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1 **Feeding choice and predation pressure of two invasive gammarids, *Gammarus***
2 ***tigrinus* and *Dikerogammarus villosus*, under increasing temperature**

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13
14
15 A running headline: Temperature effects on the diet of invasive gammarids

16

17 **Abstract**

18 In most European freshwater ecosystems, the invasive gammarids *Gammarus tigrinus* and
19 *Dikerogammarus villosus* strongly impair recipient communities through predation of a wide range of
20 native invertebrates. Due to the effects of temperature on both the physiology and the behaviour of such
21 ectotherms, understanding how global warming may influences their ecological impact is a research
22 priority. These species were therefore exposed to three different food types to determine their
23 detritivorous, herbivorous and carnivorous characters, and predation was measured characterizing the
24 Holling's functional response. The effect of increasing water temperatures (15, 20, 25 °C) on both the
25 food choice and predatory activities was investigated. Both species showed a significant preference for
26 animal tissues at all temperatures. The total food intake increased with temperature for *G. tigrinus* but
27 did not change for *D. villosus*, which may result from specific-species differences in metabolic
28 requirements. The consumption of live prey strongly increased with temperature. The main differences
29 were an increased searching efficiency in *G. tigrinus* and a decreased handling time in *D. villosus* as
30 temperature increased, which may result from differences in foraging strategies. These results suggest
31 that climate change is likely to increase the predation pressure of both invasive gammarids on prey
32 species.

33

34 *Keywords:* climate change, biological invasions, amphipods, trophic ecology, predatory impact

35

36 **Introduction**

37 In the past few decades, the establishment of invasive species has strongly disturbed the structure and
38 function of many freshwater ecosystems (Ricciardi & MacIsaac, 2011). Although environmental factors
39 are known to play a crucial role in the spread of many non-native invasive species (Leppäkoski et al.,
40 2002; Labat et al., 2011), little is known about their influence on the nature and direction of species'
41 ecological impacts (Van der Velde et al., 2009). In addition, the strength of ecological impact is a
42 growing concern in the context of current global changes (Bellard et al., 2013), as the resulting changes
43 in environmental conditions (e.g. nutrient concentrations, temperature, pollution) could significantly
44 alter the competitive balance between alien and native species (Piscart et al., 2009). The scenarios of the
45 Intergovernmental Panel on Climate Change (IPCC) predict a global increase in temperature from
46 +0.3 °C to +4.8 °C by 2100, as well as differences in thermal patterns among geographical regions
47 (IPCC, 2013). Generally, freshwater organisms will have to tolerate fluctuations in water temperature.
48 For these ectothermic organisms, even a minor increase in temperature may strongly influence both
49 physiology and behaviour (Maazouzi et al., 2011; Foucreau et al., 2014; Colinet et al., 2015). The
50 success of most invasive aquatic crustaceans correlates with their strong capacity to tolerate
51 environmental stressors and/or the flexibility of their diet (Van der Velde et al., 2000). Consequently,
52 the changes in temperature predicted by the IPCC could influence the establishment of aquatic invasive
53 species, and could modulate their impacts on recipient ecosystems (Rahel & Olden, 2008).

54 The invasive gammarids (Crustacea: Amphipoda) *Gammarus tigrinus* Sexton 1939 and
55 *Dikerogammarus villosus* (Sowinsky, 1894) have already colonized many freshwater ecosystems, and
56 are currently established in most of the large rivers of Western Europe (Pinkster et al., 1977; Bollache et
57 al., 2004; Platvoet et al., 2009a). In addition to the biological traits that determine their competitiveness
58 (e.g. ability to exploit trophic resources: Van der Velde et al., 2000; Maazouzi et al., 2009; their
59 tolerance of a wide range of environmental factors: Wijnhoven et al., 2003; Piscart et al., 2011a), these
60 gammarids are known to prey upon many aquatic invertebrates (Dick et al., 2002; Platvoet et al., 2009a),

61 and at high rates. For instance, the functional response (the relationship between resource use and
62 resource availability) (Holling, 1959a), has been shown to rise more steeply and to a higher asymptote
63 than in native gammarids (Bollache et al., 2008; Dick et al., 2013). *D. villosus* is known to have a higher
64 searching efficiency and a lower handling time than its native counterparts, and was therefore more
65 efficient in exploiting trophic resources. As such, invasive gammarids may strongly affect the structure
66 and functioning of recipient ecosystems (Bollache et al., 2004; Orav-Kotta et al., 2009; Piscart et al.,
67 2010, 2011b). The North American amphipod *G. tigrinus* is a thermophilous species capable of
68 tolerating temperatures of up to ca. 32 °C (Wijnhoven et al., 2003). In contrast, the Ponto-Caspian *D.*
69 *villosus* prefers cooler temperatures, but its sedentary lifestyle allows it to maintain a lower basal
70 metabolic rate than its native relatives, as well as high predation rates when temperature exceeds 25 °C
71 (Maazouzi et al., 2011). Thermal plasticity involves physiological modifications within individuals,
72 which increase energy requirements (Pörtner et al., 2002; Issartel et al., 2005a; 2005b; Maazouzi et al.,
73 2011). *G. tigrinus* and *D. villosus* must therefore satisfy increased energy needs if they are to persist in
74 the habitats exposed to increasing temperatures. In many aquatic ectotherms, the energetic cost resulting
75 from increasing temperature is compensated by dietary shifts. These changes may be quantitative, with
76 previous studies reporting increased food intake in aquatic ectotherms (Niu et al., 2003; Van der Velde
77 et al., 2009; Woodward et al., 2010), and/or qualitative, with consumers relying increasingly on high-
78 energy food sources such as animal tissues, when exposed to increasing water temperatures (Parmenter,
79 1980).

80 Gammarids are opportunistic feeders capable of switching their trophic regime from herbivorous
81 (Dehedin et al., 2013) to detritivorous (Piscart et al. 2011b) and carnivorous (Piscart et al. 2009)
82 components with the consumption of dead or live prey (MacNeil et al., 1997). In the context of
83 temperature increases, the main hypothesis of this study was that *G. tigrinus* and *D. villosus* will
84 prioritize carnivory, which provide more energy, and increase their overall food intake, therefore
85 increasing predation pressure on resident prey populations (e.g., other crustacean species). The primary

86 aim of this study was to detect quantitative and qualitative changes in the diet of *G. tigrinus* and *D.*
87 *villosus* exposed to a range of thermal conditions. In this context, we predicted: (i) an increase in the
88 overall quantity of food consumed as temperature increased (ii) a concomitant growing preference for
89 animal tissues. To validate predictions (i) and (ii), food choice experiments were performed, with both
90 species were offered macrophytes, leaf litter and dead chironomid larvae at three different water
91 temperatures (15, 20 and 25 °C). We also predicted (iii) that increasing temperature would enhance the
92 predatory activity of invasive gammarids, thus amplifying their impacts on prey. To examine this
93 prediction, a second experiment was conducted to determine the functional response (FR) of *G. tigrinus*
94 and *D. villosus* fed on live water fleas under the three different temperatures. We hence predicted that
95 FR parameters (i.e. searching efficiency and handling time) should be modified by increasing
96 temperature, with the *per capita* predation rate increasing more steeply and to a higher asymptote.

