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Ross Vander Vorste, Florian Mermillod-Blondin, Frédéric Hervant, R. Mons,  
T. Datry

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

3 Authors: Vander Vorste<sup>1</sup>, R., F. Mermillod-Blondin<sup>2</sup>, F. Hervant<sup>2</sup>, R. Mons<sup>1</sup> and T. Datry<sup>1</sup>

4 Affiliations:

5 <sup>1</sup>IRSTEA, UR MALY, 5 rue de la Doua, CS70077, F-69626 Villeurbanne, France

6 <sup>2</sup>LEHNA, UMR CNRS 5023, Ecologie des Hydrosystèmes Naturels et Anthropisés, Université de Lyon,  
7 Université Lyon 1, 6 rue Raphael Dubois, 69622 Villeurbanne, France

8  
9 Corresponding author:

10 R. Vander Vorste, IRSTEА, UR MALY, Centre de Lyon-Villeurbanne, 5 Rue de la Doua CS70077 69626  
11 Villeurbanne Cedex, France. Tel (33) 4.72.20.10.87 Fax (33) 4.78.47.78.75, ross.vander-vorste@irstea.fr

12 Abstract:

13 The saturated sediments below and adjacent to the riverbed (i.e., hyporheic zone) can be a refuge for biota during  
14 disturbances, such as drying. Prior to drying, organisms are constrained by abiotic and biotic factors (e.g., water  
15 temperature, competition) and may respond through vertical migration into the hyporheic zone. However, it  
16 remains unclear when these factors become harsh enough to trigger this response. Furthermore, potential  
17 consequences of using the hyporheic zone, which is often food-limited, on the survival, ecosystem function and  
18 physiology of organisms are unknown. Using 36 mesocosms, the hypotheses that i) *Gammarus pulex* migrates  
19 into the hyporheic zone to avoid increasing surface water temperature and intraspecific competition and ii)  
20 migration would have negative consequences on the survival, leaf mass consumption and energy stores of  
21 organisms were tested. Three levels of temperature (15, 20, 25°C) and species density (low, medium, high) were  
22 manipulated in a factorial design over 15 days. Increased temperature to 25°C and a 3-fold increase in density  
23 both caused *G. pulex* to migrate into the hyporheic zone, but the interaction of these factors was not synergistic.  
24 Importantly, the survival, leaf consumption and glycogen content were reduced in high temperature and density  
25 treatments, indicating tradeoffs between tolerating harsh surface conditions and limitations in the hyporheic  
26 zone. Identifying that the hyporheic zone is used by invertebrates to avoid high water temperature and

27 intraspecific competition is a key finding considering the global-scale increases in temperature and flow  
1  
2 28 intermittence, yet its capacity to provide refuge is likely temporally limited.  
3

4 29 Key words:  
5

6  
7 30 stream drying, refuge, resistance, resilience, avoidance behavior, invertebrates  
8  
9

## 10 31 **Introduction**

11  
12 32 The saturated interstitial areas beneath the riverbed and into the adjacent banks (i.e., hyporheic zone; White,  
13  
14 33 1993) have long been recognized for their potential to serve as a refuge for biota during disturbances (i.e.,  
15  
16 34 hyporheic refuge hypothesis; Palmer et al. 1992, Dole-Olivier et al. 1997, Stubbington 2012). They may also be  
17  
18 35 a major source of colonization promoting the resilience of invertebrates (i.e., capacity to recover, Stanley 1994)  
19  
20 36 following disturbances, such as flooding and drying (e.g., Holomuzki and Biggs 2007, Kawanishi et al. 2013,  
21  
22 37 Vander Vorste et al. 2015 *in review*). Despite substantial empirical evidence showing that the physical  
23  
24 38 characteristics of the hyporheic zone (e.g., % fine sediment, hydraulic conductivity, vertical hydraulic gradient)  
25  
26 39 alter its potential to serve as a refuge (e.g., Navel et al. 2010, Descoux et al. 2013, Mathers et al. 2014), the  
27  
28 40 abiotic or biotic factors invertebrates respond to through vertical migration into the hyporheic zone remain  
29  
30 41 speculative (Stubbington 2012). Among these factors, water temperature and biotic interactions are thought to  
31  
32 42 be two of the most influential in triggering a behavioral response for stream invertebrates to enter the hyporheic  
33  
34 43 zone (James et al. 2008, Wood et al. 2010, Stubbington et al. 2011).  
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36  
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38 44 Understanding the effects of increasing water temperature on the vertical migration of invertebrates into  
39  
40 45 the hyporheic zone is critical in a context of global warming and water scarcity (Postel 2000, Datry et al. 2014,  
41  
42 46 Jaeger et al. 2014). In many rivers, the increase in water temperature observed over the past 100 years ranges  
43  
44 47 between 0.009–0.077 °C y<sup>-1</sup> (Kaushal et al. 2010) and higher maximum temperatures (e.g., Mantua et al. 2010)  
45  
46 48 are exceeding the physiological tolerance of aquatic organisms (Mouthon and Daufresne 2006, Wenger et al.  
47  
48 49 2011, Stewart et al. 2013a). For example, a 1.5°C increase in mean temperature combined with historically high  
49  
50 50 summer temperatures (29.5°C max.) caused dramatic and long-lasting (>1 yr) declines in mollusk richness and  
51  
52 51 diversity in the Saône River, France (Mouthon and Daufresne 2006). Moreover, increasing water scarcity issues  
53  
54 52 and subsequent low flow, flow cessation and drying events in river systems (Postel 2000, Datry et al. 2014,  
55  
56 53 Jaeger et al. 2014) exacerbate the general trend in water temperature increase. For example, during the initial  
57  
58 54 contraction phase of drying streams, water temperature can reach above 25°C (e.g., Boulton 1989, Ludlam and  
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55 Magoulick 2010). Insect larvae (e.g., Ephemeroptera, Plecoptera, Trichoptera), and crustaceans (e.g.,  
1 Amphipoda, Isopoda) experience drastic increases in mortality between 21–25°C (Stewart et al. 2013a, Foucreau  
2 et al. 2014). To avoid thermal stress, invertebrates can migrate into the hyporheic zone, which is often several  
3  
4 57 degrees cooler and remains buffered from highly variable surface temperatures (Constantz and Thomas 1997,  
5  
6 58 Evans and Petts 1997). However, it is unknown at what temperatures invertebrates vertically migrate into the  
7  
8 59 hyporheic zone, limiting our capacity to understand and predict the effects of global change on river community  
9  
10 60 resilience. As climate change and water abstraction will continue to challenge river communities, quantifying the  
11  
12 61 potential of the hyporheic zone to act as a refuge will be a key step towards predicting future responses of  
13  
14 62 aquatic invertebrates (Keppel et al. 2015).  
15  
16 63

