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

Abstract

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

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

Introduction

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

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

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

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

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

Materials and methods

Specimen collection and maintenance

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

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

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

$$Qi = (MFi - MIi) - Di$$

Where (MIi) and (MFi) are the fresh weights of the food type (i) at the beginning and at the end of the experiment, respectively, and (Di) is the mean difference in fresh weight before and after the experiment in the control treatments.

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

$$IOIi = (100 \times Qi) / Q$$

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

Experiment 2 – functional response according to temperature

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

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

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

Statistical analyses

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

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

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

$$g(N) = aN / (1 + ahN)$$

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

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

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

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

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

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

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

All statistical analyses were performed using R 3.1.0 software.

Results

Experiment 1 – food choice according to temperature

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

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

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

Experiment 2 – functional response according to temperature

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

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

Discussion

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

Experiment 1 – food choice according to temperature

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

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

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

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

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

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

Experiment 2 – functional response

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

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

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

Conclusion

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

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

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

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

Fig. 4 Results of the backward and forward stepwise model selection procedure used to compare the functional responses obtained at three different water temperatures for two invasive gammarids, *Gammarus tigrinus* (a) and *Dikerogammarus villosus* (b). The model parameters *a* and *h* estimate predator searching efficiency and prey handling time, respectively. We considered the simplest model assuming the same parameter values regardless of the temperature (model a,h), the most complex model 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

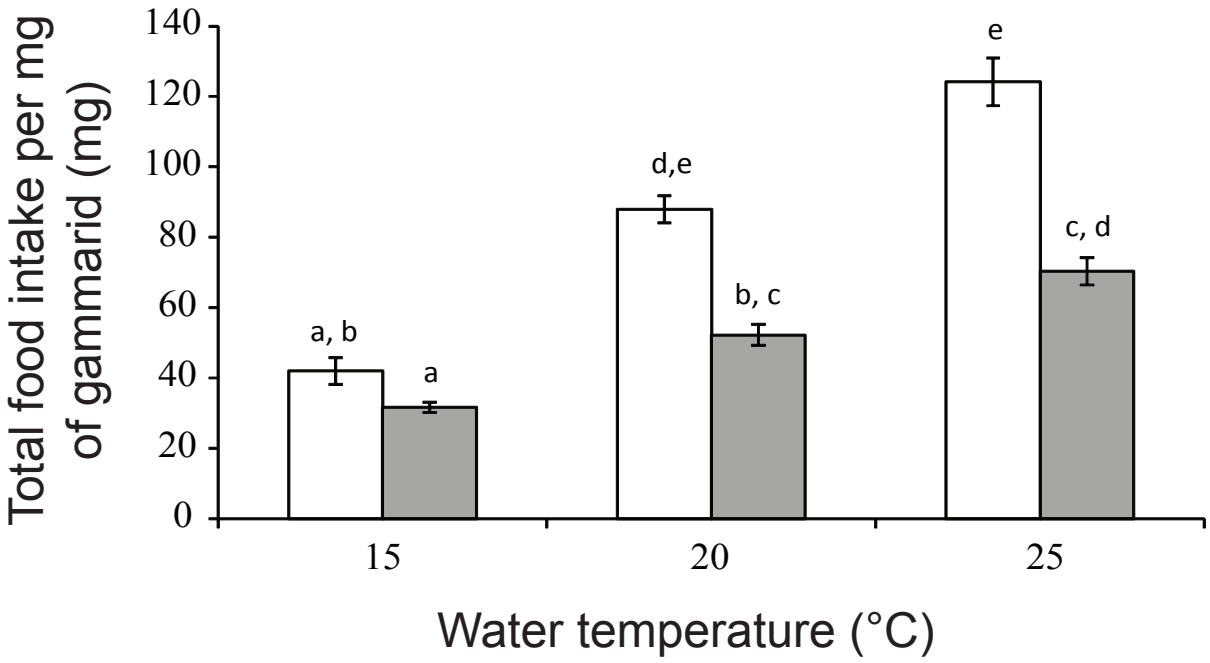
530 and 3=25°C), and all the intermediate models. The numbers above the models are the Akaike criterion
531 (AIC_c) values. The models selected based on the AIC_c values are in grey and the best model is in black.
532 Bold arrows show the next model along the procedure and dots indicate the end of the procedure; when
533 the selected model did not perform better that 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