97

98 **Materials and methods**

99 *Specimen collection and maintenance*

100 Experiments were performed with specimens collected by kick sampling from the Brivet River near
101 Saint Nazaire (47° 19' 21.0822" N, 2° 11' 41.9136" W) from February 2014 to March 2014 for *G.*
102 *tigrinus*, and from the Loire River near Bourgeuil (47°14'10.83" N, 0°9'2.18"E) in April 2014 for *D.*
103 *villosus*. The two sites, approx. 180 km apart, have been inhabited by invasive species for at least the
104 last decade (Piscart et al., 2010) and experience the same climatic conditions. To avoid any body size
105 effect, only adult males and females with intermediate size ranges (8-12 mm for *G. tigrinus* and 12-16
106 mm for *D. villosus*) were used. Given that adults do not exhibit a distinct sexual dimorphism except
107 when females are ovigerous, they were captured during the precopula mate guarding period and
108 carefully separated in the field. Conversely, *D. villosus* exhibit a distinct sexual dimorphism (males
109 having more robust gnathopods than females and the second antenna have dense 'brush-like' tufts of
110 setae (Piscart & Bollache, 2012) and were captured at any conditions. Since parasite infection can

111 modify gammarids' FR (Dick et al., 2010), those harbouring symptomatic parasites such as
112 acanthocephalans and muscle-wasting microsporidians (when distinguishable) were excluded. Sampled
113 organisms were then transferred to controlled conditions. The two species were maintained separately at
114 15 °C in 10-L tanks filled with aerated site water under a 12:12 h light:dark. Animals were fed *ad*
115 *libitum* with vegetation and fauna from the sampling sites, except during the starvation period (see
116 below).

117

118 *Experiment 1- food choice according to temperature*

119 The consumption of different food types by *G. tigrinus* and *D. villosus* was monitored at three water
120 temperatures: 15 °C, corresponding to the thermal optimum for both species (Wijnhoven et al., 2003;
121 Maazouzi et al., 2011); 20 °C, the mean temperature often observed at the sampling sites in summer
122 (DREAL Bretagne, 2014); and 25 °C, to simulate the 5 °C increase in temperature predicted by the
123 IPCC (2013) worst-case scenario.

124 We used three diets to encompass the various feeding modes used by gammarids: herbivory with
125 fresh macrophytes (*Apium nodiflorum* (L.) Lag. 1821) collected from the Yaigne River (Vern-sur-Seiche,
126 Bretagne, 49°02'03.9"N, 1°34'08.0"W); detritivory with conditioned leaf litter (*Corylus avellana* (L.)
127 1753) also collected from the Yaigne; and carnivory with thawed dead dipteran larvae (*Chironomus*
128 *riparius* (Meigen, 1804)). We used dead chironomids to exclude the energetic cost of capturing live prey,
129 which could hide a potential shift towards carnivory under the assumption of temperature-induced
130 changes in energy needs.

131 Prior to experimentation at 20 and 25 °C, gammarids were acclimatized to the experimental
132 temperature for 24 h, by gradually increasing the temperature in one degree steps from 15 °C to the
133 required temperature (Foucreau et al., 2014). After the temperature acclimatization period, gammarids
134 starved individually for 24 h without food, to increase appetite and reduce food residue in the gut
135 (standardization of hunger). We check that no cannibalism appear by counting the

136 After 48 h (i.e. 24 h of acclimatization and 24 h of starvation), 20 gammarids (10 males and 10
137 females) of each species were placed into separate 20-cm diameter glass petri dishes filled with 180 mL
138 filtered water from the sampling sites for 48 h, under a 12:12 h light:dark regime. This duration was
139 considered short enough to avoid any effect of thermal conditions on food (e.g. macrophyte necrosis
140 macrophyte at higher temperatures) and long enough to take into account temporal variation in food
141 consumption (Piscart et al., 2011b). Daily measures of dissolved oxygen concentrations were realized
142 randomly in petri dishes to be sure that no oxygen depletion occurred during the experiment, especially
143 at high temperatures. Five 6-mm diameter macrophyte discs, five 6-mm diameter leaf litter discs, and 15
144 chironomid larvae were randomly placed into each petri dish. As a result, each food type covered a
145 similar area of the petri dish. To avoid food depletion, partially or entirely consumed leaf discs or larvae
146 were replaced 24 h after the beginning of the experiment. For each temperature, three control treatments
147 consisting of a petri dish filled with water and the three food types but without gammarids were
148 performed to estimate food consumption related to bacterial and fungal activities.

149 The fresh weight of each food type was measured before and after the experiment (Ohaus®
150 Analytical Plus balance, Ohaus AP250D) to estimate the quantity (Q_i) of the food type (i) that was
151 consumed per mg of gammarid after 48 h, as follows:

$$152 \quad Q_i = (M_{Fi} - M_{Li}) - D_i$$

153 Where (M_{Li}) and (M_{Fi}) are the fresh weights of the food type (i) at the beginning and at the end of the
154 experiment, respectively, and (D_i) is the mean difference in fresh weight before and after the experiment
155 in the control treatments.

156 The food preference of gammarids was assessed by measuring the index of relative importance
157 (IOI) of each food type (i) as follows (modified version of Kurian, 1977):

$$158 \quad IOI_i = (100 \times Q_i) / Q$$

159 Where (Q) is the total quantity of food consumed per mg of gammarid over 48 h.

160

161 *Experiment 2 – functional response according to temperature*

162 The functional response of *G. tigrinus* and *D. villosus* fed on water fleas (*Simocephalus exspinosus* (De
163 Geer, 1778)) at 15, 20 and 25 °C was determined. The prey were entirely consumed by gammarids
164 during this experiment, facilitating prey counting and the calculation of predation rates (Stoffels et al.,
165 2011).

166 Water fleas were collected from a pond located on the campus of the University of Rennes 1 (campus de
167 Beaulieu, Rennes, France) (48°07'08.0"N, 1°38'22.1"W). Gammarids and prey were gradually
168 acclimatized to the temperature of 20 or 25 °C over 24 h as for the experiment 1. After this
169 acclimatization period, gammarids were starved for 24 h.

170 After 48 h (i.e. 24 h of acclimatization and 24 h of starvation), gammarids were placed into
171 individual plastic cups (7 cm diameter) filled with 60 mL of filtered water from their sampling site and
172 containing 1, 3, 6, 12, 18 or 24 water fleas (prey density, *N*), without shelter for prey or gammarids. The
173 duration of the experiment was 8 h based on preliminary tests and consumed prey were continuously
174 counted and replaced immediately following consumption, to avoid prey depletion. For each
175 temperature, prey density and gammarid species were replicated with three males and three females,
176 giving a total of 216 predation tests. Six replicates of each prey density but without gammarids were
177 used to control for prey mortality.

178

179 *Statistical analyses*

180 The total quantity of food consumed by gammarids exposed to the different temperatures was compared
181 using analyses of variance (ANOVA) tests, with temperature and sex as fixed factors. Data were log- or
182 square root- transformed to meet assumptions of homoscedasticity and normality. Pairwise comparisons
183 between temperatures were performed using Tukey's HSD tests. The respective contribution of each
184 food type, represented by the index of relative importance (IOI), was compared for the different
185 temperatures using ANOVA models. Since the transformed values of IOI did not meet the normality

186 assumption, Friedman's tests were used to check for significant differences in IOI values between the
187 food types for each temperature. Pairwise comparisons were then performed using Wilcoxon signed
188 rank tests. Kruskal-Wallis tests were performed to identify differences between the IOI values among
189 temperatures for each food type. Pairwise comparisons were then conducted using Wilcoxon signed
190 rank tests.

