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

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## Abstract

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

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

## Introduction

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

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

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

Few studies have concurrently considered the effects of hydrological variability on benthic and interstitial invertebrates (but see Belaidi et al., 2004, Wood et al., 2010, Stubbington et al., 2011, Datry, 2012), and the present study is the first to compare the responses of these contiguous communities in a limnocrene spring. The study was conducted during the latter stages of a supra-seasonal drought (Marsh, 2007) in which extremely high surface water temperatures were linked to invertebrate migrations into the hyporheic zone in lotic reaches downstream of the spring (Wood et al., 2010). It was predicted that: i) benthic invertebrates would not migrate into interstitial sediments in the spring unless adverse conditions developed in the surface water; and ii) densities of mobile taxa would fluctuate in response to migrations between the spring and downstream reaches, if unfavourable conditions affected the latter.

## Materials and methods

### *Study site*

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

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

### *Field sampling*

Paired samples of benthic and interstitial invertebrate assemblages were collected at monthly intervals between April and November 2006 inclusive. Five sampling points were located in shallow (<0.5 m) areas of the spring, this number of replicates being selected to minimize impacts on the sensitive ecosystem (Cantonati et al., 2006). Benthic invertebrates were collected using a Surber sampler (0.1 m$^2$ frame, 250 μm mesh net) by manually disturbing the sediments within the frame to a depth of ~5 cm for 30 seconds. A current was generated manually to carry disturbed invertebrates into the net. Large clasts located within the sample area were inspected individually and attached invertebrates included in the sample.

Prior to the study, a stainless steel T-bar was used to insert one open-ended PVC tube (internal diameter 19 mm) 20 cm into the sediments at each sampling point, to act as a semi-permanent interstitial habitat sampling well (Boulton & Stanley, 1995, Stubbington et al., 2009). Wells were sealed with bungs between sampling occasions to prevent colonisation by benthic fauna. Each month, 6 L (three 2 L aliquots) of interstitial water were extracted from the base of each well by manually operating a bilge pump, as detailed in Boulton & Stanley (1995). The extracted water was filtered through a 90 µm-mesh sieve to retain invertebrates. All invertebrate samples were preserved in the field using a 4 % formaldehyde solution.

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

### *Laboratory analysis*

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

### *Data analysis*

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

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

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

To examine use of subsurface sediments by benthic invertebrates, the proportion of a population present in interstitial habitat (i.e. the *interstitial proportion* of the population, analogous to the hyporheic proportion *sensu* Stubbington et al., 2011) was determined for each month. The interstitial proportion was calculated by dividing a taxon's interstitial abundance by its total (benthic + interstitial) abundance at a sampling point, facilitating comparison of assemblages sampled using contrasting methods. The interstitial proportion was calculated for TIA and for individual taxa which: i) were found predominantly in benthic samples; ii) comprised >1 % of all interstitial invertebrates, and; iii) were not insects subject to development-related seasonal changes in vertical distribution. The calculated proportions were arcsine square-root transformed prior to inclusion in ANOVA tests to analyse temporal change in the use of interstitial habitat by benthic taxa.

## Results

### *Environmental conditions*

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

### *Invertebrate communities*

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

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

### *Temporal change in the benthic invertebrate community*

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

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

*Temporal change in interstitial invertebrate community*

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

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

*Proportion of benthic invertebrates in interstitial habitats*

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

## Discussion

*Environmental conditions*

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

*The spring as an invertebrate refuge*

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

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

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

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

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

### *Subsurface sediments as an invertebrate refuge*

Wood et al. (2010) noted invertebrate migrations into the hyporheic zone during a period of elevated surface water temperatures in the stream, but the thermal stability of the spring removed this migration trigger. It was therefore predicted that no thermally-driven migrations into deeper sediments would occur at the spring, and accordingly, no significant changes in the interstitial abundance and/or interstitial proportion of common, predominantly benthic, non-insect taxa were observed during peak temperatures. However, the interstitial proportion of the *G. pulex* population did vary considerably between months, increasing gradually from 0.11 in April to 0.60 in July, which coincided with a moderate temperature increase. This correlation may indicate vertical range extension stimulated by increased activity rather than refuge-seeking behaviour (Hultin, 1971, Stubbington, 2012), although a link between temperature and burrowing activity has not been confirmed. The migration of the entire *G. pulex* population into interstitial habitat in October occurred during moderate hydrological conditions, after flow had begun to recover (Wood et al., 2010); this migration remains unexplained and contradicts previous research suggesting that upwelling groundwater impedes downward movements into subsurface sediments (Stubbington et al., 2011).