List of Tables

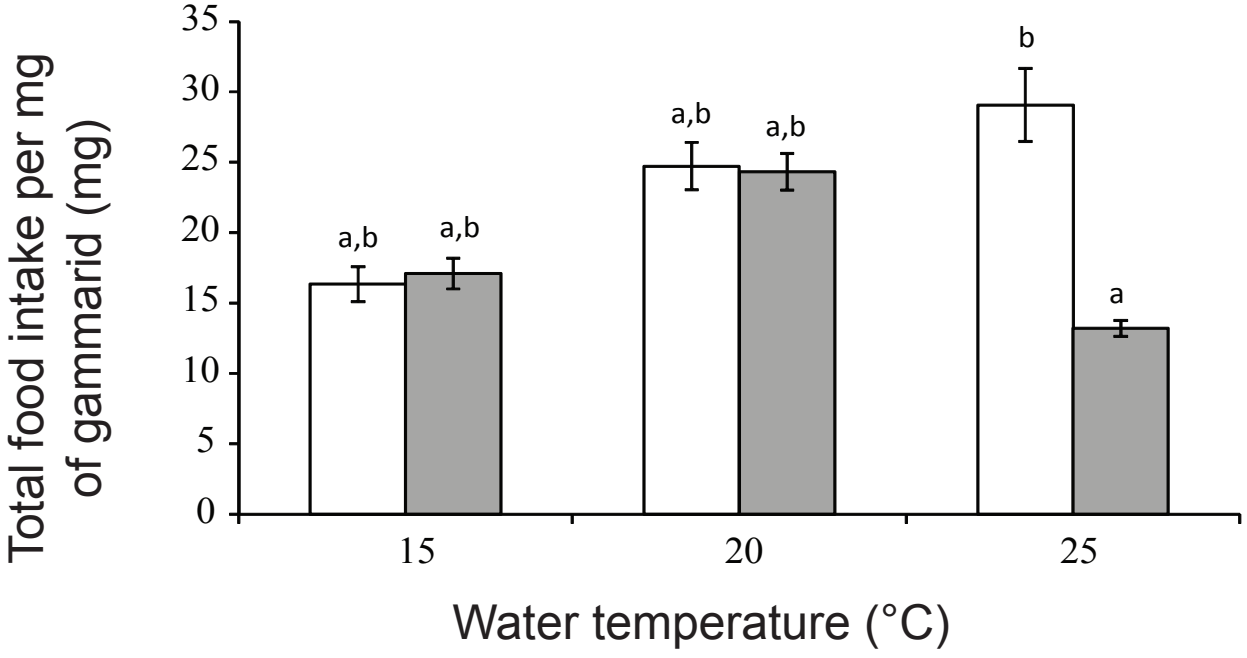
Table 1 Values of predator searching efficiency (*a*) and the prey handling time (*h*) with 95 % confidence intervals estimated for the two invasive gammarids *Gammarus tigrinus* and *Dikerogammarus villosus* feeding on water fleas at three water temperatures. The estimates were obtained by fitting the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