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18  
19 64 In addition to increased water temperature, vertical migration may occur when levels of biotic  
20  
21 65 interactions increase, resulting notably from the contraction of aquatic habitat occurring during low flow, flow  
22  
23 66 cessation and river drying (e.g., Power et al. 1985, Ludlam and Magoulick 2010). In particular, intraspecific  
24  
25 67 competition for space and food can increase greatly following flow cessation (Lake 2003), as invertebrate  
26  
27 68 densities reach up to 35 000 individuals (ind.) m<sup>-2</sup> (e.g., Acuña et al. 2005). High density of *Chironomus riparius*  
28  
29 69 (Diptera) resulted in up to 75% mortality of early instars, delayed development and increased migration to avoid  
30  
31 70 competition (Silver et al. 2000). The hyporheic zone may provide refuge from intraspecific competition  
32  
33 71 occurring on the surface (James et al. 2008, Stubbington et al. 2011); particularly because invertebrate densities  
34  
35 72 in the hyporheic zone are comparatively lower (Datry 2012, Capderrey et al. 2013). Furthermore, biotic  
36  
37 73 interactions may increase (e.g., Scherr et al. 2010), decrease (e.g., Jiang and Morin 2004), or remain unaffected  
38  
39 74 (e.g., Wooster et al. 2011) at high water temperatures, subsequently affecting vertical migration of surface-  
40  
41 75 dwelling invertebrates into the hyporheic zone. Yet the constant interplay between water temperature and  
42  
43 76 intraspecific competition in the natural environment render their effects difficult to disentangle using field  
44  
45 77 surveys (Heino et al. 2015). Experimental approaches (e.g., mesocosms) can advance our understanding of the  
46  
47 78 responses of invertebrates to multiple abiotic and biotic factors (Stewart et al. 2013b), and have been crucial to  
48  
49 79 understanding vertical distribution of invertebrates at surface water and hyporheic zone interface (e.g., Nogaro et  
50  
51 80 al. 2009, Navel et al. 2010, Vadher et al. 2015).  
52  
53

54 81 Despite the potential for invertebrates to seek refuge in the hyporheic zone to avoid the harmful effects  
55  
56 82 of water temperature and/or biotic interactions occurring on the surface, their survival, ecosystem function and  
57  
58 83 physiology may be jeopardized because food resources are often limited or of poor quality (Hervant et al. 1997,  
59  
60 84 Burrell and Ledger 2003, Danger et al. 2012). For example, the surface invertebrate, *Gammarus fossarum*,

85 subjected to starvation showed immediate hyperactivity and experienced mortality after 20 days (Hervant et al.  
1 86 1997). Unless invertebrates can return to the surface to consume leaf litter (e.g., Elliott 2005, Navel et al. 2010),  
2  
3 87 the decomposition of leaf litter on the surface will be considerably reduced when surface detritivores enter the  
4  
5 88 hyporheic zone. Moreover, at the physiological level, invertebrate triglycerides and glycogen contents, two  
6  
7 89 major energy stores involved in reproductive physiology and defense against environmental stress, may be  
8  
9 90 considerably reduced within 1–2 weeks of the absence of food (Hervant et al. 1999). Therefore, use of a food-  
10  
11 91 limited environment (the hyporheic zone) by invertebrates to avoid high temperatures and biotic interactions on  
12  
13 92 the surface represents a tradeoff that may undermine the capacity of the hyporheic zone to provide refuge during  
14  
15 93 disturbances.  
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18  
19 94 In this study, we measured the effect of temperature and intraspecific competition on the vertical  
20  
21 95 migration of the common stream shredding detritivore, *Gammarus pulex* (Crustacea: Amphipoda) into the  
22  
23 96 hyporheic zone. We hypothesized that the hyporheic zone would be used as a refuge by this species to avoid  
24  
25 97 high water temperature and intraspecific competition. We also hypothesized that migration into hyporheic zone  
26  
27 98 would have negative effects on the survival, leaf consumption and energy stores of organisms. Based on these  
28  
29 99 hypotheses, we predicted that: i) a higher proportion of organisms would migrate into the hyporheic zone as  
30  
31 100 water temperature and species density increased, and ii) that the survival, leaf mass consumption rate and energy  
32  
33 101 stores would decrease at the highest temperature and species densities. We also examined the potential  
34  
35 102 interaction effect (synergistic, antagonistic, additive) of water temperature and intraspecific competition on the  
36  
37 103 vertical migration of *G. pulex* into the hyporheic zone.  
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39

## 40 104 **Methods**

### 41 42 43 105 *Study organism and collection site*

44  
45 106 *Gammarus pulex* is a widespread and common surface-dwelling shredder that is important in leaf litter  
46  
47 107 degradation across European streams (MacNeil et al. 1997, Dangles and Malmqvist 2004, Piscart et al. 2011).  
48  
49 108 All individuals were collected from a small stream near Dijon, France (47°24'13"N, 04°52'57"E), where species  
50  
51 109 identity was previously confirmed through DNA analysis (Foucreau et al. 2013). During collection, sieves  
52  
53 110 between 2.5–5.0 mm were used to select similar-sized individuals. Individuals were returned to a temperature-  
54  
55 111 controlled ( $15 \pm 2^\circ\text{C}$ ) room and allowed to acclimatize to laboratory temperature, water quality and food source  
56  
57 112 for a 14-day period (Navel et al. 2010) before the start of the experiment. Water temperature was kept constant  
58  
59 113 ( $15 \pm 2^\circ\text{C}$ ) using a thermostatic water pump (TECO, Ravenna, Italy) and oxygen concentrations were maintained  
60  
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62  
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114 near saturation with oxygen bubblers. During this time, individuals were fed alder leaves (*Alnus glutinosa*)  
115 collected in the autumn at a nearby river bank, air-dried and stored at room temperature.

