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RESEARCH ARTICLE

Gammarus pulex (Crustacea: Amphipoda) avoids increasing water temperature and intraspecific competition through vertical migration into the hyporheic zone: a mesocosm experiment

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Abstract The saturated interstices below and adjacent to the riverbed (i.e., the hyporheic zone) can be a refuge for biota during low flows, flow cessation and river drying. Prior to complete drying, organisms are constrained by abiotic and biotic factors (e.g., water temperature, competition) and may respond through vertical migration into the hyporheic zone. However, it remains unclear when temperature and competition become harsh enough to trigger migration. Furthermore, potential consequences of using the hyporheic zone, which is often food-limited, on the survival, effects on ecosystem function and physiology of organisms are unknown. We tested the hypotheses that (1) *Gammarus pulex*, a widespread detritivore, migrates into the hyporheic zone to avoid increasing surface water temperature and intraspecific competition and (2) that these factors would reduce their survival, leaf mass consumption and energy stores. Using 36 mesocosms, three temperature (15, 20, 25 °C) and species density levels (low, medium, high) were manipulated in a factorial design over 15 days. Increasing temperature to 25 °C and a threefold increase in density both caused *G. pulex* to vertically migrate, and the interaction of these factors was additive, rather than antagonistic or synergistic. Importantly, survival, leaf consumption and glycogen content were reduced in high temperature and density treatments, suggesting tradeoffs between tolerating harsh surface conditions and limitations of inhabiting the hyporheic zone. Identifying that the hyporheic zone is used by *G. pulex* to avoid high water temperature and intraspecific competition is a key finding considering the global-scale increases in temperature and flow intermittence.

Keywords Stream drying · Refuge · Resistance · Resilience · Avoidance behavior · Invertebrates

Introduction

The saturated interstices beneath the riverbed and into the adjacent banks (i.e., the hyporheic zone; White 1993) can serve as a refuge for biota during disturbances, such as flooding and drying (i.e., hyporheic refuge hypothesis; Palmer et al. 1992; Dole-Olivier et al. 1997; Stubbington 2012). They may also be a major source of colonists promoting invertebrate community resilience (i.e., capacity to recover, Stanley et al. 1994) following disturbances (e.g., Holomuzki and Biggs 2007; Vander Vorste et al. 2015). However, the abiotic and biotic factors invertebrates respond to through vertical migration into the hyporheic zone remain speculative (Stubbington 2012). Among these factors, water temperature and biotic interactions are thought to be two of the most influential in triggering vertical migrations of benthic invertebrates (James et al. 2008; Wood et al. 2010; Stubbington et al. 2011).

Understanding the effects of increasing water temperature on the vertical migration of invertebrates into the hyporheic zone is critical in a context of global warming and water scarcity (Postel 2000; Datry et al. 2014; Jaeger et al. 2014). In many rivers, higher maximum temperatures are exceeding the physiological tolerance of aquatic organisms (Mouthon and Daufresne 2006; Stewart et al.

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2013a). For example, historically high summer temperatures (29.5 °C max.) led to dramatic and long-lasting (>1 year) declines in mollusk richness and diversity in the Saône River, France (Mouthon and Daufresne 2006). To avoid thermal stress, invertebrates can migrate into the hyporheic zone, which is often several degrees cooler and remains buffered from highly variable surface temperatures (Constantz and Thomas 1997). However, it is unknown at what temperatures invertebrates are triggered to vertically migrate, limiting our capacity to understand and predict the effects of global change on river community resilience.

In addition to increased water temperature, vertical migration may occur when levels of biotic interactions increase, resulting notably from the contraction of aquatic habitat occurring during low flows, flow cessation and surface water loss in rivers (McGrath et al. 2007; Stubbington et al. 2011). In particular, intraspecific competition for space and food can increase greatly following flow cessation (Covich et al. 2003), as invertebrate densities reach up to 35,000 individuals (ind.) m^{-2} (e.g., Acuña et al. 2005). The hyporheic zone may provide refuge from intraspecific competition occurring on the surface (Stubbington et al. 2011), particularly because invertebrate densities in the hyporheic zone are lower (Olsen and Townsend 2003). Yet the constant interplay between water temperature and intraspecific competition in the natural environment render their effects difficult to disentangle using field surveys (Heino et al. 2015). Experimental approaches (e.g., mesocosms) can advance our understanding of invertebrate responses to multiple abiotic and biotic factors (Stewart et al. 2013b), and have been crucial to understanding benthic invertebrate migration into the hyporheic zone (e.g., Navel et al. 2010; Vadher et al. 2015).

