also recorded stress responses at >25 °C. In addition, amphipods acclimatize to
350 prevailing temperatures (Lagerspetz & Vainio, 2006, Maazouzi et al., 2011) and so
351 may not tolerate rapid-onset extremes (Buchanan et al., 1988). Given that
352 temperatures recorded in the Little Stour were close to the tolerance thresholds of *G.*
353 *pulex*, its increased abundance in the cooler sediments of both the spring and the
354 river's hyporheic zone (Wood et al., 2010) may partly reflect refuge-seeking
355 behaviour. However, there is little existing evidence that amphipods follow thermal
356 gradients into cooler waters, and the proposed vertical and longitudinal migrations
357 would also have involved atypical movement against a DO gradient (Henry &
358 Danielopol, 1998). Therefore, migrations in both vertical and longitudinal dimensions
359 may reflect a temperature-induced increase in activity. Positive rheotaxis combined
360 with increased activity levels may have manifested as upstream migrations (Hultin,
361 1971, Stanley et al., 1994) and the consequent entrance of amphipods into the
362 headwater spring.

363

364 Seasonal changes may also have contributed to the August peaks in amphipod
365 abundance (Fig. 3). While several studies have noted stable *G. pulex* abundance
366 during summer (Macan & Mackereth, 1957, Mortensen, 1982, Stubbington et al.,
367 2011), late summer peaks may also occur (Welton, 1979), and in the Little Stour high
368 temperatures may have promoted reproduction and population expansion. Sutcliffe et
369 al. (1981) noted maximum juvenile growth rates at 20 °C, and Moenickes et al. (2011)
370 reported comparable maxima. Considering: i) that mean brood size may exceed 30
371 eggs in *G. pulex* and 70 eggs in *C. pseudogracilis* (Sutcliffe, 1993); ii) the positive
372 relationship between brood development time and water temperatures up to 20 °C (*G.*
373 *pulex*; Sutcliffe, 1993) or 25 °C (*C. pseudogracilis*; Sutcliffe & Carrick, 1981) and;
374 iii) a development time as short as 16 days (*G. pulex*; Welton & Clarke, 1980), high
375 reproductive activity may have contributed to the amphipod population increase
376 recorded between July and August. However, no such increase was observed in
377 downstream reaches (Wood et al., 2010).

378

379 Regardless of temporal changes in abundance, the spring provided a passive refuge
380 from extreme temperatures for all invertebrates present. Of the 55 taxa recorded in the
381 benthic zone of the spring, 34 were also found at the nearest downstream site (Fig. 1;
382 Table 1) and only eight taxa were not noted further downstream (Wood et al., 2010).
383 This community nestedness indicates that, despite contrasting environmental
384 characteristics, the lentic spring acted as a refuge for a substantial subset of the stream
385 fauna, in which taxa persisted during the drought, and from which individuals could
386 recolonize downstream reaches following the end of the disturbance.

387

388 *Subsurface sediments as an invertebrate refuge*

389 Wood et al. (2010) noted invertebrate migrations into the hyporheic zone during a
390 period of elevated surface water temperatures in the stream, but the thermal stability
391 of the spring removed this migration trigger. It was therefore predicted that no
392 thermally-driven migrations into deeper sediments would occur at the spring, and
393 accordingly, no significant changes in the interstitial abundance and/or interstitial
394 proportion of common, predominantly benthic, non-insect taxa were observed during
395 peak temperatures. However, the interstitial proportion of the *G. pulex* population did
396 vary considerably between months, increasing gradually from 0.11 in April to 0.60 in
397 July, which coincided with a moderate temperature increase. This correlation may

398 indicate vertical range extension stimulated by increased activity rather than refuge-
399 seeking behaviour (Hultin, 1971, Stubbington, 2012), although a link between
400 temperature and burrowing activity has not been confirmed. The migration of the
401 entire *G. pulex* population into interstitial habitat in October occurred during moderate
402 hydrological conditions, after flow had begun to recover (Wood et al., 2010); this
403 migration remains unexplained and contradicts previous research suggesting that
404 upwelling groundwater impedes downward movements into subsurface sediments
405 (Stubbington et al., 2011).