191 FR parameters, namely predator searching efficiency and prey handling time, were estimated by
192 fitting the observed predation rates to the Holling's type II FR model (Holling, 1959b):

$$193 \quad g(N) = aN / (1 + ahN)$$

194 where $g(N)$ is the *per capita* predation rate, N is the prey density, a is a measure of the searching
195 efficiency and h is the handling time.

196 Non-linear regressions were performed with the *nls* function of R software (R Development Core
197 Team 2010). Confidence intervals of the parameter estimates were obtained using a bootstrap method
198 applied to residuals, to avoid making a normality assumption.

199 To test the influence of increasing temperature on gammarid FR, FR parameter estimates
200 obtained at each temperature were compared with a backward and forward stepwise model selection
201 procedure designated as *Dynamics Likelihood Ratio Tests* by Posada and Crandall (2001). A set of
202 models including the simplest model assuming the same parameter values for all temperatures (two
203 parameters), the most complex model assuming different parameter values per temperature (six
204 parameters) and all intermediate models was considered and the following forward/backward
205 procedures were applied:

- 206 1. Start from the simplest/most complex model. This is the current model.
- 207 2. Consider all the alternative models with one additional/less parameter.
- 208 3. Select the alternative model that leads to the greater decrease/smaller increase of the residual sum
209 of squares (RSS).
- 210 4. Compare the current model and the selected model using a likelihood ratio test.

211 5. If the fitting improvement/degradation is significant/insignificant ($\alpha = 5\%$), repeat steps 2-3 using
212 the selected model as the current model.

213 In addition, the confidence regions (Beale, 1960) for the parameter estimates when the three FRs per
214 gammarid species were considered separately were constructed and represented. These confidence
215 regions were defined as the set of parameter values such that the RSS stays below a given threshold:

$$216 \quad RSS(\theta) < RSS_{\min} [1 + p/(n-p)F_{1-\alpha}(p, n-p)]$$

217 All statistical analyses were performed using R 3.1.0 software.

218

219 **Results**

220 *Experiment 1 – food choice according to temperature*

221 For *G. tigrinus*, sex factor had a significant effect on the quantity of food consumed by gammarid
222 (ANOVA, $F_{53,1} = 24.05$; $p < 0.001$), and females consumed a greater quantity of food than males at 20
223 and 25 °C (Tukey's HSD test, $p < 0.05$, Fig. 1a). Moreover, the total quantity of food consumed by *G.*
224 *tigrinus* increased with temperature (ANOVA, $F_{53,2} = 40.86$; $p < 0.001$, Fig. 1a). The food intake was
225 two to three times higher at 20 or 25 °C than at 15 °C, for both sexes (Tukey's HSD test, $p < 0.05$, Fig.
226 1a). For *D. villosus*, sex had no significant effect on the quantity of food consumed with respect to
227 temperature (ANOVA, $F_{50,1} = 1.61$; $p = 0.21$), except at 25 °C where the females consumed more food
228 than the males (Tukey's HSD test, $p < 0.05$, Fig. 1b). In contrast to *G. tigrinus*, the food intake for *D.*
229 *villosus* was not influenced by temperature (ANOVA, $F_{53,2} = 2.67$; $p = 0.08$, Fig. 1b). The total quantity
230 of food consumed by *D. villosus* was the same at all temperatures (23.38 ± 6.46 mg of food per mg of
231 gammarid for females and 18.22 ± 5.64 mg of food per mg of gammarid for males).

232 All food types were consumed by both species, and both preferentially consumed chironomid
233 larvae at all three temperatures (Friedman's test, $p < 0.001$, Fig. 2). The IOI of macrophytes was
234 significantly higher at 20 and 25 °C than at 15 °C for *G. tigrinus* (Kruskal-Wallis test, $\chi^2 = 36.38$; $df = 2$;
235 $p < 0.001$, Fig. 2a), whereas the opposite pattern was observed for the IOI of chironomid larvae

236 (Kruskal-Wallis test, $\chi^2 = 29.30$; $df = 2$; $p < 0.001$, Fig. 2a). For *D. villosus*, the IOI of macrophytes was
237 significantly higher at 20°C than at 15 and 25 °C (Kruskal-Wallis test, $\chi^2 = 25.24$; $df = 2$; $p < 0.001$, Fig.
238 2b).

239

240 *Experiment 2 – functional response according to temperature*

241 Prey showed no mortality in the control treatments, suggesting that deaths during the experiments were
242 due to gammarid predation only. The plot of the consumption rate as a function of prey density (Fig. 3)
243 showed an increasing but decelerating relationship for each temperature and each gammarid species,
244 supporting the assumption of a type II FR. However, the shape of these relationships seemed different
245 according to the temperature and the gammarid species, suggesting an impact of these factors on the FR
246 parameter values. The fits of the Holling's type II FR model to the data are also shown in Fig. 3, while
247 the corresponding parameter estimates and their 95 % confidence intervals are detailed in Table 1.
248 Regardless of the gammarid species, both the backward and forward dynamics likelihood ratio test
249 procedures converged to the same alternative model (Fig. 4). For *G. tigrinus*, an alternative model with
250 five parameters was retained (Fig. 4a). The searching efficiency a was equivalent between 15 and 20 °C,
251 and was significantly higher at 25 °C (see Fig. 4a for the results of the stepwise procedure and the
252 associated statistics: LRT p-values and AIC_c values). The handling time h was highest at 15 °C and
253 increased between 20 and 25 °C (Fig. 4a, Table 1). For *D. villosus*, an alternative model with four
254 parameters was retained (Fig. 4b). The searching efficiency was significantly higher at 20 °C and did
255 not differ between 15 and 25 °C (see Fig. 4b for the results of the stepwise procedure and the associated
256 statistics). The handling time was equivalent between 15 and 20 °C, and significantly decreased at 25 °C
257 (Fig. 4b, Table 1). For *G. tigrinus*, the projections of the confidence regions showed little overlap on the
258 y-axis suggesting three distinct values of handling time, and a strong overlap only between 15 and 20 °C
259 on the x-axis suggesting a higher searching efficiency at 25 °C (Fig. 5a). For *D. villosus*, only the value

260 of the handling time at 25 °C differed from the others on the y-axis, and only the value of the searching
261 efficiency at 20 °C differed from the others on the x-axis (Fig. 5b).

262

263 **Discussion**

264

265 Our results clearly highlighted that temperature significantly alters the feeding behaviour of invasive
266 gammarids (Van der Velde et al., 2009); probably because thermal tolerance increases energy needs
267 (Wijnhoven et al., 2003; Maazouzi et al., 2011; Foucreau et al., 2014).

268

269 *Experiment 1 – food choice according to temperature*

270 Consistent with their omnivorous status (Poje et al., 1988; Platvoet et al., 2009b), all food types
271 provided were consumed by both species during the experiments. The effect of sex on food intake of *G.*
272 *tigrinus*, and to a lesser extent *D. villosus*, may be explained by differences in energy metabolism among
273 sexes (Foucreau et al., 2013). Energy requirements are higher for females than males due to their more
274 energy-expensive reproductive cycle (Sutcliffe, 2010) and their higher metabolic rate related to their
275 lower body weight (Normant et al., 2007). These differences in energy metabolism coupled to the
276 energetic demand under increasing temperature might explain the higher food consumption observed in
277 females at 20 °C and/or 25 °C. At 15 °C, the total quantity of food ingested by *G. tigrinus* was twice as
278 high as that consumed by *D. villosus*, suggesting that *G. tigrinus* is more voracious than *D. villosus*.