#### 116 *Mesocosm description*

117 Mesocosms (n = 36) were constructed from opaque PVC tubing (70 cm length × 25 cm diameter, 2 mm  
118 thickness) with a PVC end cap, forming a vertical column (Fig. 1). To enumerate the individuals that migrated  
119 into the hyporheic zone, mesocosms were constructed in two parts, a 30-cm surface zone and a 40-cm hyporheic  
120 zone. These parts were joined during the experiment using PVC flanges (25 cm diameter) and allowed quick  
121 separation at the end of the experiment. Mesocosms were filled to a height of 50 cm with gravel substrate (10–14  
122 mm) extracted from the Rhône River, France, leaving 10 cm of substrate in the surface zone (Fig. 1).  
123 Dechlorinated tap water was continuously pumped from a 1000-L tank into the bottom of the mesocosms using  
124 two 24-channel peristaltic pumps at a rate of 1.25 L h<sup>-1</sup>, creating a slightly positive vertical hydraulic gradient  
125 (i.e., upwelling movement of water) and constituting a complete renewal of mesocosm water volume in 24 h  
126 (Fig. 1). Water drained through a hole (2 cm diameter), screened (0.5 cm mesh) to prevent invertebrates from  
127 escaping, located 5 cm below the top of each column. Surface water was aerated using an oxygen bubbler to  
128 keep dissolved oxygen (O<sub>2</sub>) concentrations between 8.5–9.5 mg L<sup>-1</sup>. A 12:12-h light:dark cycle was applied to  
129 the surface water zone using GroLux (35 W, 8500 K, Sylvania Inc., Noida, India) aquarium lights above  
130 mesocosms (Fig. 1).

#### 131 *Experimental design*

132 Surface water temperature and species density were manipulated at 3 levels each in a factorial design over a 15-  
133 day period. Three treatments of temperature (15, 20 and 25°C) were tested (n= 12 mesocosms per temperature).  
134 To heat the surface water, a 10-m long heated cable (0.5 cm diameter) (Hydrokable, Hydor Inc. Sacramento, CA  
135 USA) was buried into the surface substrate and coiled around the inner wall of the mesocosms to the top of the  
136 surface zone. Surface water temperature was controlled using an electronic thermostat (± 0.1°C) (Hobby, Dohse  
137 Aquaristik GmbH & Co., Graftschaff, Germany). Surface and hyporheic water temperature was recorded hourly  
138 using iButton loggers (Maxim Integrated, San Jose, CA USA). For the unheated temperature treatment (15°C)  
139 (see below), an equally sized cable was similarly installed to account for the possible effect cables might have on  
140 vertical migration. Water temperature in the hyporheic zone was kept at 15.5 ± 0.5°C (mean ± SD) throughout  
141 the experiment, representing an approximate mean temperature reported from several rivers and providing a  
142 thermal refuge for organisms (Constantz and Thomas 1997, Evans and Petts 1997, Stubbington et al. 2011). For

143 the first 24 hours of the experiment, water temperature was kept constant ( $15.2 \pm 0.3^\circ\text{C}$ ) across all treatments.

144 After this acclimatization period, temperatures were increased to the treatment level at a rate of  $0.2^\circ\text{C h}^{-1}$  for  
145  $20^\circ\text{C}$  and  $0.4^\circ\text{C h}^{-1}$  for  $25^\circ\text{C}$  treatments over a 24-h period to avoid thermal shock of the organisms (Stewart et  
146 al. 2013a). Surface water temperature was then kept constant until the end of the experiment.

147 Three species density treatments, based on previous field surveys reporting *G. pulex* densities (Welton  
148 1979, Elliott 2005), were tested ( $n= 12$  mesocosms per density). A low density treatment of  $40 \text{ ind. mesocosm}^{-1}$ ,  
149 corresponding to  $815 \text{ ind. m}^{-2}$ , was used to represent density treatments having little or no intraspecific  
150 competition. Density was increased 3-fold to  $120 \text{ ind. mesocosm}^{-1}$  ( $2444 \text{ ind. m}^{-2}$ ) to induce moderate levels of  
151 intraspecific competition (i.e., medium density). A high density treatment of  $500 \text{ ind. mesocosm}^{-1}$  ( $10\ 183 \text{ ind.}$   
152  $\text{m}^{-2}$ ) was used to induce high levels of intraspecific competition. For each treatment, individuals were counted by  
153 hand before being transferred into mesocosms using a small-hand net at the start of the experiment.

#### 154 *Proportion of individuals migrated into the hyporheic zone*

155 The proportion of individuals that migrated into the hyporheic zone was quantified after 15 days by separating  
156 the surface and hyporheic zones of the mesocosms. For this, mesocosms were placed into a  $60 \times 80 \times 40 \text{ cm}$   
157 plastic wash basin, with care taken to avoid agitation of the surface water that may cause organisms to  
158 redistribute vertically. The hyporheic zone was isolated from the surface zone by removing the stainless steel  
159 bolts that attached the two parts of the mesocosm and rapidly sliding the surface zone into the large basin,  
160 leaving the hyporheic zone of the mesocosm undisturbed. During this process, the water level in the columns  
161 was maintained until the moment of separation to avoid incidental migration of organisms into hyporheic zone.  
162 Substrate from each section was then sieved ( $500 \mu\text{m}$ ) separately to recover all individuals from their respective  
163 zone.

#### 164 *Survival of organisms*

165 Upon collection, all individuals were placed in white sorting trays and visually inspected for any movement.  
166 Individuals that did not survive the experiment were counted and separated from living individuals so they were  
167 not used for assays of triglycerides and glycogen (see below). Because *G. pulex* is known to feed on its dead  
168 conspecifics (MacNeil et al. 1997), individuals not found at the end of the experiment were presumed to be dead  
169 and consumed. Few individuals ( $<1\%$ ) appeared to be killed during the sampling effort (i.e. sieving); however,

170 these individuals could not be reliably separated from individuals that did not survive the experimental  
171 treatments.