406

407 *C. pseudogracilis* occurred at very low abundance in interstitial habitat throughout the
408 study. Previous research has recorded *C. pseudogracilis* in subsurface habitats (Martin
409 et al., 2009), populations may be groundwater-adapted (Gibson et al., 2008), and the
410 taxon may migrate into deeper sediments to survive surface drying in temporary
411 waters (Holsinger & Dickson, 1977, Harris et al., 2002). In addition, the larger *G.*
412 *pulex* predates *C. pseudogracilis* (Dick, 1996), and subsurface sediments are a
413 potential refuge from biotic interactions (Stubbington et al., 2011). However, despite
414 their morphological similarity, the habitat preferences of *C. pseudogracilis* and *G.*
415 *pulex* differ (MacNeil et al., 1999), and the scarcity of the former taxon in interstitial
416 sediments suggests that some undetermined feature of this habitat (e.g. water
417 chemistry or pore size distribution) was unsuitable.

418

419 Droughts increase groundwater residence times in deep sediments (Manga, 1999,
420 McGuire et al., 2002) and resultant hypoxia may trigger the migration of stygobionts
421 into shallower substrates (Wood et al., 2010). Accordingly, Wood et al. (2010)
422 recorded a significant increase in groundwater Crustacea in the Little Stour hyporheic
423 zone in July, which coincided with peak temperatures as flows receded. Similar
424 evidence of upward migrations at the spring is limited: *N. aquilex* abundance peaked
425 in August, *P. cavaticus* was only recorded in July, and *C. subterraneus* was most
426 abundant in May; no temporal changes were significant. Stygobionts are tolerant of
427 low oxygen availability (Danielopol et al., 1994, Malard & Hervant, 1999) and so
428 may have been unaffected by changes in water chemistry at a site dominated by
429 upwelling groundwater.

430

431 *Refuges at the stream scale*

432 The interstitial habitat of the hyporheic zone has been posited as a vital component in
433 the suite of refuges available to benthic invertebrates at sub-reach scales (Stubbington,
434 2012). However, while individual invertebrates always act at the smallest spatial
435 scales (Lancaster, 2008), recolonisation following a disturbance can occur at the
436 segment scale and, over time, at the stream scale, particularly in small systems such as
437 chalk streams (Dole-Olivier, 2011). It is therefore appropriate to place individual
438 refuges in a stream context, and the conceptual model presented in Fig. 5 outlines the
439 survival options available to invertebrates at disturbance onset. Using the drought-
440 impacted Little Stour as an example, tolerant, eurytopic and sedentary taxa may
441 remain in affected habitats such as warm, shallow riffles in preference to expending
442 energy (Fig. 5). Other individuals may actively follow thermal gradients (Wood et al.,
443 2010) into subsurface sediments, but interstitial habitats present their own challenges
444 to inhabitation and invertebrates may therefore remain in the benthic zone (James et
445 al., 2008). Near the sediment surface, refuge-seeking behaviour includes localized
446 lateral movements into habitats such as deep pools (Covich et al., 2003), and mobile
447 taxa such as amphipods may undertake longitudinal migrations. While active and
448 passive drift to downstream refuges are less energetically expensive, positively
449 rheotactic behaviour favours active upstream migrations (Stanley et al., 1994), and

450 during drought, such movements may be promoted by elevated temperatures (Hultin,
451 1971) and facilitated by slow flow velocities (Hughes, 1970).

452

453 **Conclusion**

454 The few studies that have considered concurrent benthic and interstitial (hyporheic)
455 invertebrate responses to environmental variability have noted divergent trajectories
456 of community change (Belaidi et al., 2004, Wood et al., 2010, Stubbington et al.,
457 2011, Datry, 2012). While our study was unique in examining these communities in a
458 limnocrene spring, the typical pattern was observed: a temporally homogeneous
459 interstitial community including both benthic and stygobiont taxa (Fig. 4b), and a
460 diverse, temporally variable benthic fauna (Fig. 4a). In addition, comparison with
461 published data from downstream lotic reaches (Wood et al., 2010) identified
462 contrasting benthic community responses to drought in adjacent spring and stream
463 habitats. With climate change scenarios predicting increases in air temperature and
464 drought frequency (Solomon et al., 2007, Kundzewicz et al., 2008), this study
465 highlights perennial headwaters and interstitial habitats as vital refuges that promote
466 invertebrate persistence at the stream-system scale (Frissell et al., 1986). The
467 importance of these habitats should be recognized in management and rehabilitation
468 activities seeking to enhance community survival during drought disturbances.