279 Moreover, the total quantity of food consumed per mg of gammarid increased with temperature
280 in *G. tigrinus* but not in *D. villosus*. The first prediction suggesting a positive effect of temperature on
281 the quantity of food consumed by gammarids was confirmed only for *G. tigrinus*. Compared to *G.*
282 *tigrinus*, which exhibits a considerable swimming activity (personal observation), *D. villosus* is an
283 ambush predator that stays motionless (Platvoet et al., 2009b), and whose swimming activity is low and
284 not influenced by increasing temperature (Maazouzi et al., 2011). In addition, previous studies have

285 highlighted that *D. villosus* has a lower basal metabolism than many other amphipods (Wijnhoven et al.,
286 2003; Maazouzi et al., 2011). The behaviour and the lower metabolic rate of *D. villosus* hence require
287 less energy compared to *G. tigrinus* at high temperature. Therefore, *D. villosus*, compared to *G. tigrinus*,
288 needs less energy to significantly increase food intake. In addition, the non-linear relationship that exists
289 between temperature and performance in ectotherms may result in differential effects of any thermal
290 increase if the performance curves of the species do not perfectly overlap (Colinet et al., 2015). *G.*
291 *tigrinus* is a thermophilous species (Wijnhoven et al., 2003), and may therefore exhibit a more
292 pronounced response to thermal changes due to a high amplitude of its thermal performance curve.
293 However, additional experiments with measurements of the metabolic rates and swimming activities of
294 gammarids are needed to confirm this hypothesis.

295 Contrary to the second prediction, food preferences did not changed significantly with increasing
296 temperature. The proportion of each food type varied only slightly with an important consumption of
297 dead chironomids. This is not surprising as dead chironomids were the most energy-rich food source in
298 this study. This result is consistent with previous investigations (MacNeil & Platvoet, 2005; Platvoet et
299 al., 2009a; Van der Velde et al., 2009), and confirms the high level of carnivory of these invasive
300 gammarids. However, the overconsumption of dead prey under laboratory conditions does not mean that
301 gammarids are strong predators in nature. For instance, Médoc et al. (2011) found that the consumption
302 of isopods (*Asellus aquaticus*) by *Gammarus roeseli* was significantly reduced when the prey were alive.
303 Additional food-choice experiments are needed to test whether the cost of capturing live prey changes
304 gammarids' food preferences.

305 Maximum consumption of macrophytes was observed at 20 °C in both species, and also at 25 °C
306 in *G. tigrinus*. These results might be due to the macrophyte discs floating and therefore constituting a
307 food resource as well as a habitat for the gammarids, while chironomid larvae and leaf litter remained at
308 the bottom of the experimental units. The gammarids appeared less mobile under high temperature and
309 could increase the time spent on substrates that can be eaten to save energy, which might explain the

310 growing contribution of floating macrophytes to the diet. Another explanation might be that with
311 increasing temperature, increase in both epilithic biofilm production and microbial decomposition of the
312 leaves made them more palatable to the gammarids (Díaz Villanueva et al., 2011a,b).

313

314 *Experiment 2 – functional response*

315 The number of prey consumed increased with water temperature in both species, which agrees with the
316 third prediction and the results of previous studies (Van der Velde et al., 2009; Maier et al., 2011;
317 Stoffels et al., 2011). These data seem not congruent with the total food intake of *D. villosus*, observed
318 in the first experiment, which did not increase with temperature over 48 h. However, the first
319 experiment was conducted with dead chironomids, whereas *D. villosus* is known to be aggressive (Dick
320 et al., 2002), and its attacks on live prey may have increased with its increased activity. Significant
321 differences in FR parameters were observed among temperatures. Searching efficiency increased with
322 temperature and was highest at 25 °C for *G. tigrinus* and at 20 °C for *D. villosus*. The searching
323 efficiency of *D. villosus* was hence maximal at the intermediate temperature (20 °C) and not at the
324 highest temperature (25 °C) as for *G. tigrinus*. These results may be due to differences in the foraging
325 strategy of the two gammarids. Prey mobility might have increased with temperature (Gerritsen, 1982),
326 thereby promoting predator-prey encounters and explaining the increase in searching efficiency between
327 15 and 20 °C. Beyond a given level of prey mobility, the ambush predator might be expected to become
328 less successful in catching prey, which could explain the decrease in searching efficiency between 20
329 and 25 °C for *D. villosus*. Alternatively, *D. villosus*, which has a more restricted thermal plasticity than
330 *G. tigrinus*, is likely to be more stressed at 25 °C and its efficiency could be reduced by the stressful
331 thermal conditions (Stoffels et al., 2011).

332 For both species, the handling time decreased with temperature and was the lowest at 20 °C for
333 *G. tigrinus* and at 25 °C for *D. villosus*. Gammarids probably displayed a stronger predatory behaviour
334 with reduced handling times and quicker intakes to forage more and satisfy the temperature-induced

335 increase in energetic needs. *G. tigrinus* is much smaller than *D. villosus*, and therefore probably less
336 successful in handling mobile prey. This could explain the increase in the handling time of *G. tigrinus*
337 between 20 and 25 °C when prey mobility was expected to be highest.

338

339 *Conclusion*

340 To conclude, no evidence was found on for a qualitative change in the diet of *G. tigrinus* and *D. villosus*
341 under increasing temperature, with a preference for animal tissues regardless of the experimental
342 temperature. However, the food intake increased with temperature, suggesting that predation pressure by
343 both invasive species on resident prey is likely to increase with ongoing global warming, with slight
344 differences depending on the foraging strategy. *G. tigrinus* actively forages and its searching efficiency
345 increased with temperature, causing potential impacts at low prey densities. *D. villosus* is an ambush
346 predator whose handling time decreased with temperature, causing potential impacts at high prey
347 densities. Due to its predatory behaviour and aggressiveness, *D. villosus* receives much attention
348 compared to other invasive species such as *G. tigrinus* (Dick & Platvoet, 2000; Dick et al., 2002;
349 MacNeil & Platvoet, 2005; MacNeil et al., 2010). Although *D. villosus* is a large predator capable of
350 consuming more prey than *G. tigrinus*, the total quantity of food consumed by *G. tigrinus* was three
351 times that of *D. villosus* at 25 °C when considering equivalent biomass. In this way, the dietary response
352 of *G. tigrinus* to increasing temperature can be viewed as more pronounced than that of *D. villosus*. Ours
353 study hence suggests that global warming needs to be carefully considered in the study of biological
354 invasions. Global warming is a factor that could strongly strengthen the impact of invasive species on
355 native fauna and also modify the relative impact of the different invasive species.

356

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361

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504

505 **List of figures**

506

507 **Fig. 1** Total quantity of food consumed (mean \pm SE) per mg of gammarid for females (white bars) and
508 males (grey bars) of two invasive gammarids fed on macrophytes, leaf litter and dead chironomid larvae
509 at 15, 20 and 25°C: (a) *Gammarus tigrinus* and (b) *Dikerogammarus villosus*. Different letters indicate
510 significant differences ($p < 0.05$).