Despite the potential for invertebrates to avoid the harmful effects of water temperature and/or biotic interactions by migrating into the hyporheic zone, their survival, effects on ecosystem function (e.g., leaf litter decomposition) and physiology may be jeopardized because hyporheic food resources are often limited or of poor quality (Hervant et al. 1997; Burrell and Ledger 2003; Danger et al. 2012). For example, Danger et al. (2012) found that buried leaf litter had lower nutritional quality and palatability than on the surface. Unless invertebrates can return to the surface to consume leaf litter, decomposition in rivers could become considerably reduced when benthic detritivores enter the hyporheic zone. Moreover, at the physiological level, invertebrate triglycerides and glycogen contents, two major energy stores involved in reproductive physiology and defense against environmental stress, may be considerably reduced within 1–2 weeks of the absence of food (Hervant et al. 1999). Therefore, use of a food-limited environment (the hyporheic zone) by invertebrates to avoid high temperatures and biotic interactions on the surface represents a tradeoff that may reduce the capacity of the hyporheic zone to provide a refuge during disturbances.

We measured the effect of temperature and intraspecific competition on the migration of the benthic invertebrate, *Gammarus pulex* (Crustacea: Amphipoda) into the hyporheic zone. We hypothesized that *G. pulex* would migrate to avoid high temperature and intraspecific competition; therefore we predicted a higher percent of organisms would be present in the hyporheic zone at increased temperatures and species densities (i.e. prediction 1). We also hypothesized that these factors would negatively affect survival, leaf consumption and energy stores of organisms either directly or indirectly through migration into the hyporheic zone; therefore, we predicted that that the survival, leaf mass consumption rate and energy stores would decrease at increased temperatures and species densities (i.e. prediction 2). We also examined the potential interaction effect (synergistic, antagonistic, additive) of water temperature and intraspecific competition on the vertical migration of *G. pulex* into the hyporheic zone.

Methods

Study organism and collection site

Gammarus pulex is a widespread detritivore across European streams and plays an important role in leaf litter degradation (MacNeil et al. 1997; Dangles and Malmqvist 2004). All individuals were collected from a first-order stream near Dijon, France (47°24′13″N, 04°52′57″E), where species identity has previously been confirmed through DNA analysis (Foucreau et al. 2013). During collection, sieves between 250 and 500 µm were used to select similar-sized adult individuals which were returned to a temperature-controlled (15 ± 2 °C, mean ± SE) laboratory and allowed to acclimatize to temperature, water quality and food source in aquaria (40 × 22 × 25 cm) for a 14-day period (Navel et al. 2010) before the start of the experiment. Water temperature was kept constant (15 ± 2 °C) using a thermostatic water pump (TECO, Ravena, Italy) and oxygen concentrations were maintained near saturation with air bubblers. During the acclimatization period, individuals were fed ad libitum alder leaves (*Alnus glutinosa*) collected in the autumn at a nearby river bank, air-dried and stored at room temperature. Leaf litter was pre-conditioned in the laboratory by immersing in river water for 10 days to allow for microbial colonization and improve leaf palatability (Navel et al. 2010).

Mesocosm description

Mesocosms (n = 36) were constructed from opaque PVC tubing (70 cm length × 25 cm diameter, 2 mm thickness) with a PVC end cap, forming a vertical column (Fig. 1). To enumerate the individuals that migrated into the hyporheic zone, mesocosms were constructed in two sections, a 30-cm surface section and a 40-cm hyporheic section, joined using PVC flanges (25 cm diameter) that allowed separation at the end of the experiment. Mesocosms were filled to a height of 50 cm with gravel substrate (10–14 mm) from the Rhône River, France that was washed and air dried to reduce organic matter content (Fig. 1). Dechlorinated tap water was continuously pumped from a 1000-L tank into the bottom of the mesocosms using peristaltic pumps at a rate of 1.25 L h^{-1}, creating a slightly positive vertical hydraulic gradient (i.e., upwelling movement of water) and constituting a complete renewal of mesocosm water volume every 24 h (Fig. 1). Water drained through a hole (2 cm diameter), screened (500 µm mesh) to prevent invertebrates from escaping, located 5 cm below the top of each column. Surface water was aerated using an air bubbler to keep dissolved oxygen (O_2) concentrations between 8.5 and 9.5 mg L^{-1}. A 12:12-h light:dark cycle was applied using Grolux (35 W, 8500 K, Sylvania Inc., Noida, India) aquarium lights above mesocosms (Fig. 1). A video describing mesocosm construction and experimental design is available at http://dx.doi.org/10.6084/m9.figshare.1544573.