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

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

### *Refuges at the stream scale*

The interstitial habitat of the hyporheic zone has been posited as a vital component in the suite of refuges available to benthic invertebrates at sub-reach scales (Stubbington, 2012). However, while individual invertebrates always act at the smallest spatial scales (Lancaster, 2008), recolonisation following a disturbance can occur at the segment scale and, over time, at the stream scale, particularly in small systems such as chalk streams (Dole-Olivier, 2011). It is therefore appropriate to place individual refuges in a stream context, and the conceptual model presented in Fig. 5 outlines the survival options available to invertebrates at disturbance onset. Using the drought-impacted Little Stour as an example, tolerant, eurytopic and sedentary taxa may remain in affected habitats such as warm, shallow riffles in preference to expending energy (Fig. 5). Other individuals may actively follow thermal gradients (Wood et al., 2010) into subsurface sediments, but interstitial habitats present their own challenges to inhabitation and invertebrates may therefore remain in the benthic zone (James et al., 2008). Near the sediment surface, refuge-seeking behaviour includes localized lateral movements into habitats such as deep pools (Covich et al., 2003), and mobile taxa such as amphipods may undertake longitudinal migrations. While active and passive drift to downstream refuges are less energetically expensive, positively rheotactic behaviour favours active upstream migrations (Stanley et al., 1994), and during drought, such movements may be promoted by elevated temperatures (Hultin, 1971) and facilitated by slow flow velocities (Hughes, 1970).

## Conclusion

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

## Acknowledgements

This research was funded by a Natural Environment Research Council Urgency Grant (NE/E001769/1) entitled 'The response of aquatic invertebrate fauna to supra-seasonal drought and drying in a largely perennial chalk stream'. We thank Sally Little for assistance in the field and laboratory and Stuart Ashby for technical support. Air temperature data were kindly provided by the British Atmospheric Data Centre. We also thank two anonymous reviewers for their insightful comments, which greatly improved an earlier draft of this manuscript.

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

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

| | | | | | |
|---|---|---|---|---|---|
| Elmidae | *Elmis aenea* | <0.1 | | 0.2 | |
| | *Elmis aenea* (larvae) | <0.1 | | 0.6 | 0.4 |
| | *Limnius volckmari* (larvae) | | | 0.4 | |
| | *Oulimnius* spp. (larvae) | | | <0.1 | |
| Haliplidae | *Brychius elevatus* | | | <0.1 | |
| | *Haliplus confinis* | <0.1 | | | |
| | *Haliplus lineatocollis* | 2.0 | <0.1 | | |
| | Unidentified (larvae) | 2.8 | 0.2 | <0.1 | |
| Hydrophilidae | *Berosus affinis* | <0.1 | | | |
| Hygrobiidae | *Hygrobia hermanni* | <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 | |
| Ephydridae | | <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 | | | |