511

512 **Fig. 2** Values (mean \pm SE) of the index of importance (see text for details) of three food types: leaf litter
513 (white bars), macrophytes (light grey bars) and dead chironomid larvae (dark grey bars) consumed by
514 two invasive gammarids at 15, 20 and 25°C: (a) *Gammarus tigrinus* and (b) *Dikerogammarus villosus*.
515 Different letters indicate significant differences ($p < 0.01$)

516

517 **Fig. 3** Relationship between the number of live water fleas consumed by the two invasive gammarids
518 *Gammarus tigrinus* (a) and *Dikerogammarus villosus* (b) and prey density (i.e. the functional response),
519 at three water temperatures: 15°C (dots and large-dashed line), 20°C (triangles and small-dashed curve)
520 and 25°C (squares and full curve). The symbols are direct observations (six replicates per prey density)
521 and the curves are the fits of the theoretical function corresponding (see text for details and Table 1 for
522 the estimates of a and h).

523

524 **Fig. 4** Results of the backward and forward stepwise model selection procedure used to compare the
525 functional responses obtained at three different water temperatures for two invasive gammarids,
526 *Gammarus tigrinus* (a) and *Dikerogammarus villosus* (b). The model parameters a and h estimate
527 predator searching efficiency and prey handling time, respectively. We considered the simplest model
528 assuming the same parameter values regardless of the temperature (model a,h), the most complex model
529 assuming different parameter values for each temperature (model $a_1, a_2, a_3, h_1, h_2, h_3$ with 1=15°C, 2=20°C

530 and 3=25°C), and all the intermediate models. The numbers above the models are the Akaike criterion
531 (AIC_c) values. The models selected based on the AIC_c values are in grey and the best model is in black.
532 Bold arrows show the next model along the procedure and dots indicate the end of the procedure; when
533 the selected model did not perform better than the current model, which becomes the best model. The
534 results of the likelihood ratio tests used to compare current and selected models are on the arrows
535

536 **Fig. 5** Confidence regions (grey area) of the estimated parameters (predator searching efficiency (*a*) and
537 prey handling time (*h*), see text for details) of the functional response of the two invasive gammarids
538 *Gammarus tigrinus* (a) and *Dikerogammarus villosus* (b) feeding on live water fleas at 15, 20 and 25°C

539 **List of Tables**

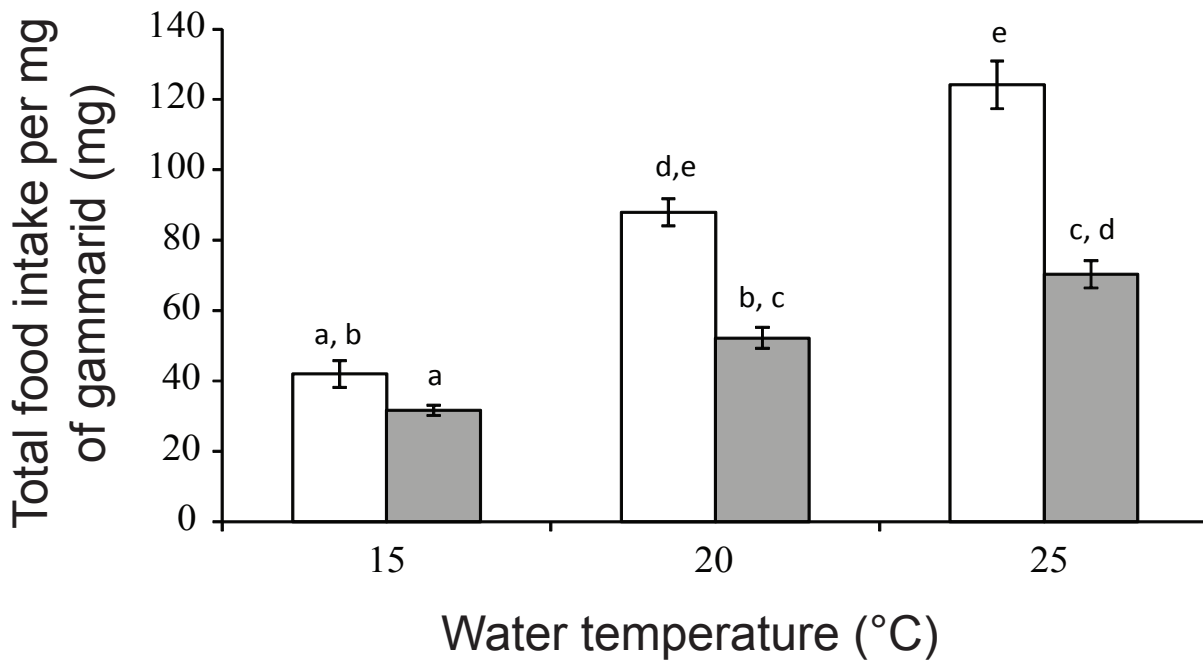
540

541 **Table 1** Values of predator searching efficiency (a) and the prey handling time (h) with 95 %
 542 confidence intervals estimated for the two invasive gammarids *Gammarus tigrinus* and
 543 *Dikerogammarus villosus* feeding on water fleas at three water temperatures. The estimates were
 544 obtained by fitting the Holling's type II functional response model to the number of prey eaten (see the
 545 Materials and Methods section for further details).

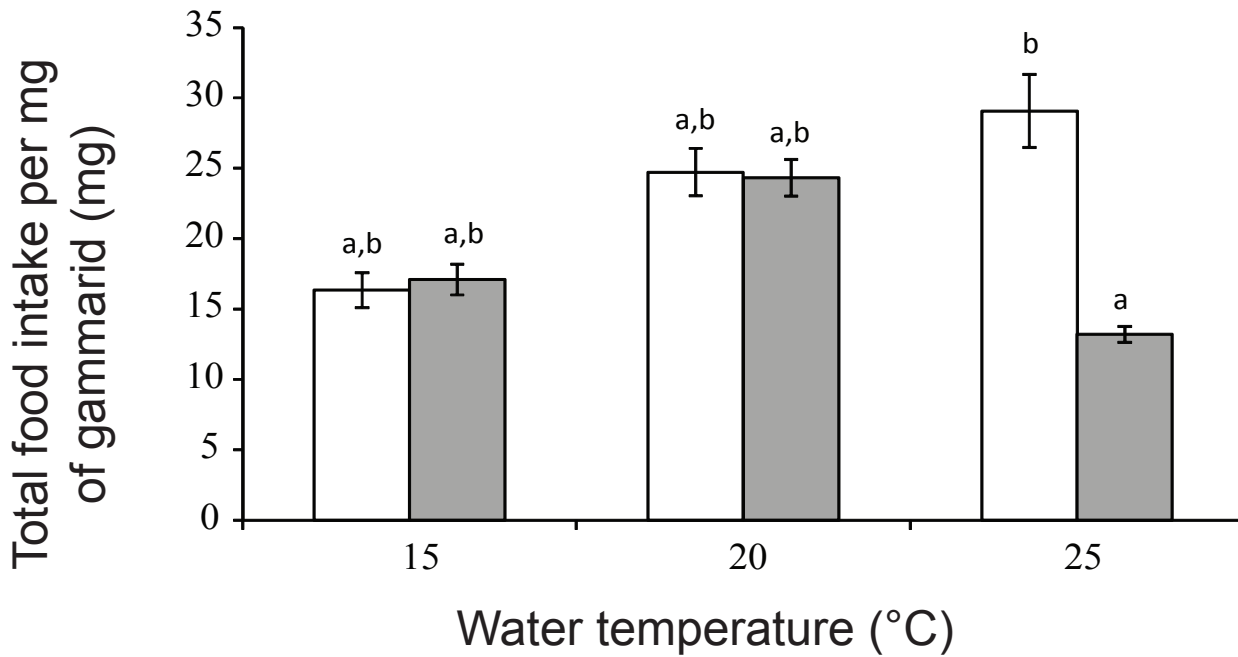
	Water temperature (°C)	Predator searching efficiency (a) [95 % CI]	Prey handling time (h) [95 % CI]
<i>G. tigrinus</i>	15	0.6313 [0.1847; 4.7401]	0.4979 [0.2327; 0.7304]
	20	0.6202 [0.3580; 1.3960]	0.0717 [0.0717; 0.1315]
	25	20.290 [5.3700; 269.18]	0.2109 [0.1799; 0.2404]
<i>D. villosus</i>	15	1.8920 [1.3428; 2.7926]	0.1037 [0.0868; 0.1197]
	20	2.9434 [2.0145; 4.1390]	0.0960 [0.0839; 0.1090]
	25	1.2759 [0.8456; 1.8611]	0.0690 [0.0465; 0.0885]