Experimental design

Surface water temperature and species density were manipulated at 3 levels each in a factorial design over a 15-day period. Three temperature treatments (15, 20 and 25 °C) were tested (n = 12 mesocosms per temperature; http://dx.doi.org/10.6084/m9.figshare.1544573). To heat the surface water, a 10-m long heated cable (0.5 cm diameter) (Hydrokable, Hydor Inc. Sacramento, CA USA) was buried into the surface substrate to a depth of 10 cm and coiled around the inner wall of the mesocosms to the top of the surface section. Surface water temperature was controlled using an electronic thermostat (±0.1 °C) (Hobby, Dohse Aquaristik GmbH & Co., Grafschaft, Germany). Surface and hyporheic water temperature was recorded hourly using iButton loggers installed on the substrate surface and bottom of the mesocosms, respectively (Maxim Integrated, San Jose, CA USA). For the unheated temperature treatment (15 °C), an equally sized cable was similarly installed to account for the possible effect cables might have on vertical migration. Water temperature in the hyporheic zone was kept at 15.5 ± 0.2 °C throughout the experiment, representing an approximate mean temperature reported from several rivers and providing a thermal refuge for organisms (Constantz and Thomas 1997; Wood et al. 2010). For the first 24 h of the experiment, surface water temperature was kept at 15.2 ± 0.2 °C across all treatments. After this acclimatization period, surface water temperatures were increased to

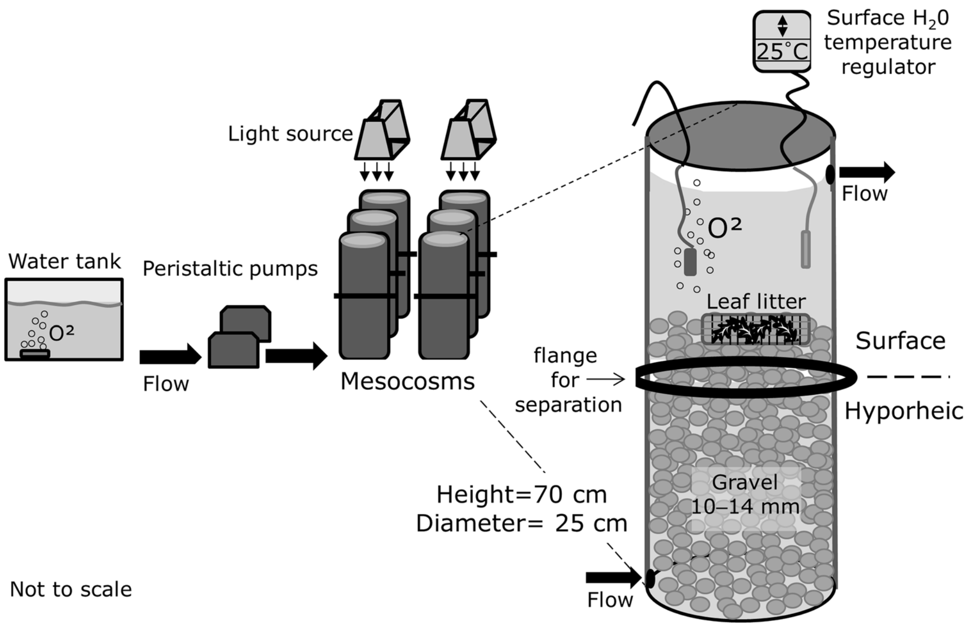

Fig. 1 Experimental set-up of mesocosms (n = 36) used to test the effect of water temperature, species density and their interaction on the migration of *Gammarus pulex* into the hyporheic zone

the treatment level at a rate of 0.2 °C h^{-1} for 20 °C (20.2 ± 0.1 °C) and 0.4 °C h^{-1} for 25 °C (25.3 ± 0.1 °C) treatments over a 24-h period to avoid thermal shock of the organisms (Stewart et al. 2013a). Surface water temperature was then kept constant until the end of the experiment.

Three species density treatments, based on previous field surveys reporting benthic *G. pulex* densities (Welton 1979; Elliott 2005), were tested (n = 12 mesocosms per density, n = 4 per density and temperature level). A low density treatment of 40 ind. $mesocosm^{-1}$, corresponding to 815 ind. m^{-2}, was used to represent density treatments having little or no intraspecific competition. Density was increased threefold to 120 ind. $mesocosm^{-1}$ (2444 ind. m^{-2}, medium density) to induce moderate levels of intraspecific competition. A high density treatment of 500 ind. $mesocosm^{-1}$ (10 183 ind. m^{-2}) was used to induce high levels of intraspecific competition. Selection of organisms included individuals of both sexes. For each treatment, individuals were counted then transferred into mesocosms using a small-hand net at the start of the experiment.

Alder leaves (2.2 ± 0.1 g) with primary veins removed, dried at 60 °C for 24 h, were enclosed in 15 × 6-cm plastic mesh (10 mm diameter) bags to provide a food source for *G. pulex*. This mesh size allowed *G. pulex* to enter the bags freely and consume leaf litter. One leaf litter bag, pre-conditioned as previously described, was placed on the substrate surface before the start of the experiment.