## Figure legends

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

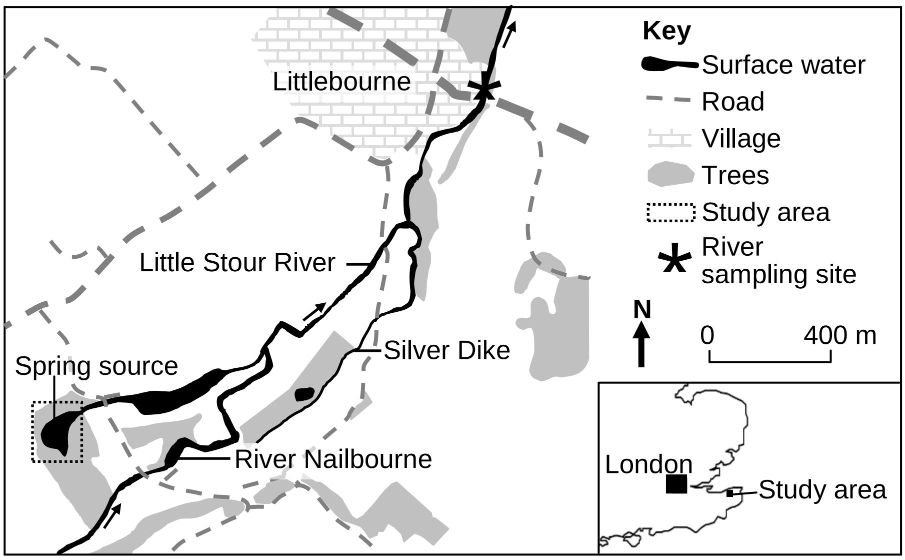
Fig. 2: Maximum daily air temperature at Manston (51º 34.6'N 1º 33.4'E) and mean surface and interstitial water temperature in the Little Stour River spring source, April to November 2006. 1 SE was ≤0.22 $^{o}$C in all cases.

Fig. 3: Mean ± 1 SE benthic and interstitial abundance of a) *Gammarus pulex* and b) *Crangonyx pseudogracilis* between May and November 2006. Abundance data are presented as individuals per sample for both benthic (0.1 $m^2$) and interstitial (6 L) samples.

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

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

## Figures



R Stubbington and P. J. Wood.