546

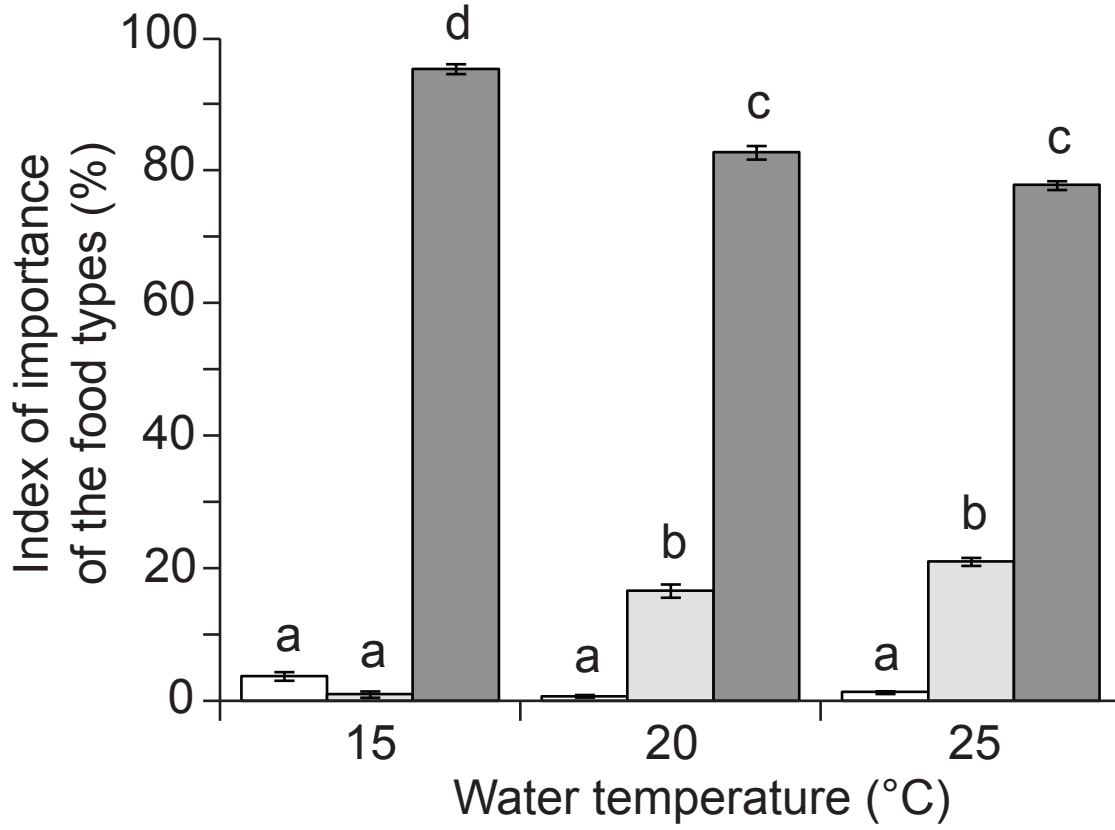
a) *Gammarus tigrinus*



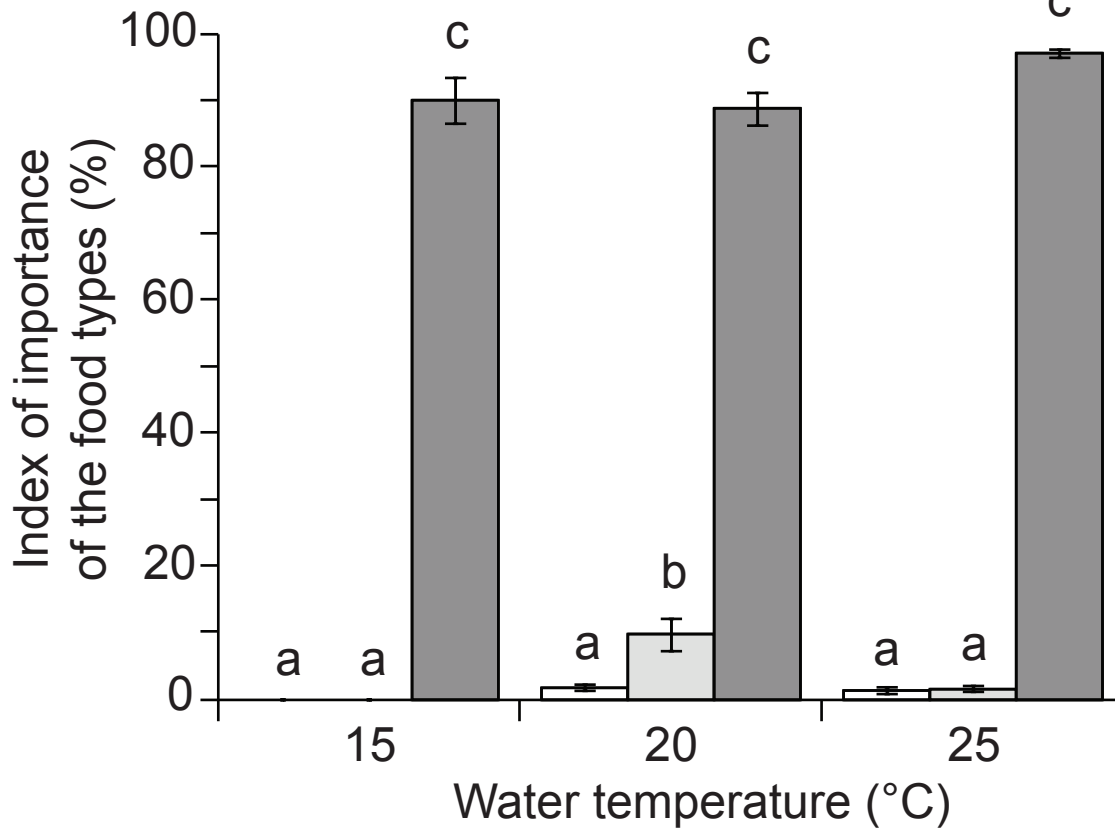
b) *Dikerogammarus villosus*



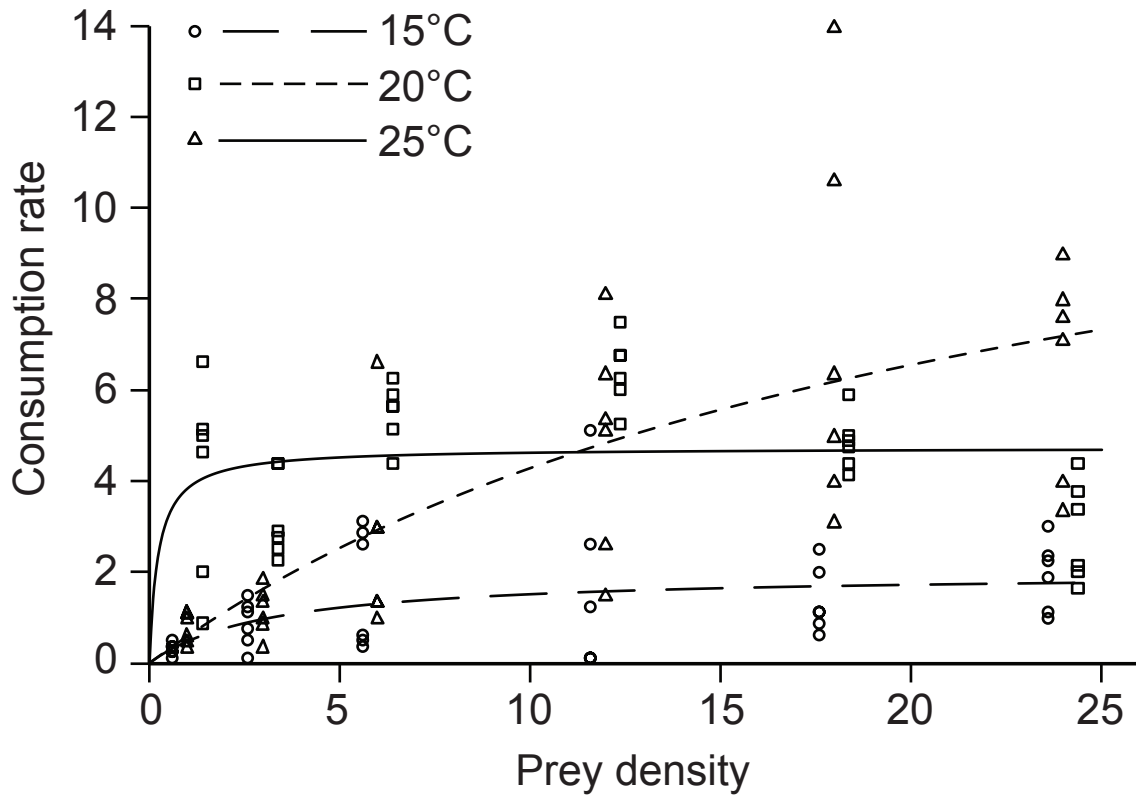