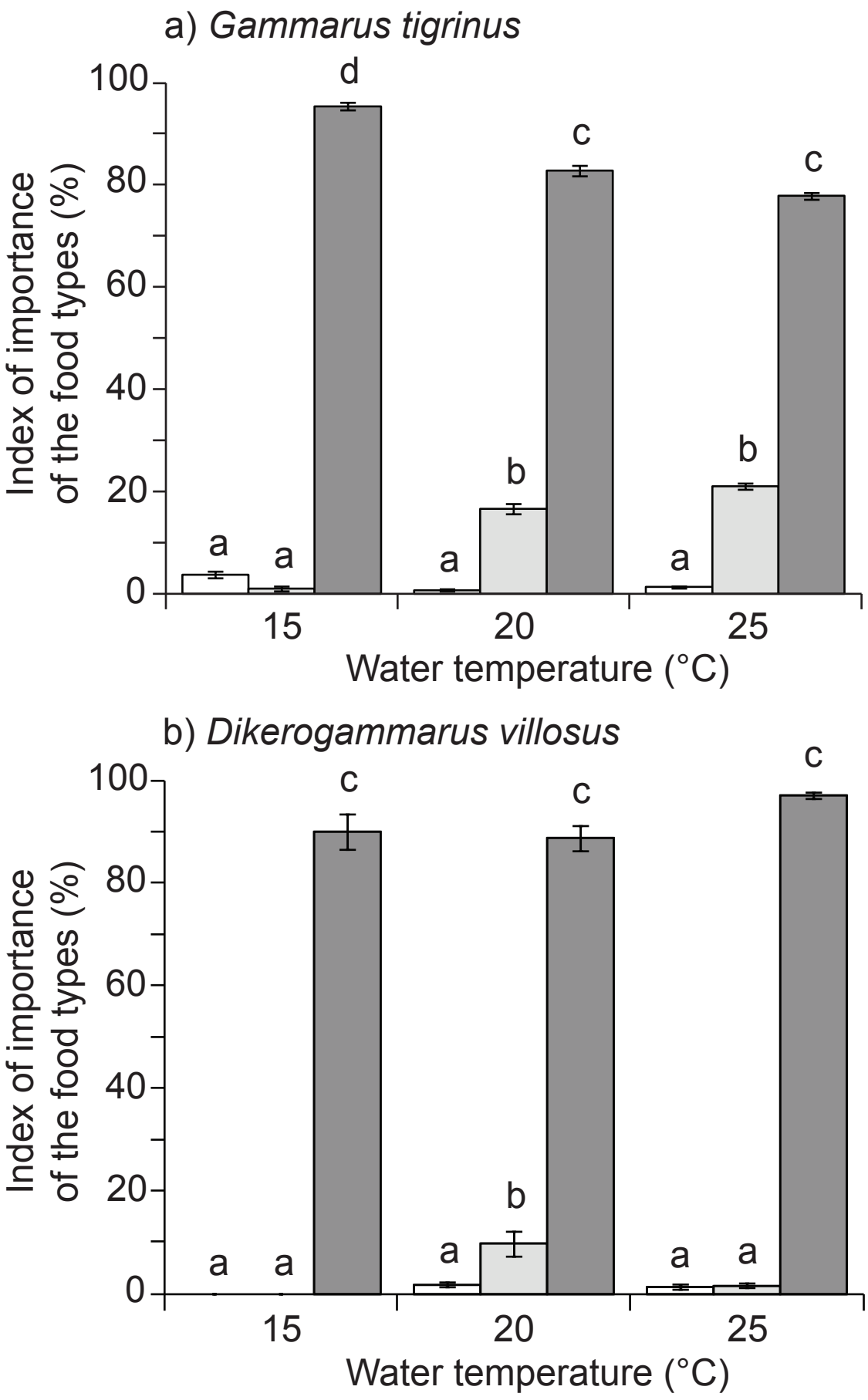
	Water temperature (°C)	Predator searching efficiency (*a*) [95 % CI]	Prey handling time (*h*) [95 % CI]
G. tigrinus	15	0.6313 [0.1847; 4.7401]	0.4979 [0.2327; 0.7304]
	20	0.6202 [0.3580; 1.3960]	0.0717 [0.0717; 0.1315]
	25	20.290 [5.3700; 269.18]	0.2109 [0.1799; 0.2404]
D. villosus	15	1.8920 [1.3428; 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]