Percent of individuals that migrated into the hyporheic zone

The percent of individuals that migrated into the hyporheic zone was quantified after 15 days by separating the surface and hyporheic sections of the mesocosms. For this, individual mesocosms were placed into a 60 × 80 × 40 cm container, with care taken to avoid agitation of the surface water that may have caused organisms to redistribute vertically. The hyporheic section was isolated from the surface section by removing the stainless steel flange bolts that attached the two sections of the mesocosm and rapidly sliding the surface section into the large basin, leaving the hyporheic section undisturbed. During this process, the water level in the column was maintained until the moment of separation to avoid any subsequent migration of organisms into the hyporheic section. Substrate from each section was then sieved (500 µm) separately to recover all individuals from their respective section.

Survival of *G. pulex*

Upon collection, all individuals were placed in sorting trays and inspected for movement. Individuals that did not survive the experiment were counted and separated from living individuals so they were not used for assays of triglycerides and glycogen (see below). Because *G. pulex* is known to feed on its conspecifics (MacNeil et al. 1997), individuals not found at the end of the experiment were presumed to be dead and consumed. Few individuals (<1 %) appeared to be killed during sieving; however, these individuals could not be reliably separated from individuals that did not survive the experimental treatments.

Measuring leaf litter consumption rate

Following the experiment, leaves were dried at 60 °C for 24 h and re-weighed. Leaf consumption rates (mg. $ind.^{-1}$ day^{-1}) were calculated as [(initial dry leaf mass) − (final dry leaf mass)/(*N* ind.)*15 days]. To correct final leaf mass consumption for leaching and microbial consumption not attributable to *G. pulex*, a temperature-specific correction factor was calculated based on the leaf mass loss in bags (n = 9) immersed in additional columns void of *G. pulex* for 15 days at each temperature level (Navel et al. 2010). Consumption rates were calculated based on both the initial number and final number of individuals per mesocosm to account for survivorship and ensure that analyses testing the effects water temperature and species density on this metric were not biased by the calculation method.

Measuring triglycerides and glycogen contents

For triglycerides and glycogen assays, individuals collected at the end of the experiment were dried using an absorbent cloth, freeze-dried and weighed in groups of 3–4 individuals. Three replicate groups from each mesocosm were analyzed to establish mean triglycerides and glycogen contents, regardless of location in the mesocosm. Groups were weighed (±0.1 mg) and then ground into powder with a small mortar in pre-weighed glass tubes. Triglycerides and glycogen (µmol g^{-1} dry mass) were extracted using standard enzymatic methods with prepared solutions (Sigma-Aldrich, Saint-Quentin Fallavier, France) described in further detail in Salin et al. (2010). Assays were made using an Aquamate spectrophotometer (Thermo Scientific Inc., Waltham, MA, USA) at 25 °C.

Data analysis

Difference in the mean percent of *G. pulex* that migrated into the hyporheic zone, percent survivorship, leaf mass consumption rate and triglycerides and glycogen contents between treatments was tested using a two-way analysis of variance (ANOVA). The design was a 3 (temperature levels: 15, 20, 25 °C) × 3 (species density levels: low, medium, high) factorial design with interactions. Post hoc Tukey HSD multiple comparisons were used to compare mean levels within temperature and density treatment factors. Plotted residual variances and Levene's test were used to check for normality and homogeneity of variance, subsequently, all percentages were arc($\sqrt{-x}$)-transformed and leaf mass consumption rates and triglycerides and glycogen content values were $\log_{10}$(x)-transformed to meet these assumptions. ANOVAs and post hoc comparisons were made using *R* (version 3.1.1; R Project for Statistical Computing, Vienna, Austria).

Results

Effect of water temperature and species density on *G. pulex* migration into the hyporheic zone

The percent of individuals that migrated into the hyporheic zone increased as water temperature and species density increased (ANOVA, temperature effect: $F_{2,27} = 4.28$, $P = 0.024$; density effect: $F_{2,27} = 11.354$, $P < 0.001$; Table 1, Fig. 2). The percent of organisms that migrated was higher in the 25 °C treatments than at 15 °C (Tukey HSD, $P = 0.030$; Fig. 2), but not different from the percent measured in the 20 °C treatments. At high species density, the percent of organisms that migrated was greater than in medium density (Tukey HSD, $P = 0.003$) and low density treatments (Tukey HSD, $P < 0.001$; Fig. 2), while medium and low density treatments did not differ. The effect of water temperature on the percent of individuals that migrated did not change at high density (ANOVA, temperature × density effect: $F_{4,27} = 1.65$, $P = 0.191$; Table 1; Fig. 2).