469

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478

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764

765 *Table 1: Taxa present in the benthic zone (BZ) and interstitial habitat (IH) of the*
766 *lentic headwater spring of the Little Stour River and a lotic reach 1.5 km downstream*
767 *(Fig. 1); data from the latter site have been published by Wood et al. (2010). Numbers*
768 *indicate the % contribution of a taxon to total invertebrate abundance (TIA), where*
769 *TIA = 17771 individuals in the spring BZ, 2747 in the spring IH, 8231 in the river*
770 *BZ, and 1152 in the river IH.*

Group	Species	Headwater spring		River site	
		BZ	IH	BZ	IH
TURBELLARIA					
Planariidae		4.2		1.3	0.9
OLIGOCHAETA		18.5	2.2	7.3	2.7
HIRUDINEA					
Erpobdellidae	<i>Erpobdella octoculata</i>	0.7		1.4	<0.1
Glossiphoniidae	<i>Glossiphonia complanata</i>	0.1		0.5	
	<i>Glossiphonia heteroclita</i>	<0.1			
	<i>Helobdella stagnalis</i>	<0.1	<0.1	0.4	
	<i>Theromyzon tessulatum</i>			<0.1	
Piscicolidae	<i>Piscicola geometra</i>	<0.1	<0.1	<0.1	<0.1
MOLLUSCA					
Hydrobiidae	<i>Potamopyrgus antipodarum</i>			<0.1	
Lymnaeidae	<i>Lymnaea peregra</i>	0.2		<0.1	
Physidae	<i>Physa fontinalis</i>	<0.1		<0.1	<0.1
Planorbidae	<i>Planorbarius corneus</i>			<0.1	
	<i>Planorbis planorbis</i>			<0.1	
	<i>Planorbis vortex</i>			0.1	
Sphaeriidae		<0.1		0.7	0.3
Valvatidae	<i>Valvata cristata</i>		<0.1	<0.1	
	<i>Valvata piscinalis</i>	0.3	<0.1	<0.1	
HYDRACARINA		4.9	1.6	0.4	0.2
CRUSTACEA					
Cladocera			1.0	<0.1	<0.1
Copepoda		0.7	13.6	<0.1	3.1
Ostracoda		11.9	59.1	2.9	3.6
Asellidae	<i>Asellus aquaticus</i>	2.7	0.2	0.3	0.9
	<i>Proasellus meridianus</i>	2.7	0.3	0.1	1.2
	<i>Proasellus cavaticus</i>		<0.1		
Crangonyctidae	<i>Crangonyx pseudogracilis</i>	4.5	0.1	<0.1	<0.1
	<i>Crangonyx subterraneus</i>	0.3	0.6		
Gammaridae	<i>Gammarus pulex</i>	4.0	6.4	47.8	44.4
Niphargidae	<i>Niphargus aquilex</i>	2.2	0.8		2.5
	<i>Niphargus fontanus</i>	<0.1			
PLECOPTERA					
Nemouridae	<i>Nemurella picteti</i>	<0.1			
EPHEMEROPTERA					
Baetidae		<0.1	0.1	1.8	1.6
Caenidae	<i>Caenis horaria</i>			1.6	0.2
	<i>Caenis</i> sp. (<i>luctuosa</i> group)			0.2	<0.1
Ephemerellidae	<i>Serratella ignita</i>	<0.1		1.6	0.7
TRICHOPTERA					
Glossosomatidae	<i>Agapetus fuscipes</i>	<0.1		21.9	23
Goeridae	<i>Silo nigricornis</i>	<0.1	0.4	0.2	
Hydropsychidae	<i>Hydropsyche siltalai</i>	<0.1		1.3	0.2
Hydroptilidae	<i>Hydroptila</i> spp.	<0.1	<0.1	0.2	
	<i>Oxyethira</i> spp.	0.1			
Leptoceridae	<i>Athripsodes</i> spp.			0.2	0.2
	<i>Mystacides</i> spp.			<0.1	0.2
Limnephilidae	<i>Drusus annulatus</i>	0.2	0.7		
	<i>Limnephilus lunatus</i>	0.2	0.3	<0.1	
Polycentropodidae	<i>Plectrocnemia conspersa</i>	<0.1			
	<i>Polycentropus flavomaculatus</i>	<0.1			
Psychomyiidae	<i>Tinodes waeneri</i>			<0.1	
Rhyacophilidae	<i>Rhyacophila dorsalis</i>			<0.1	
Sericostomatidae	<i>Sericostoma personatum</i>	0.1		0.3	<0.1
ANISOPTERA		<0.1			
MEGALOPTERA					
Sialidae	<i>Sialis lutaria</i>	0.2		<0.1	
HEMIPTERA					
Corixidae		<0.1			
Pleidae	<i>Plea leachi</i>	<0.1			
COLEOPTERA (adult unless otherwise stated)					
Curculionidae		<0.1			
Dytiscidae	<i>Stictotarsus duodecimpustulatus</i>	<0.1			
	Unidentified (larvae)	<0.1	<0.1		