#### 172 *Measuring leaf consumption rate*

173 In each mesocosm,  $220 \pm 10$  mg of alder leaves with primary veins removed, dried at  $60^{\circ}\text{C}$  for 24 hours, were  
174 enclosed in  $15 \times 6$ -cm plastic mesh (10 mm diameter) bags. This mesh size allowed *G. pulex* to enter the bags  
175 freely and consume leaf litter. Leaf litter was pre-conditioned by immersing in river water for 10 days to allow  
176 for microbial colonization (mainly aquatic hyphomycetes) and improve leaf palatability (Navel et al. 2010).  
177 After conditioning, one leaf litter bag was placed on the substrate surface of each mesocosm before the start of  
178 the experiment. Following the experiment, leaves were collected, dried at  $60^{\circ}\text{C}$  for 24 h and re-weighed. Leaf  
179 consumption rates ( $\text{mg. ind.}^{-1} \text{ day}^{-1}$ ) were calculated as  $((\text{initial dry leaf mass}) - (\text{final dry leaf mass})) / ((\# \text{ of}$   
180  $\text{individuals}) * 15 \text{ days})$ . To correct final leaf mass consumption for leaching and microbial consumption not  
181 attributable to *G. pulex*, a temperature-specific correction factor was calculated based on the leaf mass loss in  
182 bags ( $n = 9$ ) immersed in additional columns void of *G. pulex* for 15 days at each temperature level (Navel et al.  
183 2010). Consumption rates were calculated based on the initial number and also the final number of individuals  
184 per mesocosm to account for survivorship and ensure that analyses of water temperature and species density  
185 effects on consumption rate were not biased by the method of calculating consumption rate.

#### 186 *Measuring triglycerides and glycogen contents*

187 For triglycerides and glycogen assays, individuals collected at the end of the experiment were dried using an  
188 absorbent cloth, freeze-dried and weighed in groups of 3–4 individuals. Three replicate groups from each  
189 mesocosm were collected to establish mean triglycerides and glycogen contents. Groups were weighed ( $\pm 0.1$   
190 mg) and then ground into powder with a small mortar in pre-weighed glass tubes. Triglycerides and glycogen  
191 ( $\mu\text{mol.g}^{-1}$  dry mass) were extracted using standard enzymatic methods with prepared solutions (Sigma-Aldrich,  
192 Saint-Quentin Fallavier, France) described in further detail in Hervant et al. (1995) and Salin et al. (2010).  
193 Assays were made using an Aquamate spectrophotometer (Thermo Scientific Inc., Waltham, MA, USA) at  $25^{\circ}\text{C}$ .

#### 194 *Data analysis*

195 Differences in the mean proportion of *G. pulex* that migrated into the hyporheic zone, percent survivorship, leaf  
196 mass consumption rate and triglycerides and glycogen contents between treatments were tested using a two-  
197 factor (two-way) analysis of variance (ANOVA). The design was a 3 (temperature levels: 15, 20,  $25^{\circ}\text{C}$ )  $\times$  3

198 (species density levels: low, medium, high) factorial design with interactions. Post hoc Tukey HSD multiple  
199 comparisons were used to compare mean levels within temperature and density treatment factors. Plotted  
200 residual variances and Levene's test were used to check for homogeneity of variance and normality and  
201 subsequently all percentages were  $\text{arc}(\sqrt{x})$ -transformed and leaf mass consumption rates and triglycerides and  
202 glycogen content values were  $\log_{10}(x)$ -transformed to meet these assumptions. ANOVA and post hoc  
203 comparisons were made using *R* (version 3.1.1; R Project for Statistical Computing, Vienna, Austria).

## 204 **Results**

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

206 The proportion of individuals that migrated into the hyporheic zone tended to increase as water temperature and  
207 species density increased (ANOVA, temperature effect:  $F_{2,27} = 4.28$ ,  $P = 0.024$ , density effect:  $F_{2,27} = 11.354$ ,  $P$   
208  $< 0.001$ ; Table 1, Fig. 2). The proportion of organisms that migrated was higher in the 25°C treatments than at  
209 15°C (Tukey HSD,  $P = 0.030$ ; Fig. 2), but not different than the proportion measured in the 20°C treatments. At  
210 high species density, the proportion of organisms that migrated was greater than in medium density (Tukey  
211 HSD,  $P = 0.003$ ) and low density treatments (Tukey HSD,  $P < 0.001$ ; Fig. 2). The effect of water temperature on  
212 the proportion of individuals that migrated did not increase at high density (ANOVA, temperature  $\times$  density  
213 effect:  $F_{4,27} = 1.65$ ,  $P = 0.191$ ; Table 1, Fig. 2).

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

215 The proportion of organisms that survived the experiment decreased as water temperature increased and there  
216 was an interaction effect of water temperature and species density (ANOVA, temperature  $\times$  density effect:  $F_{2,27}$   
217  $= 5.64$ ,  $P = 0.002$ ; Table 1, Table 2). The proportion of survival across low and medium densities was lowest at  
218 25°C compared to survival measured at 15 and 20°C (Tukey HSD,  $P < 0.001$  for all), whereas survival in the  
219 high density treatment at 25°C differed from survival in the high density treatment at 15°C (Tukey HSD,  $P =$   
220  $0.02$ ; Table 2) but not the high density treatment at 20°C.

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

222 Leaf consumption rate of *G. pulex* based on the initial density was affected by water temperature, species density  
223 and their interaction (ANOVA, temperature  $\times$  density effect:  $F_{2,26} = 15.93$ ,  $P < 0.001$ , Table 1, Fig. 3).

224 Consumption rate in the 20°C and 25°C treatments was lowest at medium and high species densities compared

225 to low density (Tukey HSD,  $P < 0.001$  for all) but at 15°C, different consumption rates were only detected  
226 between low and high density treatments (Tukey HSD,  $P < 0.001$ , Fig. 3). Similarly, consumption rate based on  
227 the final density was also affected by the interaction of water temperature and species density (results shown in  
228 Appendix SI).

#### 229 *Triglycerides and glycogen contents of organisms using the hyporheic zone*

230 Mean triglycerides content did not differ among the levels of water temperature, species density nor by the  
231 interaction of these factors (Table 1). For mean glycogen content, the effect of water temperature was not  
232 consistent across different levels of species density (ANOVA, temperature  $\times$  density effect:  $F_{4,26} = 4.013$ ,  $P =$   
233 0.012; Table 1; Fig. 4). Glycogen content at high and medium densities was lower than glycogen content at low  
234 density in the 20°C treatment (Tukey HSD,  $P = 0.004$  for both), whereas there was no difference in glycogen  
235 content among low, medium and high densities at 15°C.