Effect of water temperature and species density on survival of *G. pulex*

The percent of organisms that survived the experiment decreased as water temperature increased and there was an interaction effect of water temperature and species density (ANOVA, temperature × density effect: $F_{2,27} = 5.64$, $P = 0.002$; Tables 1, 2). The percent survival across low and medium densities was lowest at 25 °C compared to

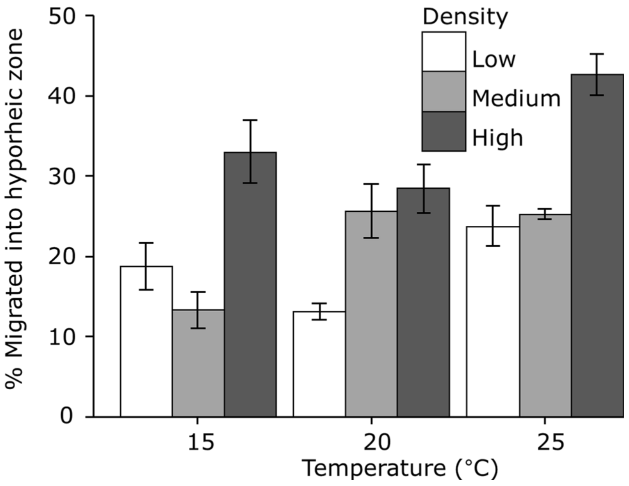

Fig. 2 Mean (±SE) percent of *Gammarus pulex* that migrated into the hyporheic zone in different water temperature and species density treatments, based on the initial species density

Table 1 The effect of temperature and species density and their interaction on dependent variables related to *Gammarus pulex*, determined using two-way ANOVA tests

Dependent variable	Factor	d.f.	MSS	*F*	*P*
% Migrated	Temperature (T)	2	0.045	4.280	**0.024**
	Density (D)	2	0.119	11.354	**<0.001**
	T × D	4	0.017	1.650	0.191
% Survivorship	Temperature (T)	2	0.343	65.869	**<0.001**
	Density (D)	2	0.008	1.559	0.229
	T × D	4	0.029	5.640	**0.002**
Leaf mass consumption	Temperature (T)	2	1.975	38.091	**<0.001**
	Density (D)	2	1.087	15.120	**<0.001**
	T × D	4	1.734	15.931	**<0.001**
Triglycerides content	Temperature (T)	2	0.959	0.727	0.493
	Density (D)	2	0.940	0.459	0.637
	T × D	4	1.082	1.240	0.319
Glycogen content	Temperature (T)	2	2.869	2.461	0.105
	Density (D)	2	2.517	0.563	0.577
	T × D	4	3.902	4.013	**0.012**

Percentages were arcsin($\sqrt{-x}$)-transformed and leaf mass consumption rate, triglycerides and glycogen contents were log10(x)-transformed. Statistical significance is indicated in bold

Table 2 Mean (±SE) percent survival of *Gammarus pulex* in different temperature and species density treatments after the 15-day experiment

Temperature (°C)	Species density	Mean (%)	Min.–Max.
15 °C	Low	61 ± 8	55–73
	Medium	62 ± 5	54–71
	High	67 ± 8	58–72
20 °C	Low	79 ± 2	73–85
	Medium	67 ± 7	62–70
	High	63 ± 7	59–67
25 °C	Low	34 ± 8	23–48
	Medium	35 ± 2	33–37
	High	48 ± 3	42–57

survival measured at 15 and 20 °C (Tukey HSD, $P < 0.001$ for all), whereas survival in the high density treatment at 25 °C differed from survival in the high density treatment at 15 °C (Tukey HSD, $P = 0.02$; Table 2) but not the high density treatment at 20 °C.

Effect of water temperature and species density on the leaf litter consumption rate of *G. pulex*

Leaf consumption rate of *G. pulex* based on the initial density was affected by water temperature, species density and their interaction (ANOVA, temperature × density effect: $F_{2,26} = 15.93$, $P < 0.001$, Table 1; Fig. 3). Consumption rate in the 20 °C and 25 °C treatments was lowest at medium and high densities compared to low density (Tukey HSD, $P < 0.001$ for all) but at 15 °C, different consumption rates were only detected between low and high density treatments (Tukey HSD, $P < 0.001$, Fig. 3). Similarly, consumption rate based on the final density was also affected by the interaction of water temperature and species density (results shown in Appendix Table 3).