a) *Gammarus tigrinus*



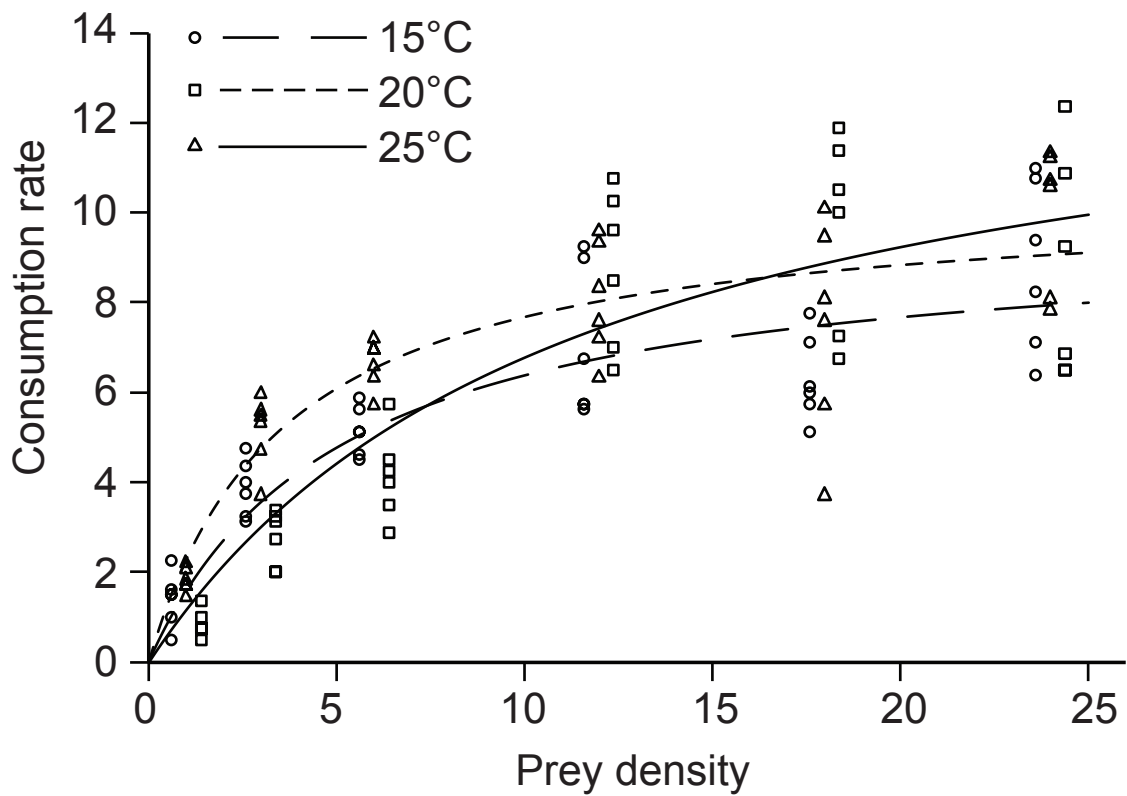
b) *Dikerogammarus villosus*



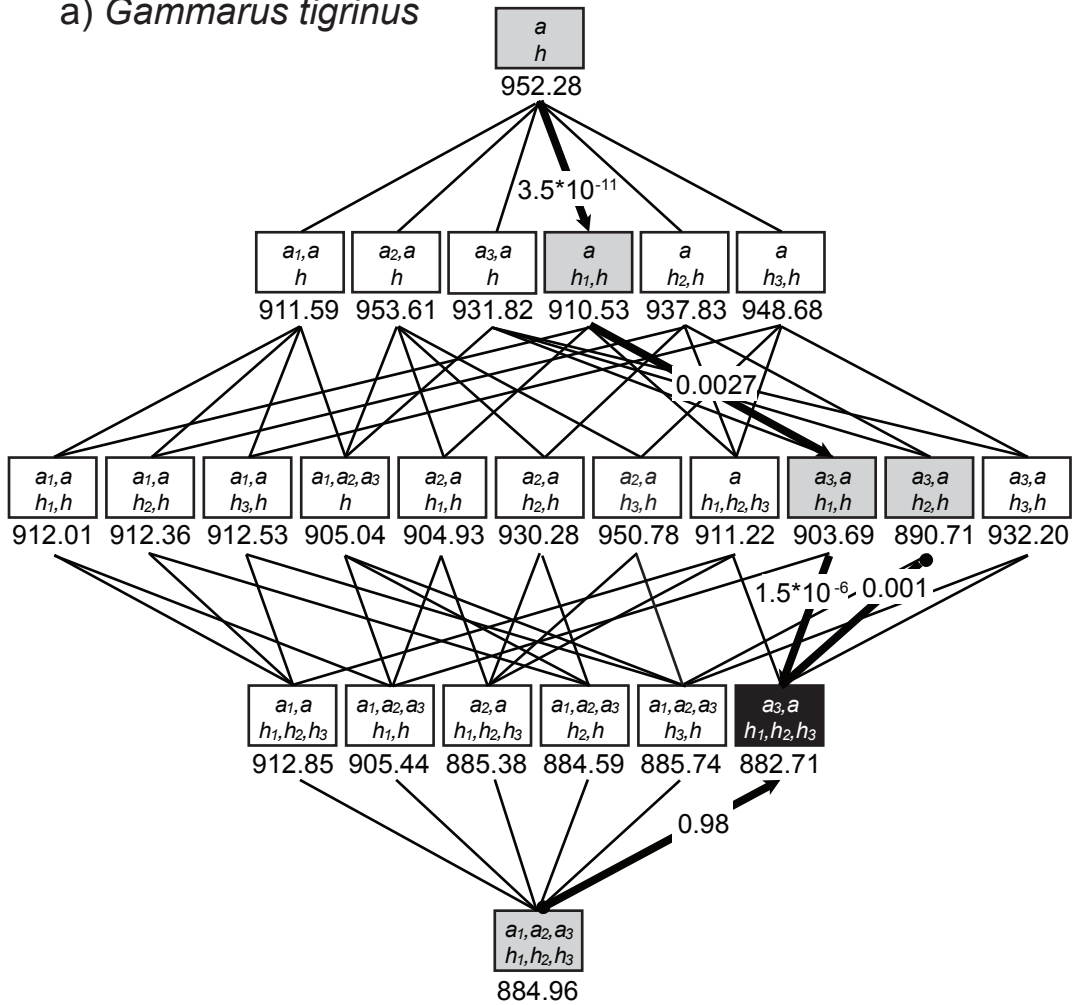
a) *Gammarus tigrinus*



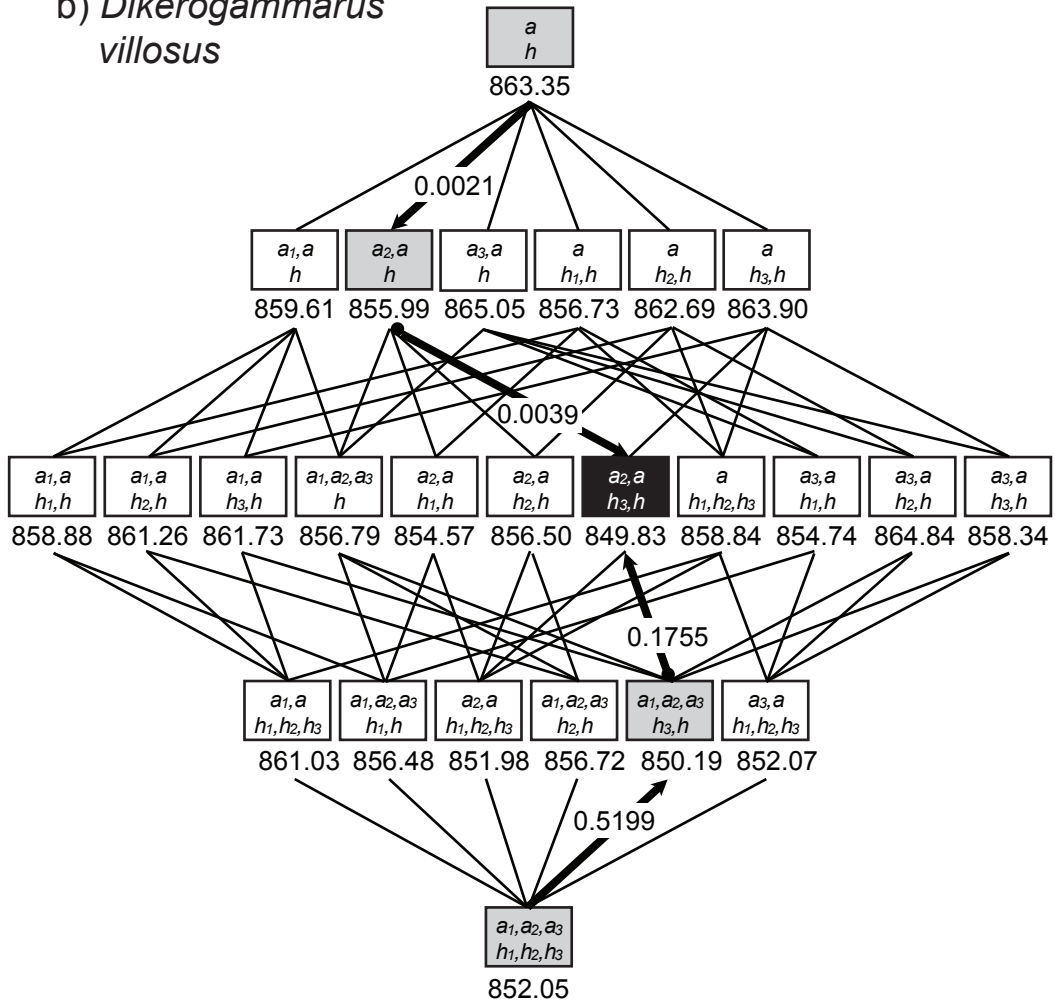