## 236 **Discussion**

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

238 In agreement with our first prediction, both increasing water temperature and intraspecific competition led to the  
239 migration of *G. pulex* into the hyporheic zone. These findings imply hyporheic refuge use is an active process in  
240 which invertebrates use abiotic and biotic cues to avoid the harsh surface conditions that coincide with low flow,  
241 flow cessation and drying events. Water temperature above 20°C caused a higher proportion of individuals to use  
242 the hyporheic zone. This threshold closely matches the temperature (24°C) when *G. pulex* survival becomes  
243 drastically reduced in short-term (10 days) enclosed exposures (Foucreau et al. 2014). For intraspecific  
244 competition, a 3-fold increase in species density (2400 ind. m<sup>-2</sup>) led to a higher proportion of individuals using  
245 the hyporheic zone. In previous behavior experiments with *G. pseudolimnaeus*, Williams and Moore (1985)  
246 found a 3.5-fold increase in species density increased the number of individuals entering the substrate. Our  
247 results mirror those from two previous studies investigating hyporheic refuge use by invertebrates (Wood et al.  
248 2010, Stubbington et al. 2011). Wood et al. (2010) reported peak invertebrate densities in the hyporheic zone  
249 when surface water temperature around 20°C was reached in the Little Stour River, UK; whereas Stubbington et  
250 al. (2011) found the highest proportion of *G. pulex* in the hyporheic zone, relative to the surface, during a low-  
251 flow period in the River Lathkill, UK, when the highest density (2449 ind. m<sup>-2</sup>) occurred. Our mesocosm  
252 approach complemented these field surveys by disentangling the individual and combined effects of water

253 temperature and intraspecific competition and identifying thresholds that will help predict the responses of  
1  
2 254 invertebrates to low flow, flow cessation and river drying.  
3

4 255 Biotic interactions (e.g., competition, predator-prey relationships) can intensify with increasing water  
5  
6 256 temperature (Burnside et al. 2014), leading to unexpected responses of species in aquatic systems (Ormerod et al.  
7  
8 257 2010). However, in this study, the effect intraspecific competition of *G. pulex* on the proportion of individuals  
9  
10 258 that migrated into the hyporheic zone did not appear to increase when temperatures were increased up to 25°C  
11  
12 259 (i.e., additive response). The absence of a synergistic response may be attributed to the behavior of *G. pulex* at  
13  
14 260 temperatures above its thermal tolerance. At temperatures above its thermal tolerance, activity rates and  
15  
16 261 metabolism can decrease sharply (e.g., Foucreau et al. 2014), which may have led to a decrease in conspecific  
17  
18 262 encounters, hence, reducing competition (Wooster et al. 2011). Therefore, biotic interactions may increase with  
19  
20 263 water temperature until the point when thermal tolerance is exceeded, which is between 21–25°C for most  
21  
22 264 aquatic invertebrates (Stewart et al. 2013a, Foucreau et al. 2014), and individuals reduce their activity in a final  
23  
24 265 attempt to conserve energy and avoid death.  
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27  
28 266 Our results, along with those from previous field studies (e.g., Wood et al. 2010, Stubbington et al.  
29  
30 267 2011), bolster evidence that the hyporheic zone is an important refuge for riverine invertebrates avoiding  
31  
32 268 increased water temperatures and biotic interactions. These results have important implications considering the  
33  
34 269 projected global-scale increases in water temperatures and flow intermittence (Postel 2000, van Vliet et al. 2013,  
35  
36 270 Datry et al. 2014). In particular, water stressed regions, such as the American Southwest, may see a 27% increase  
37  
38 271 in the median number of days of flow cessation and a 15-day increase drying event duration by mid-century,  
39  
40 272 respectively (Jaeger et al. 2014). Changes in flow regime will be coupled with average increases in global mean  
41  
42 273 and maximum river water temperatures up to 1.6°C which will, in turn, increase evaporation and drying rates  
43  
44 274 (van Vliet et al. 2013). If organisms can survive temporarily in the hyporheic zone and return to the surface when  
45  
46 275 conditions become favorable, it is likely that, in at least some systems (e.g., alluvial rivers), the hyporheic zone  
47  
48 276 can be the primary source of resilience for invertebrate communities (Vander Vorste et al. *in review*).  
49

50 277 Future experiments may aim to test the effects of different abiotic and biotic factors that could also  
51  
52 278 influence invertebrate use of the hyporheic zone. For example, dissolved oxygen saturation in receding river  
53  
54 279 pools can be as low as 6% (e.g., Boulton 1989) and when coupled with high water temperatures will likely  
55  
56 280 increase the negative effects on invertebrates and consequently, migration of invertebrates into the hyporheic  
57  
58 281 zone would be strongly increased. Depth of the water table below the riverbed may also be an important factor  
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60 282 limiting the colonization and return to surface for invertebrates (Vander Vorste et al. *in review*). Furthermore,  
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283 interspecific competition and predation often increase simultaneously in drying rivers (Lake 2003) and may  
284 trigger migration of invertebrates into the hyporheic zone (Stubbington 2012). Invertebrate migration into  
285 hyporheic zone may decrease predation risk from fish and large invertebrates (e.g., Fairchild and Holomuzki  
286 2005), and reduce top-down effects in river pools (Boersma et al. 2014). Finally, the direction of vertical  
287 hydraulic gradient (i.e., upwelling, downwelling) is likely an overriding physical force controlling vertical  
288 migration of invertebrates (Olsen and Townsend 2003, Capderrey et al. 2013, Mathers et al. 2014). In this study,  
289 the fact that mesocosms had slightly upwelling water strengthens evidence that *G. pulex* actively sought refuge  
290 in the hyporheic zone, rather than passively following the direction of water movement. In general, higher  
291 abundances of surface invertebrate are found in downwelling reaches (e.g., Dole-Olivier et al. 1997, Olsen and  
292 Townsend 2003, Capderrey et al. 2013), presumably aided by the downward movement of water. Therefore, it is  
293 expected that *G. pulex* would show a greater response to enter the hyporheic zone in downwelling river reaches.  
294 Although, vertical migration may have been related to the rheophilic nature of *G. pulex* or its ability to detect  
295 cooler temperatures in the upwelling water. Future mesocosm experiments can facilitate exploration into how  
296 these various factors will influence hyporheic zone use by invertebrates in drying rivers.