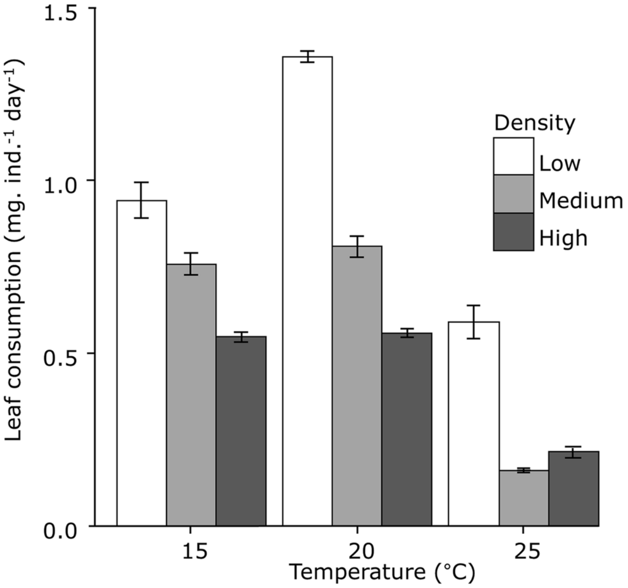

Fig. 3 Mean (±SE) leaf mass consumption rate of *Gammarus pulex* in different water temperature and species density treatments, based on the initial species density

Triglycerides and glycogen contents of *G. pulex*

Mean triglycerides content did not differ among the levels of water temperature, species density nor by the interaction of these factors (Table 1; Fig. 4a). For mean glycogen contents, the effect of water temperature was not consistent across species densities (ANOVA, temperature × density effect: $F_{4,26} = 4.013$, $P = 0.012$; Table 1; Fig. 4b). Glycogen contents at high and medium densities was lower than at low density in the 20 °C treatment (Tukey HSD, $P = 0.004$ for both) and at high density in the 25 °C treatment (Tukey HSD, $P = 0.027$), whereas there was no

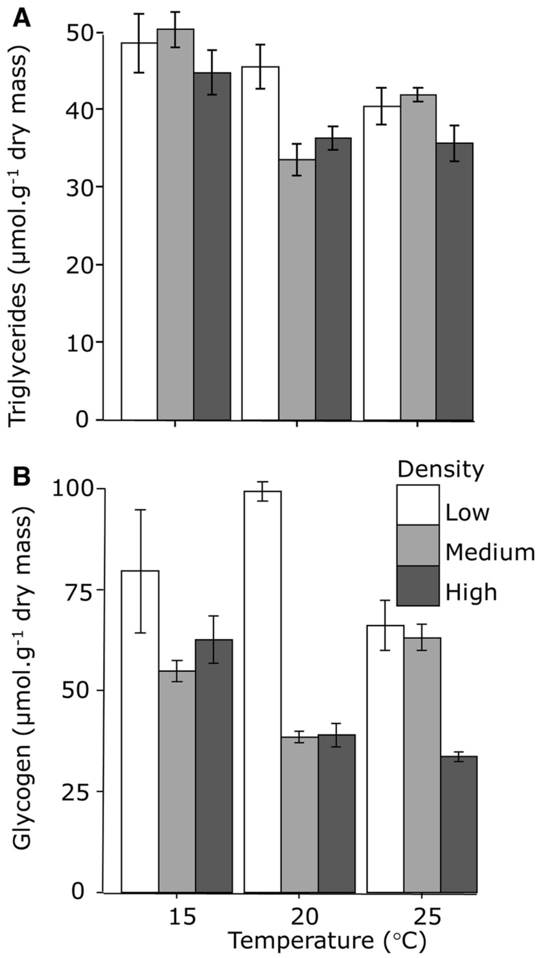

Fig. 4 Mean (±1 SE) **a** triglycerides and **b** glycogen contents of *Gammarus pulex* individuals in different temperature and species density treatments

difference in glycogen contents among low, medium and high densities at 15 °C.

Discussion

Influence of water temperature and species density on vertical migration into the hyporheic zone

In agreement with our first prediction, both increasing water temperature and species density led to the migration of *G. pulex* into the hyporheic zone. These findings imply that hyporheic refuge use is an active process, rather than a passive range extension (Stubbington 2012), in which invertebrates use abiotic and biotic cues to avoid the harsh surface conditions that coincide with low flows, flow cessation and drying events. Water temperature of 25 °C caused a higher percent of individuals to use the hyporheic zone. This threshold closely matches the temperature (24 °C) at which *G. pulex* survival becomes drastically reduced in short-term (10 days) exposures without thermal refuges (Foucreau et al. 2014). For intraspecific competition, a threefold increase in species density (2400 ind. m^{-2}) led to a higher percentage of individuals using the hyporheic zone. In previous behavior experiments with *G. pseudolimnaeus*, Williams and Moore (1985) found a 3.5-fold increase in species density increased the number of individuals on the substrate rather than in the water column. Our results also mirror those from two previous field studies investigating hyporheic refuge use by invertebrates (Wood et al. 2010; Stubbington et al. 2011). Wood et al. (2010) reported peak invertebrate densities in the hyporheic zone when surface water temperature around 20 °C was reached in the Little Stour River, UK; whereas Stubbington et al. (2011) found the hyporheic proportion of *G. pulex* peaked during a heatwave and low-flow period in the River Lathkill, UK, when the highest density occurred (2449 ind. m^{-2}; hyporheic + benthic abundance). Our mesocosm approach complemented these field surveys by disentangling the individual and combined effects of water temperature and intraspecific competition on the vertical migration of these invertebrates.