Elmidae	<i>Elmis aenea</i>	<0.1		0.2	
	<i>Elmis aenea</i> (larvae)	<0.1		0.6	0.4
	<i>Limnius volckmari</i> (larvae)			0.4	
	<i>Oulimnius</i> spp. (larvae)			<0.1	
Haliplidae	<i>Brychius elevatus</i>			<0.1	
	<i>Haliplus confinis</i>	<0.1			
	<i>Haliplus lineatocollis</i>	2.0	<0.1		
	Unidentified (larvae)	2.8	0.2	<0.1	
Hydrophilidae	<i>Berosus affinis</i>	<0.1			
Hygrobiidae	<i>Hygrobia hermanni</i>	<0.1			
DIPTERA					
Ceratopogonidae		0.1	<0.1	0.3	0.2
Chironomidae		36.3	0.4	5.8	12.0
Empididae		<0.1	<0.1	<0.1	
Ephydriidae		<0.1			
Limoniidae		<0.1		0.2	
Psychodidae		<0.1	<0.1	<0.1	
Simuliidae				0.6	0.7
Stratiomyidae				<0.1	
	Unidentified (larvae)		<0.1	<0.1	
	Unidentified (pupae)	<0.1			

771

772 **Figure legends**

773

774 Fig. 1: Map of the Little Stour River headwaters, indicating the location of the spring
775 source and a river site studied by Wood et al. (2010).

776

777 Fig. 2: Maximum daily air temperature at Manston (51° 34.6'N 1° 33.4'E) and mean
778 surface and interstitial water temperature in the Little Stour River spring source, April
779 to November 2006. 1 SE was ≤ 0.22 °C in all cases.

780

781 Fig. 3: Mean \pm 1 SE benthic and interstitial abundance of a) *Gammarus pulex* and b)
782 *Crangonyx pseudogracilis* between May and November 2006. Abundance data are
783 presented as individuals per sample for both benthic (0.1 m²) and interstitial (6 L)
784 samples.

785

786 Fig. 4: Detrended correspondence analysis (DCA) sample plots for the invertebrate
787 community of the Little Stour River spring source, between April and November
788 2006: a) benthic sediments; b) interstitial sediments.

789

790 Fig. 5: Benthic invertebrate drought refuges at the stream scale. Thick lines indicate
791 active migrations, thin lines indicate passive movements. An amphipod (Crustacea)
792 and a chironomid (Diptera) are depicted (not to scale), as representative highly mobile
793 and sedentary benthic invertebrate taxa, respectively.