Fig. 1

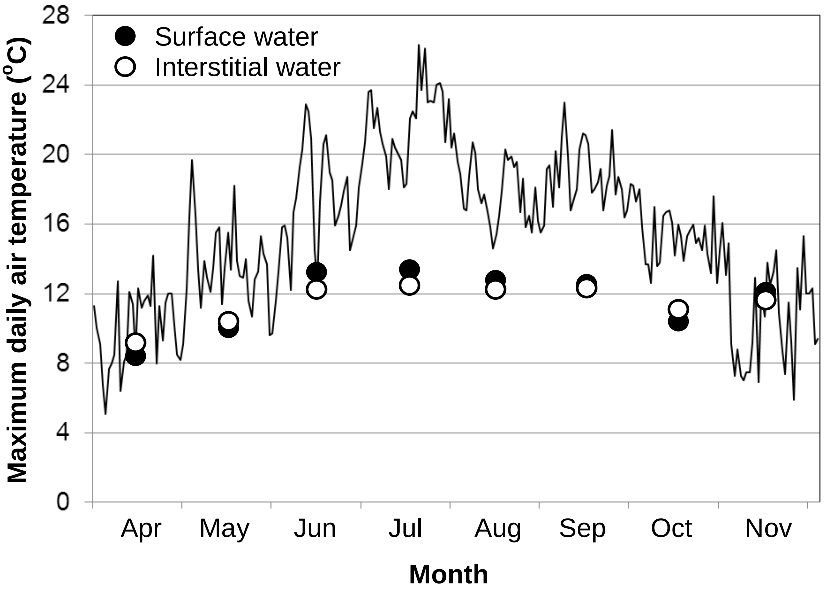


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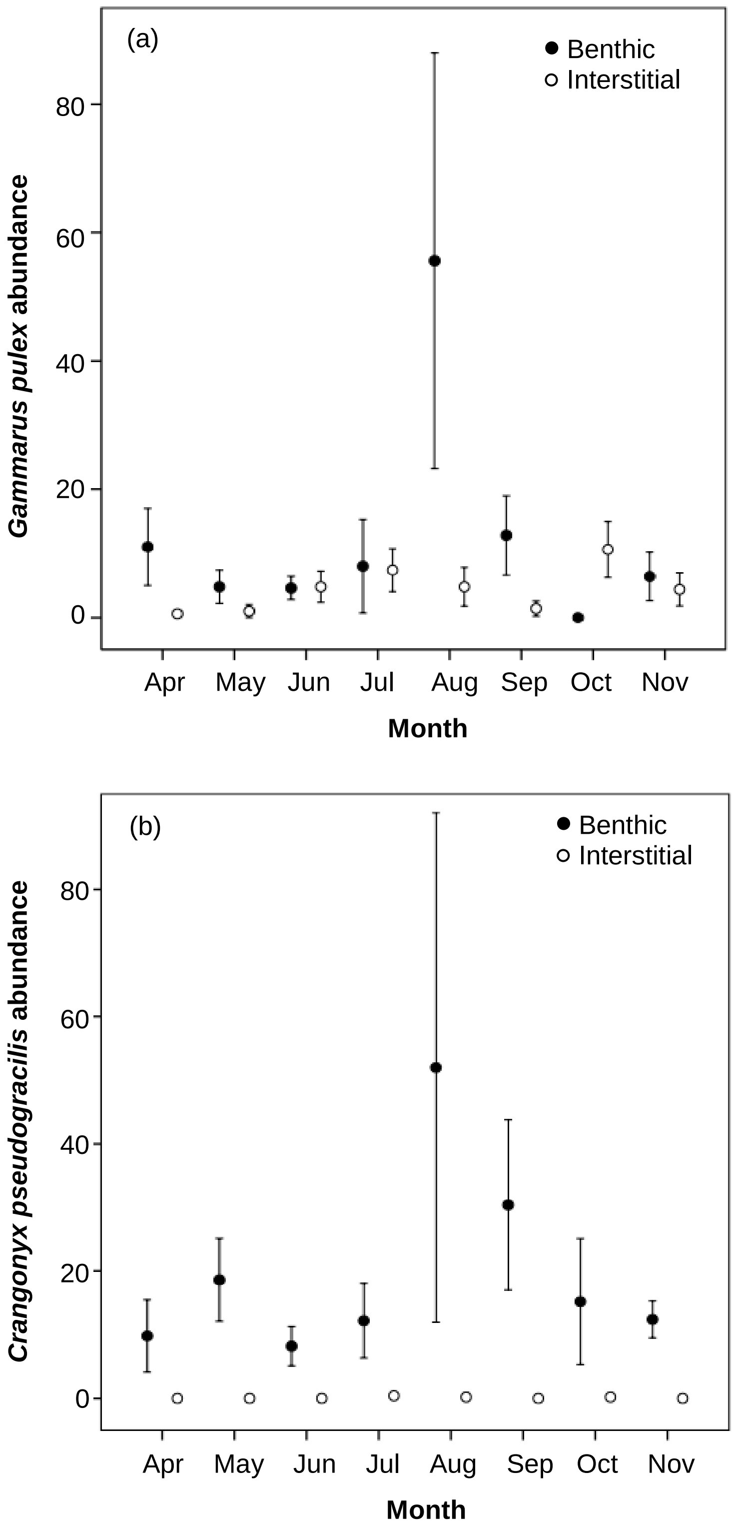
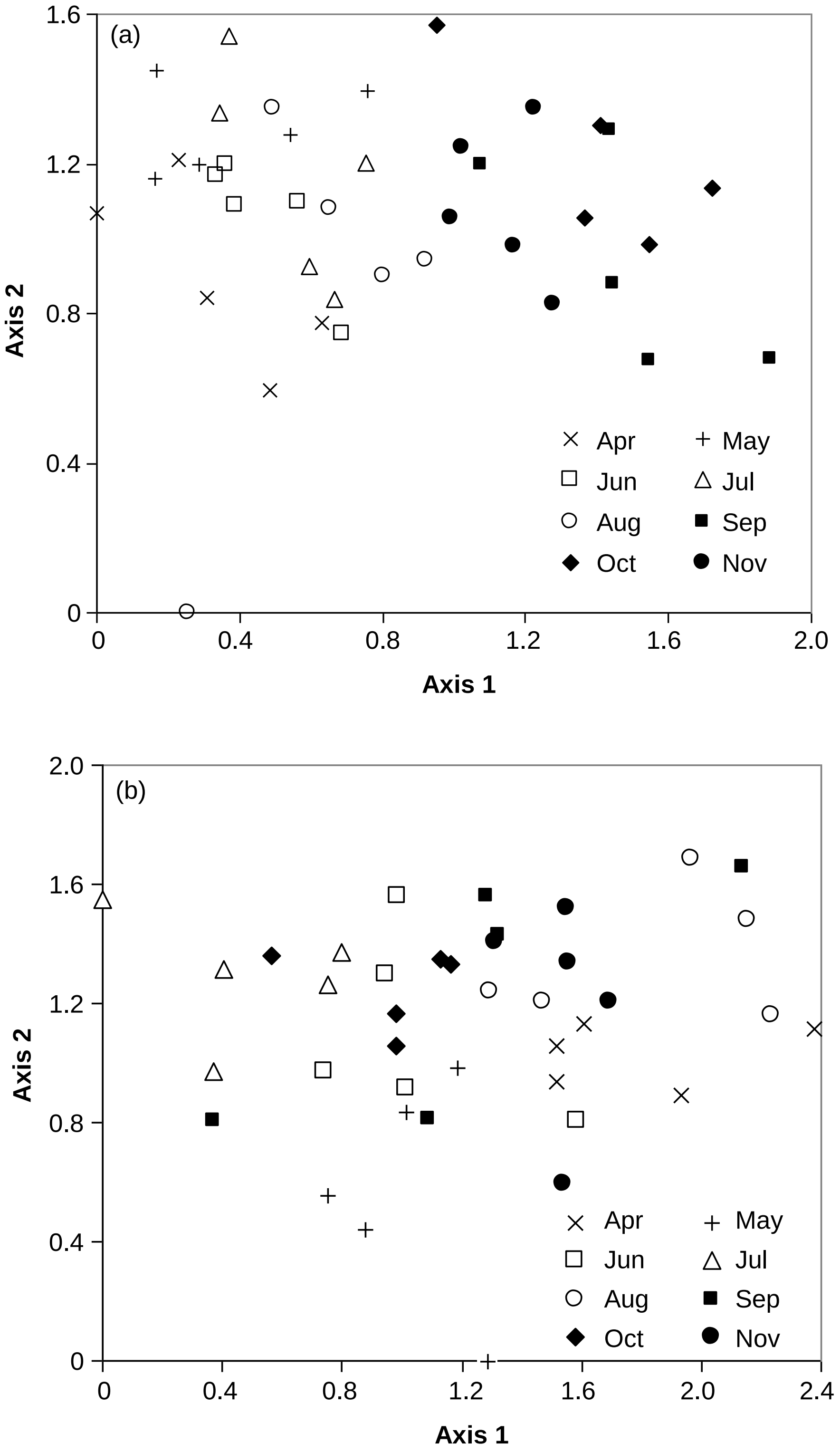
Fig. 2