Biotic interactions (e.g., competition, predator–prey relationships) can intensify with increasing water temperature due to increased metabolic rates (Burnside et al. 2014), leading to unexpected species responses in aquatic systems (Ormerod et al. 2010). However, the interaction of high water temperature and species density was not significant in this study, indicating their combined effects on the percent of *G. pulex* that migrated into the hyporheic zone was not higher or lower than expected based on their individual effects (i.e. additive response). The absence of an interaction between high temperature and species density may be attributed to the behavior of *G. pulex* at temperatures above its thermal tolerance. Above its thermal tolerance, activity and metabolic rates of *G. pulex* can decrease sharply (e.g., Foucreau et al. 2014), and this may have led to a decrease in conspecific encounters and reduced competition (Wooster et al. 2011). Therefore, biotic interactions may increase with water temperature until the point when thermal tolerance is exceeded, which is between 21 and 25 °C for many aquatic invertebrates (Stewart et al. 2013a), and individuals reduce their activity in a final attempt to conserve energy and prolong survival (Foucreau et al. 2014).

Our results, along with those from previous field studies (e.g., Wood et al. 2010; Stubbington et al. 2011), bolster evidence of hyporheic refuge use by benthic invertebrates avoiding increased water temperatures and biotic interactions. These results have important implications considering the projected global-scale increases in water temperatures and flow intermittence (Postel 2000; van Vliet et al. 2013). In particular, water-stressed regions, such as the American Southwest, may see a 27 % increase in the median number of days of flow cessation and a 15-day increase drying event duration by mid-century, respectively (Jaeger et al. 2014). Changes in flow regime will be coupled with average increases in global mean and maximum river water temperatures of up to 1.6 °C which will, in turn, increase evaporation and drying rates (van Vliet et al. 2013). If organisms can survive temporarily in the hyporheic zone and return to the surface when conditions become favorable, it is likely that, in at least some systems (e.g., alluvial rivers), the hyporheic zone can be a source of resilience for invertebrate communities (DiStefano et al. 2009; Vander Vorste et al. 2015).

Future experiments should test the effects of different abiotic and biotic factors that could also influence invertebrate use of the hyporheic zone. For example, dissolved oxygen saturation in receding river pools can be as low as 6 % (e.g., Boulton 1989) and when coupled with high water temperatures it will likely increase the negative effects on invertebrates and consequently, migration of invertebrates into the hyporheic zone could be strongly increased. Depth to the water table below the riverbed may also be an important factor limiting the colonization and return to the surface by invertebrates (Vander Vorste et al. 2015). Furthermore, interspecific competition and predation often increase simultaneously in drying rivers (Lake 2003) and may trigger migration of invertebrates into the hyporheic zone (Stubbington 2012). Invertebrate migration into hyporheic zone may decrease predation risk from fish and large invertebrates (e.g., Fairchild and Holomuzki 2005), and reduce top-down effects in river pools (Boersma et al. 2014). Finally, substrate size (Navel et al. 2010; Vadher et al. 2015) and the direction of vertical hydraulic gradient (i.e., upwelling, downwelling; Olsen and Townsend 2003; Mathers et al. 2014) are likely overriding physical factors controlling vertical migration of invertebrates. In general, higher abundances of benthic invertebrate are found in downwelling reaches (e.g., Dole-Olivier et al. 1997; Olsen and Townsend 2003; Capderrey et al. 2013), presumably aided by the downward movement of water. In this study, the fact that mesocosms had upwelling water strengthens evidence that *G. pulex* actively sought refuge in the hyporheic zone, rather than passively following the direction of water movement. Therefore, it is expected that *G. pulex* would be more likely to enter the hyporheic zone in downwelling river reaches (Dole-Olivier et al. 1997). Alternatively, vertical migration may have been related to the rheophilic nature of *G. pulex* or its ability to detect cooler temperatures in the upwelling water. Future mesocosm experiments should examine the potential for organisms to detect environmental conditions in the hyporheic zone.