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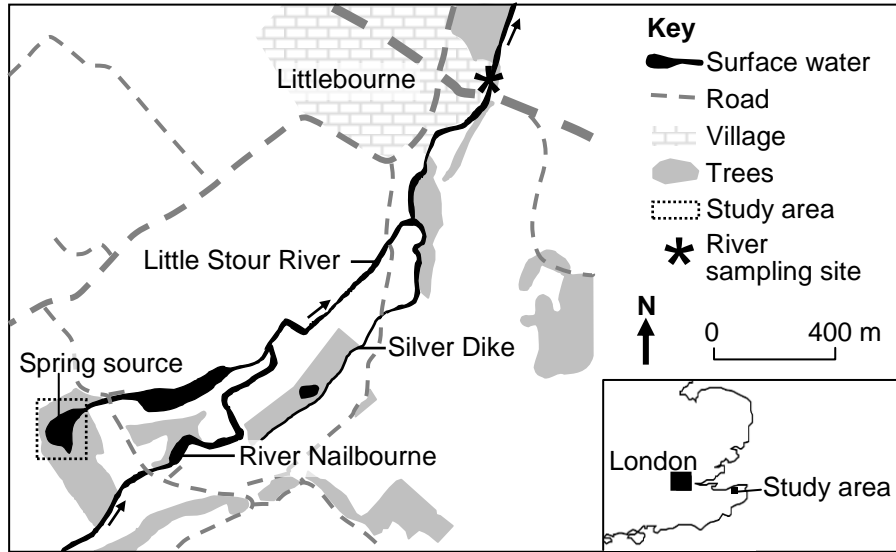
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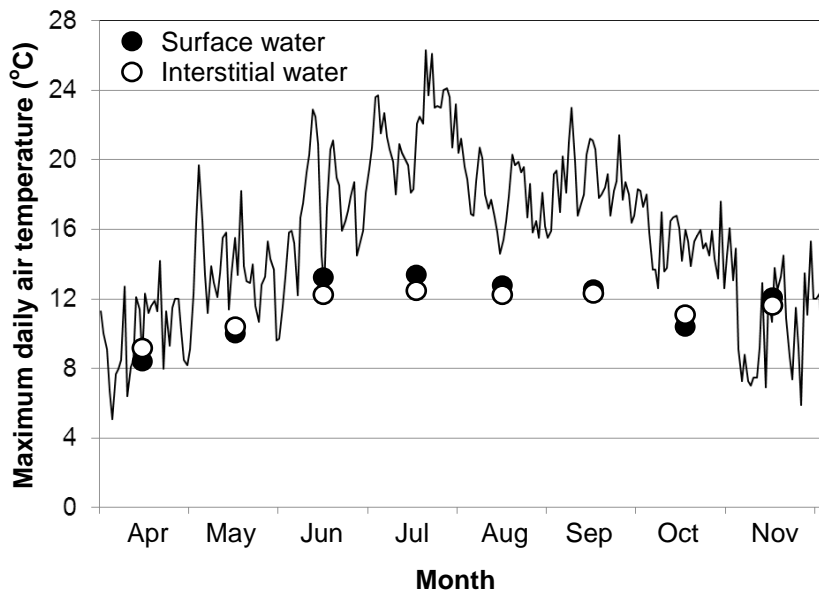
804 **Figures**

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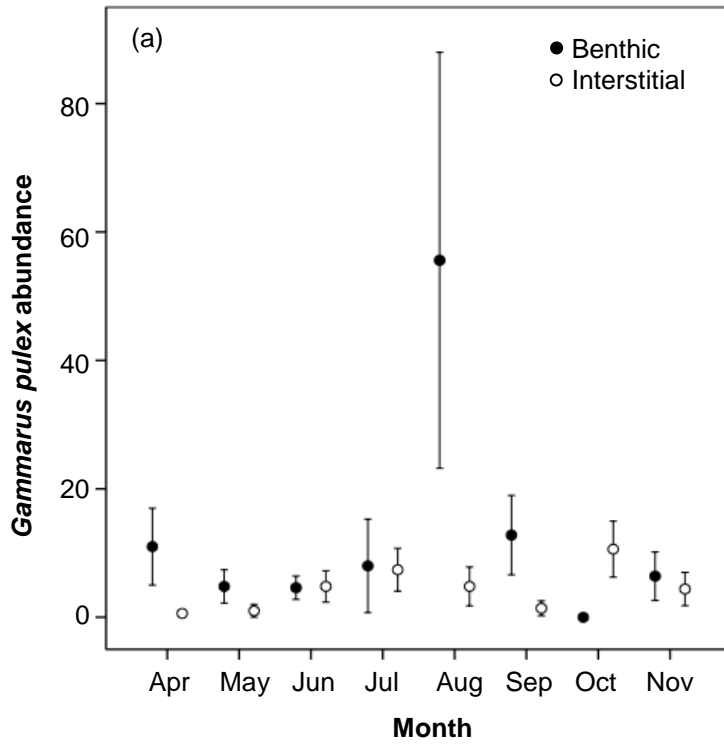
Fig. 1