R Stubbington and P. J. Wood.
Fig. 3



R Stubbington and P. J. Wood.

Fig. 4

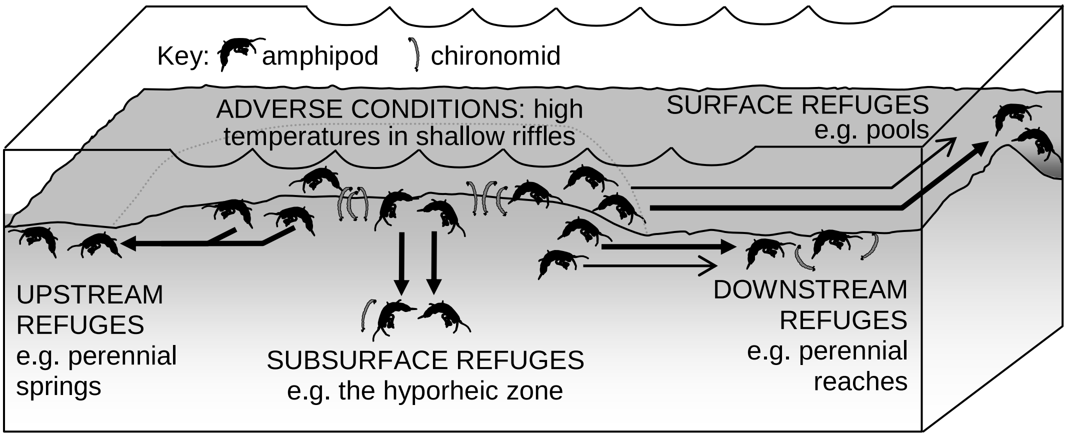


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Fig. 5