Decreased survival, leaf litter consumption rate and energy stores

In agreement with our second prediction, survival, leaf litter consumption and glycogen energy stores of *G. pulex* were reduced in high temperature and species density treatments. These reductions could be linked to either the direct or indirect effects of high temperature and intraspecific competition. For example, metabolic (Foucreau et al. 2014) and growth rates in *G. pulex* (Sutcliffe et al. 1981) are reduced at high water temperatures (~25 °C), therefore survival, consumption rates and energy stores could be reduced directly by stressful conditions. However, the high percent of *G. pulex* that migrated into the hyporheic zone shows that most individuals could avoid the direct effects of high temperature and competition. In this study, the hyporheic zone mimicked conditions in the natural streams where the availability of food resources are generally limited and/or of poor quality (Burrell and Ledger 2003; Danger et al. 2012). Consequently, once in the hyporheic zone, the lack of food and subsequent starvation could have lowered rates of survivorship, consumption and decreased glycogen content of *G. pulex*. Indeed, previous studies have shown that benthic invertebrates are highly susceptible to mortality during periods of starvation (Hervant et al. 1997, 1999). Therefore, we argue that the indirect effects of using the hyporheic zone were responsible for reductions in survival, leaf litter consumption and glycogen energy stores. This indicates that food resources may be an important limiting factor that influences invertebrate survival during disturbances despite their ability to avoid harsh conditions in the hyporheic zone.

As a consequence of migration into the hyporheic zone, the processing of leaf litter by invertebrates in the benthic zone may be substantially reduced during periods of low flow, flow cessation, and stream drying (Corti et al. 2011; Datry et al. 2011). This result expands on recent studies suggesting that leaf litter decomposition will increase with rising water temperatures due to enhanced microbial decomposition and invertebrate activity rates (e.g., Ferreira and Canhoto 2015; Mas-Martí et al. 2015). Our results suggest decomposition rates will be reduced when temperatures and/or competition increase beyond thresholds that cause a behavioral response of shredding invertebrates to enter the hyporheic zone. The large reduction in leaf litter consumption shown could have broad implications on ecosystem function in rivers considering that *G. pulex* were responsible for an estimated 13 % of leaf litter consumption in a wooded stream (Mathews 1967) and several other invertebrate shredders (e.g., Leuctridae, Leptoceridae) are known to use the hyporheic zone during disturbances (Stubbington 2012). An important next step will be to test if invertebrates are able to track diel water temperature changes, returning to the surface at night when temperatures are cooler to feed and how this may compensate for energy loss during the day.

Benthic invertebrates face a tradeoff between tolerating harsh surface conditions versus avoiding them by entering the hyporheic zone, a strategy which may not promote long-term survival. On one hand, lower water temperature, fewer conspecific interactions and the lack of large predators may entice invertebrates to migrate into the hyporheic zone during periods of low flow, flow cessation and drying. On the other hand, once in the hyporheic zone, food limitation, low oxygen concentrations (Findlay 1995) and clogging (Descloux et al. 2013), especially in rivers impacted by agricultural land use, will reduce the capacity of the hyporheic refuge. Furthermore, competitive and predatory interactions with other migrating benthic and/or established hypogean taxa (e.g., Schmid and Schmid-Araya 1997) are likely to occur, although evidence of these interactions must be explored further. These interactions may have negative or positive effects on the resilience of benthic invertebrates, depending on their outcome. Therefore, the potential cascading effects (e.g., ecosystem function) of hyporheic refuge use by invertebrates remains an important research gap that could be addressed through mesocosm experiments.

Conclusion

Understanding the influence of factors, such as water temperature and biotic interactions, which coincide with low flow, flow cessation and drying events on river communities is of increasing relevance considering that global change is exacerbating the occurrence of these events in river systems (Postel 2000; Datry et al. 2014; Jaeger et al. 2014). In many regions, once-perennial rivers are becoming intermittent (Datry et al. 2014), therefore future studies could explore differences in the response to drying among populations from perennial and naturally intermittent rivers. Although rare in freshwater ecology, experiments that simultaneously subject different populations to the same stressor (e.g. common garden experiments) have revealed strong inter-population differences in temperature tolerance within aquatic species (e.g., Foucreau et al. 2014). Therefore, such approaches could be developed to explore the responses of populations from perennial and intermittent rivers to other environmental factors associated with river contraction and drying. These experiments will in turn help refine the predictions of population and community responses to global climate change and increased water abstraction.

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Appendix

See Table 3.

Table 3 Results from 2-way ANOVA testing the effect of temperature and species density and their interaction on leaf mass consumption of *Gammarus pulex*

Dependent variable	Factor	d.f.	MSS	*F*	*P*
Leaf mass consumption	Temperature (T)	2	1.104	0.409	0.669
	Density (D)	2	1.920	9.943	**<0.001**
	T × D	4	1.649	3.390	**0.024**

Leaf mass consumption rate was log10(x)-transformed. Leaf mass consumption based on final number of individuals to account for survivorship though it was not possible to determine when organisms died during the experiment. Statistical significance is indicated in bold

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