#### 297 *Decreased survival, leaf litter consumption and energy stores*

298 In agreement with our second prediction, use of the hyporheic zone as a refuge had negative effects on survival,  
299 leaf litter consumption and energy stores of *G. pulex*. In this study, the hyporheic zone in mesocosms mimicked  
300 conditions in the natural streams, where the availability of food resources are generally limited and/or of poor  
301 quality (Burrell and Ledger 2003, Danger et al. 2012). Consequently, the low rates of survivorship ( $39 \pm 7\%$ ;  
302 mean  $\pm$  SD) and decreased glycogen content of *G. pulex* in high temperature treatments suggested that starvation  
303 could have become a factor during this 15-day experiment. Previous studies have shown surface invertebrates  
304 appear highly susceptible to mortality during periods of starvation (Hervant et al. 1997, 1999), especially  
305 compared to hypogean taxa. Therefore, food resources may be an important limiting factor that influences  
306 invertebrate survival in the hyporheic zone.

307 As a consequence of migration into the hyporheic zone, the processing of leaf litter by invertebrates on  
308 the riverbed may be substantially reduced during periods of low flow, flow cessation, and stream drying (Corti et  
309 al. 2011, Datry et al. 2011, Dehedin et al. 2013). In this study, a  $63 \pm 7\%$  reduction in leaf litter consumption per  
310 individual between 20 and 25°C suggested that *G. pulex* did not return to the surface to feed after entering the  
311 hyporheic zone. This result contrasts with recent studies suggesting that leaf litter decomposition will increase

312 with rising water temperatures due to enhanced microbial decomposition and invertebrate activity rates (e.g.,  
1 313 Ferreira and Canhoto 2015, Mas-Martí et al. 2015). However, we argue that decomposition rates will be reduced  
2  
3 314 when rising temperatures are coupled with contraction and drying of aquatic habitats and subsequent competition  
4  
5 315 for resources due to the behavioral response of shredding invertebrates to enter the hyporheic zone. Implications  
6  
7 316 of these findings are important considering *G. pulex* were responsible for an estimated 13% of leaf litter  
8  
9 317 consumption in a wooded stream (Mathews 1967) and several other invertebrate shredders (e.g., Leuctridae,  
10  
11 318 Leptoceridae) are known to use the hyporheic zone during disturbances (Stubbington 2012). An important next  
12  
13 319 step will be to test if invertebrates are able to track diel water temperature changes, returning to the surface at  
14  
15 320 night when surface temperatures are cooler to feed and how this may compensate for energy loss during the day.

16 321 River invertebrates face a tradeoff between tolerating harsh surface conditions versus avoiding them by  
17  
18 322 entering the hyporheic zone, a strategy which may not be suitable for long-term survival. On one hand, lower  
19  
20 323 water temperature, fewer conspecific interactions and the lack of large predators may entice invertebrates to  
21  
22 324 migrate into the hyporheic zone during periods of low flow, flow cessation and drying. On the other hand, once  
23  
24 325 in the hyporheic zone, food limitation, low oxygen concentration (Findlay 1995) and colmation (Descloux et al.  
25  
26 326 2013), especially in rivers impacted by agricultural land use, will reduce the capacity of the hyporheic zone to  
27  
28 327 provide refuge. Furthermore, competitive and predatory interactions with hypogean taxa (e.g., Schmid and  
29  
30 328 Schmid-Arraya 1997) are likely to occur, although quantifiable evidence must be explored further. These  
31  
32 329 interactions may have negative or positive effects on the resilience of surface invertebrates, depending on the  
33  
34 330 outcome of these interactions. Therefore, the potential cascading effects of hyporheic zone refuge use by  
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36 331 invertebrates remains an important research gap that could be addressed through mesocosm experiments.  
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### 40 332 41 42 333 *Conclusion*

43  
44 334 There is a strong need to understand the influence of factors, such as water temperature and biotic interactions,  
45  
46 335 that coincide with low flow, flow cessation and drying on river communities, especially considering global  
47  
48 336 change will continue exacerbate their negative effects on river systems (Postel 2000, Datry et al. 2014, Jaeger et  
49  
50 337 al. 2014). In many regions, once perennial rivers are now becoming intermittent (Datry et al. 2014), therefore  
51  
52 338 future studies could explore how trait variability (Violle et al. 2012) and differences in physiological tolerance  
53  
54 339 (Stoks et al. 2014) among populations from formally perennial and naturally intermittent rivers influence the  
55  
56 340 response to increased temperature and biotic interactions. Although rare in freshwater ecology, the use of  
57  
58 341 common garden experiments (i.e., simultaneously subjecting different populations to the same stressor) have  
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342 revealed strong inter-population differences in temperature tolerance within aquatic species (e.g., Foucreau et al.  
1 343 2014). Therefore, such approaches could be developed to explore the responses of populations from perennial  
2 343 3 and intermittent rivers to other environmental factors associated with river contraction and drying. These  
3 344 4 and intermittent rivers to other environmental factors associated with river contraction and drying. These  
4 344 5 experiments will in turn help refine the predictions of population and community responses to global climate  
5 345 6 change and increased water abstraction.  
6 345 7  
7 346 8 change and increased water abstraction.  
8 346 9

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18 351 21  
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547 **Tables**

548 **Table 1. Results from 2-way ANOVA testing the effect of temperature and species density and their**  
 549 **interaction on dependent variables related to *G. pulex*. Percentages were arcsin( $\sqrt{x}$ )-transformed and leaf**  
 550 **mass consumption rate, triglycerides and glycogen contents were log<sub>10</sub>(x)-transformed.**

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

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561 **Table 2. Mean ( $\pm$  SD) percent survivorship of *G. pulex* in different temperature and species density**  
562 **treatment conditions after the 15-day experiment.**