b) *Dikerogammarus villosus*



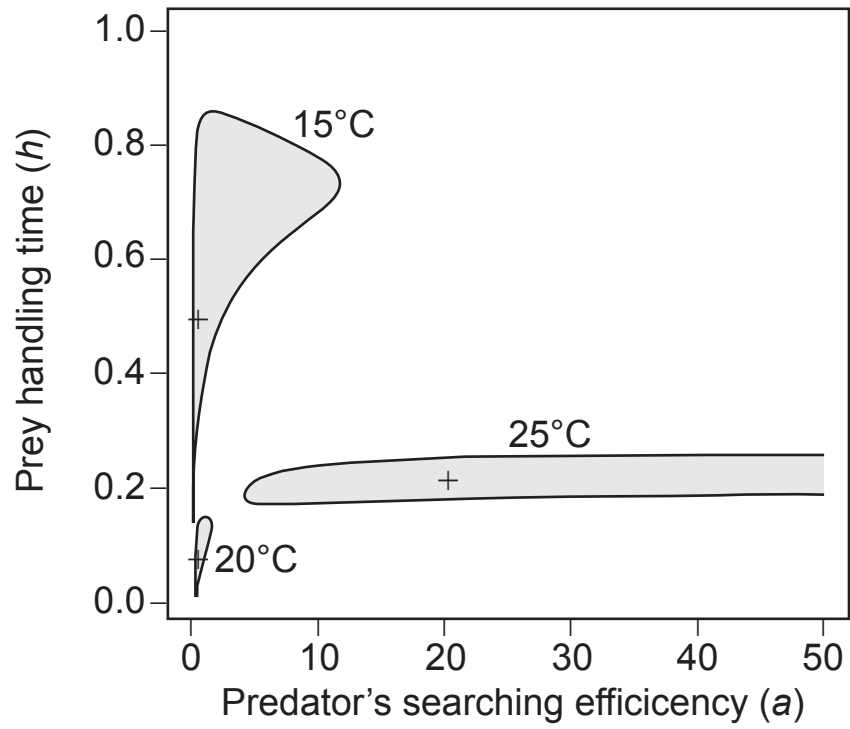
a) *Gammarus tigrinus*



b) *Dikerogammarus villosus*



a) *Gammarus tigrinus*



b) *Dikerogammarus villosus*