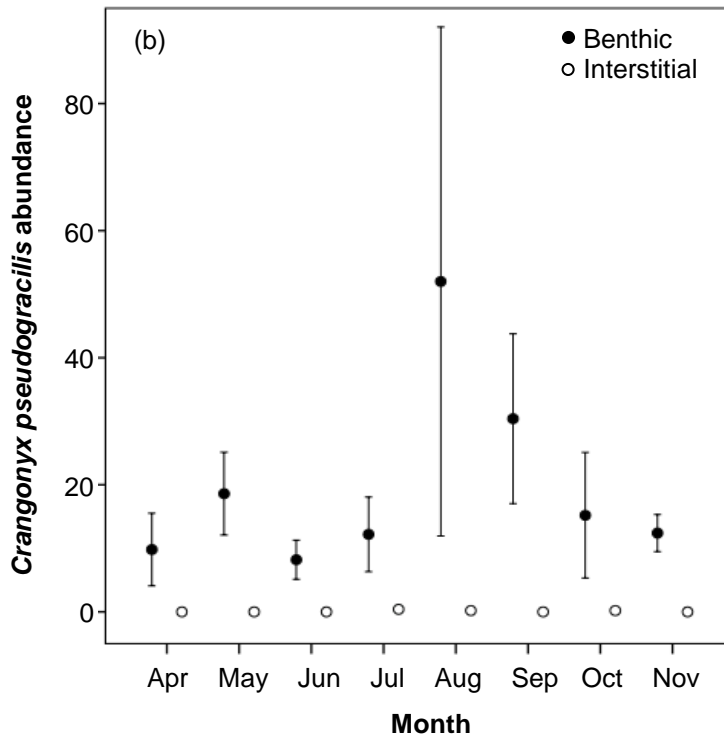
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Fig. 2

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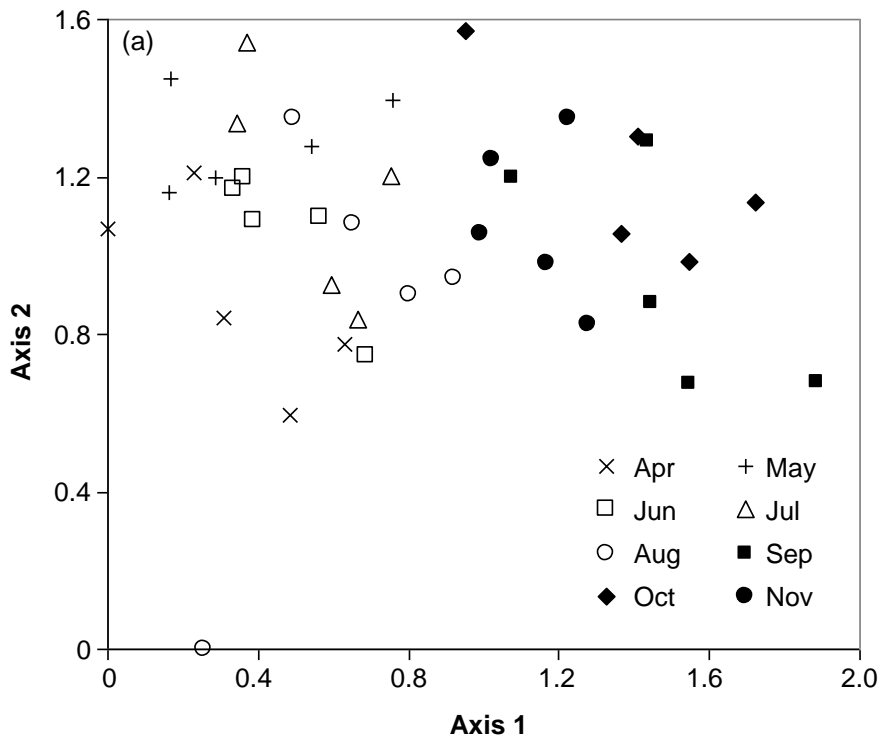