Temperature	Species density	Mean	Min. – Max.
15°C	low	61 $\pm$ 8	55 – 73
	medium	62 $\pm$ 8	54 – 71
	high	67 $\pm$ 6	58 – 72
20°C	low	79 $\pm$ 5	73 – 85
	medium	67 $\pm$ 4	62 – 70
	high	63 $\pm$ 3	59 – 67
25°C	low	34 $\pm$ 12	23 – 48
	medium	35 $\pm$ 2	33 – 37
	high	48 $\pm$ 7	42 – 57

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567 **Figure Legends**

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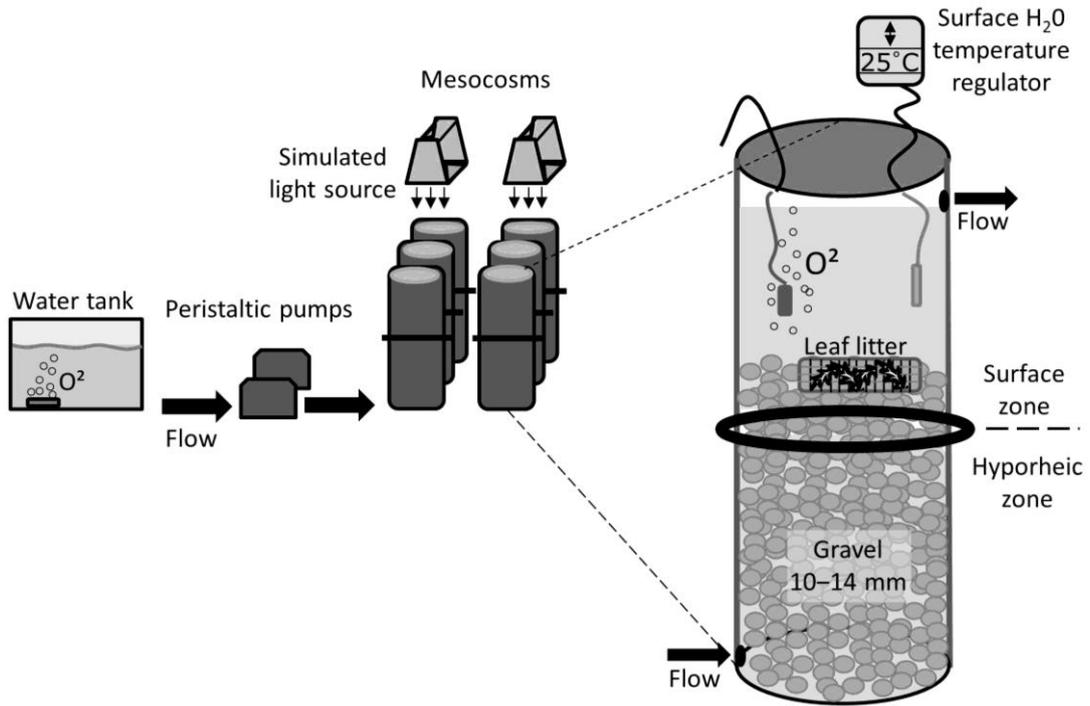
568 **Figure 1. Experimental set-up of mesocosms (n = 36) used to test the effect of water temperature, species**  
569 **density and their interaction on the migration of *G. pulex* into the hyporheic zone.**

570 **Figure 2. Mean ( $\pm$  SE) proportion (percent) of *G. pulex* that migrated into the hyporheic zone at different**  
571 **temperature and species density treatment conditions. Percent migrated into hyporheic zone is based on**  
572 **the initial species density.**

573 **Figure 3. Mean ( $\pm$  SE) leaf mass consumption rate of *G. pulex* (mg. ind.<sup>-1</sup> day<sup>-1</sup>) at different temperature**  
574 **and species density treatment conditions. Calculation based on initial species density.**

575 **Figure 4. Mean ( $\pm$  1 S.E) individual triglycerides ( $\mu$ mol.g<sup>-1</sup> dry mass) (A) and glycogen content ( $\mu$ mol.g<sup>-1</sup>**  
576 **dry mass) (B) of *G. pulex* at different temperature and species density treatment conditions.**