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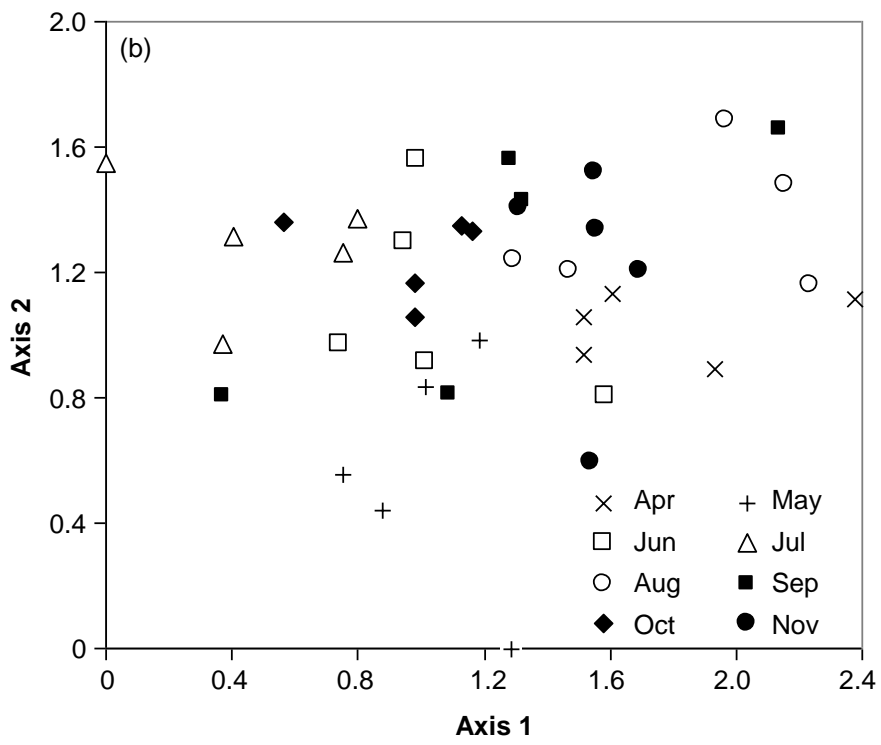


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Fig. 3



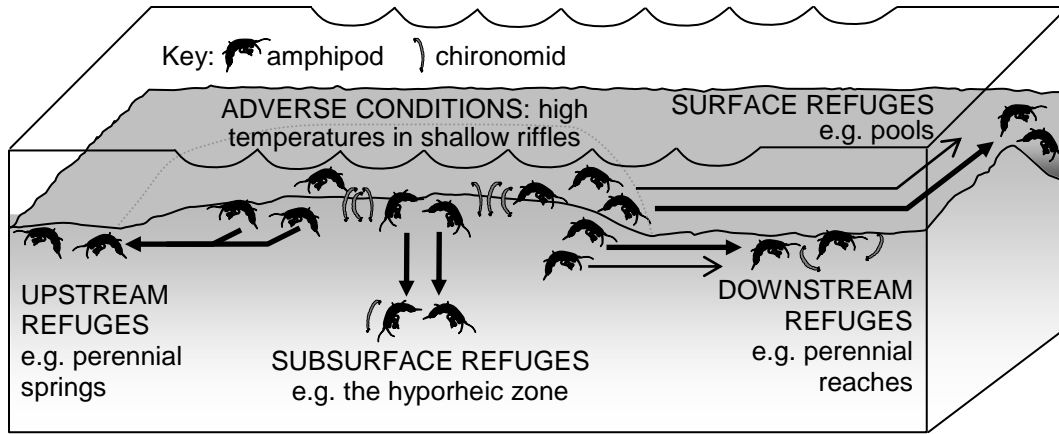
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Fig. 4

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Fig. 5