590 **Figure 1.**



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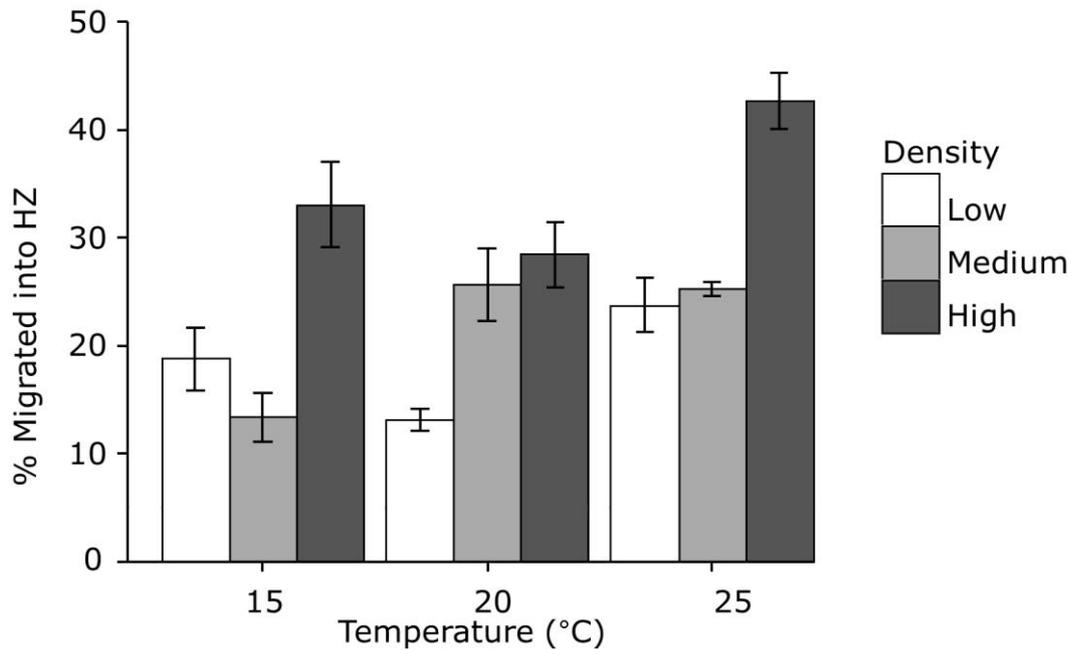
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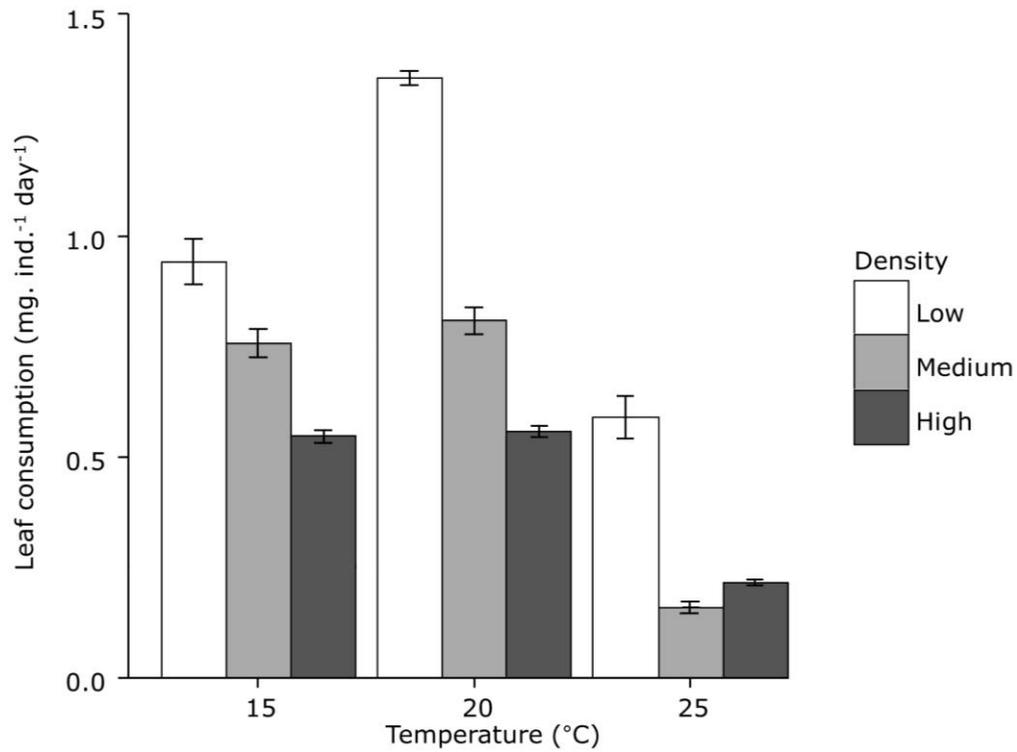
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603 **Figure 2.**

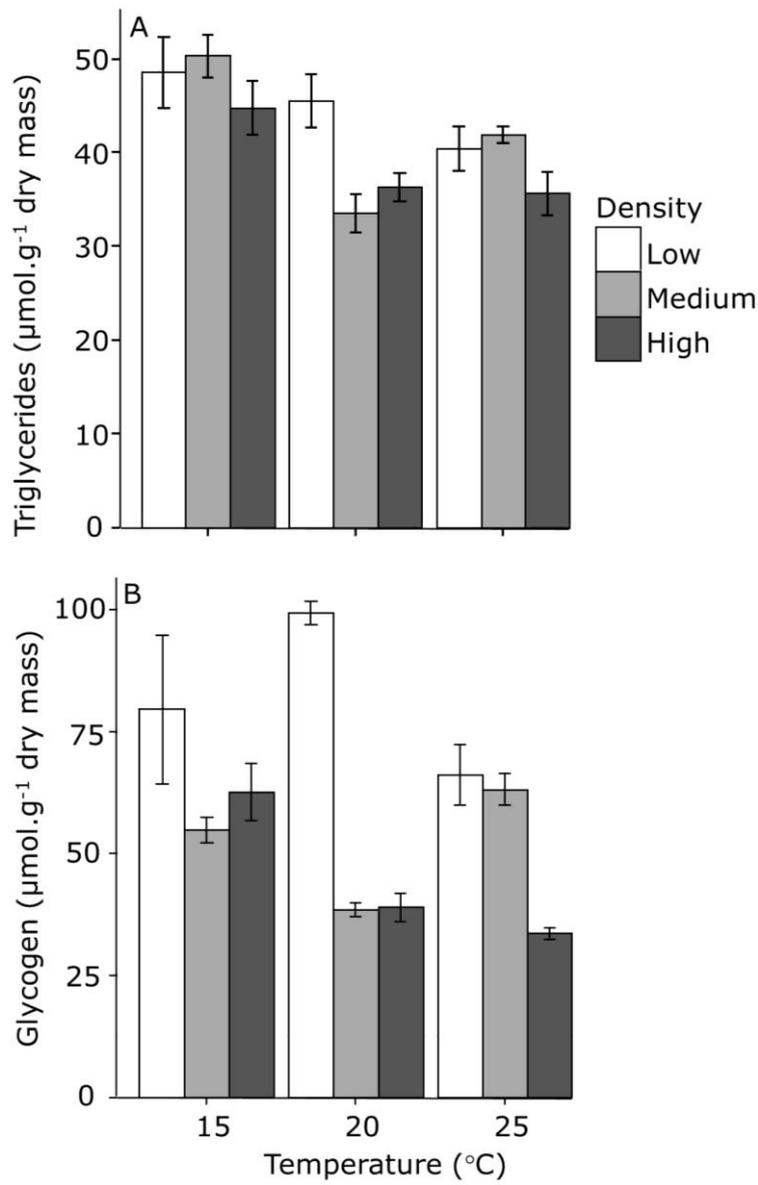


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607 **Figure 3.**



619 **Figure 4.**



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626 **Appendix SI**

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3 627 **Results from 2-way ANOVA testing the effect of temperature and species density and their interaction on**  
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5 628 **leaf mass consumption of *G. pulex*. Leaf mass consumption rate was log<sub>10</sub>(x)-transformed. Leaf mass**  
6  
7 629 **consumption based on final number of individuals to account for survivorship though it was not possible**  
8  
9 630 **to determine when organisms died during the experiment.**

Dependent variable	Factor	d.f.	MSS	<i>F</i>	<i>P</i>
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

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