a) *Gammarus tigrinus*

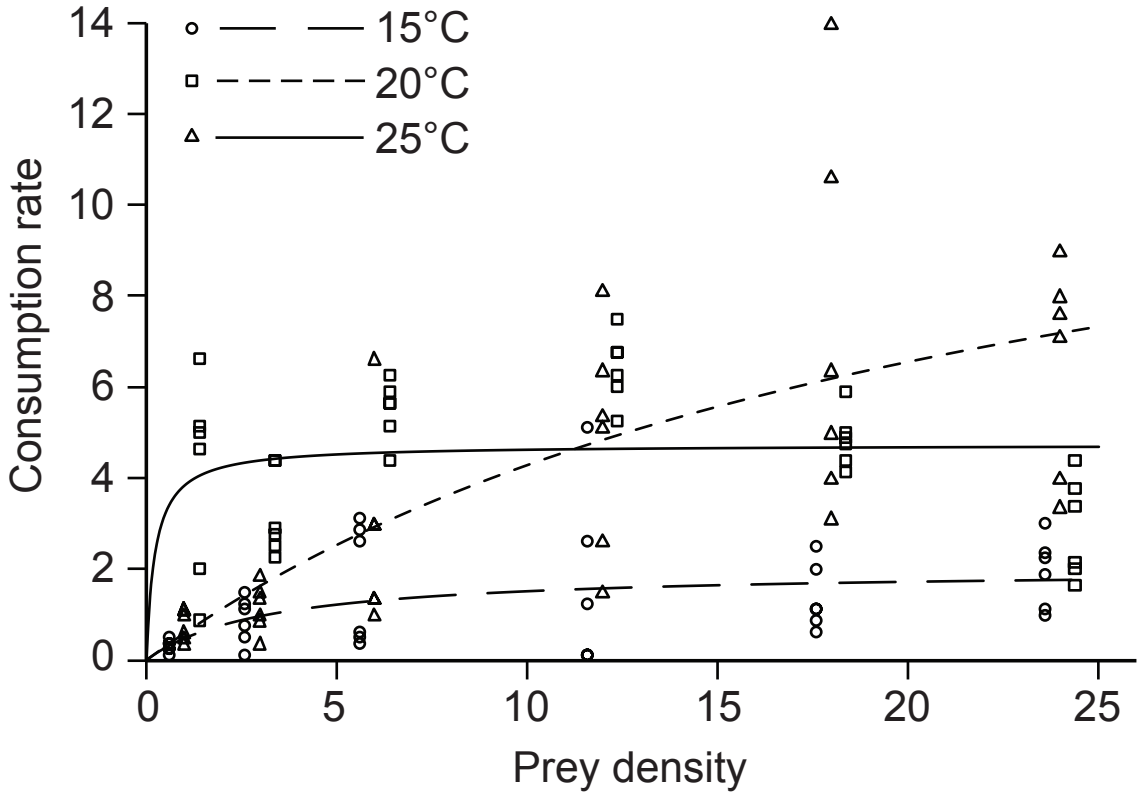


b) *Dikerogammarus villosus*

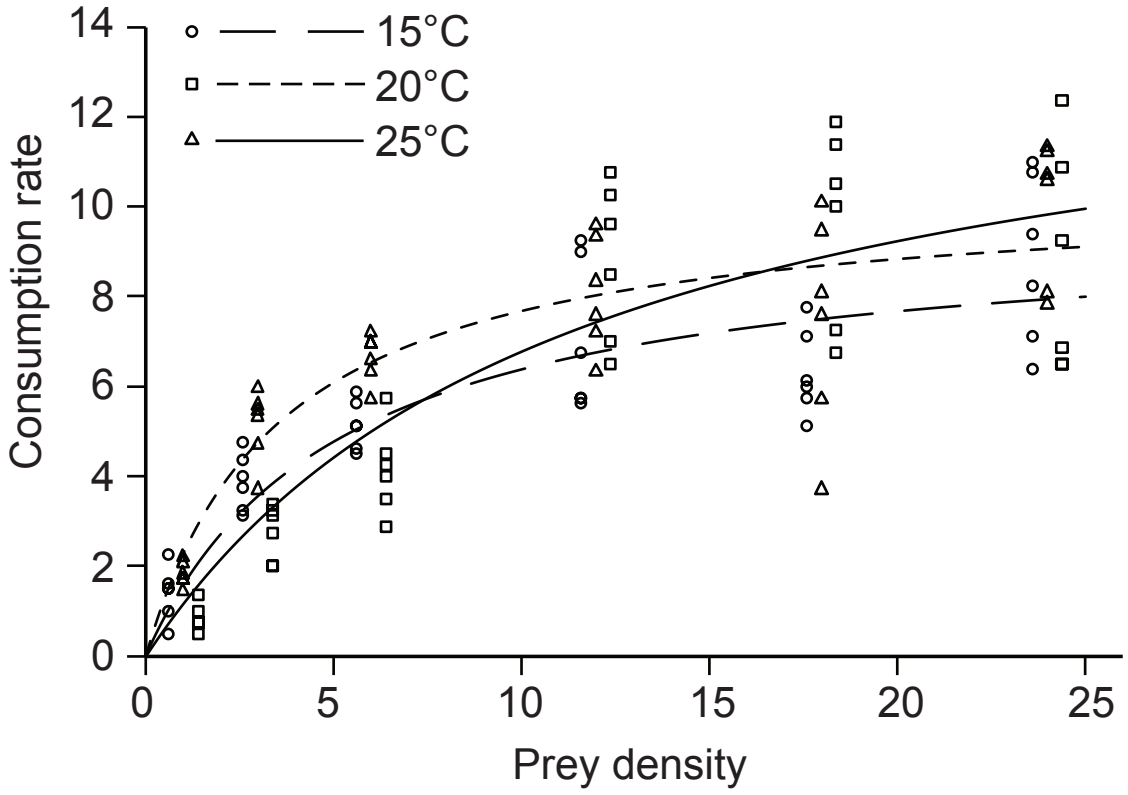


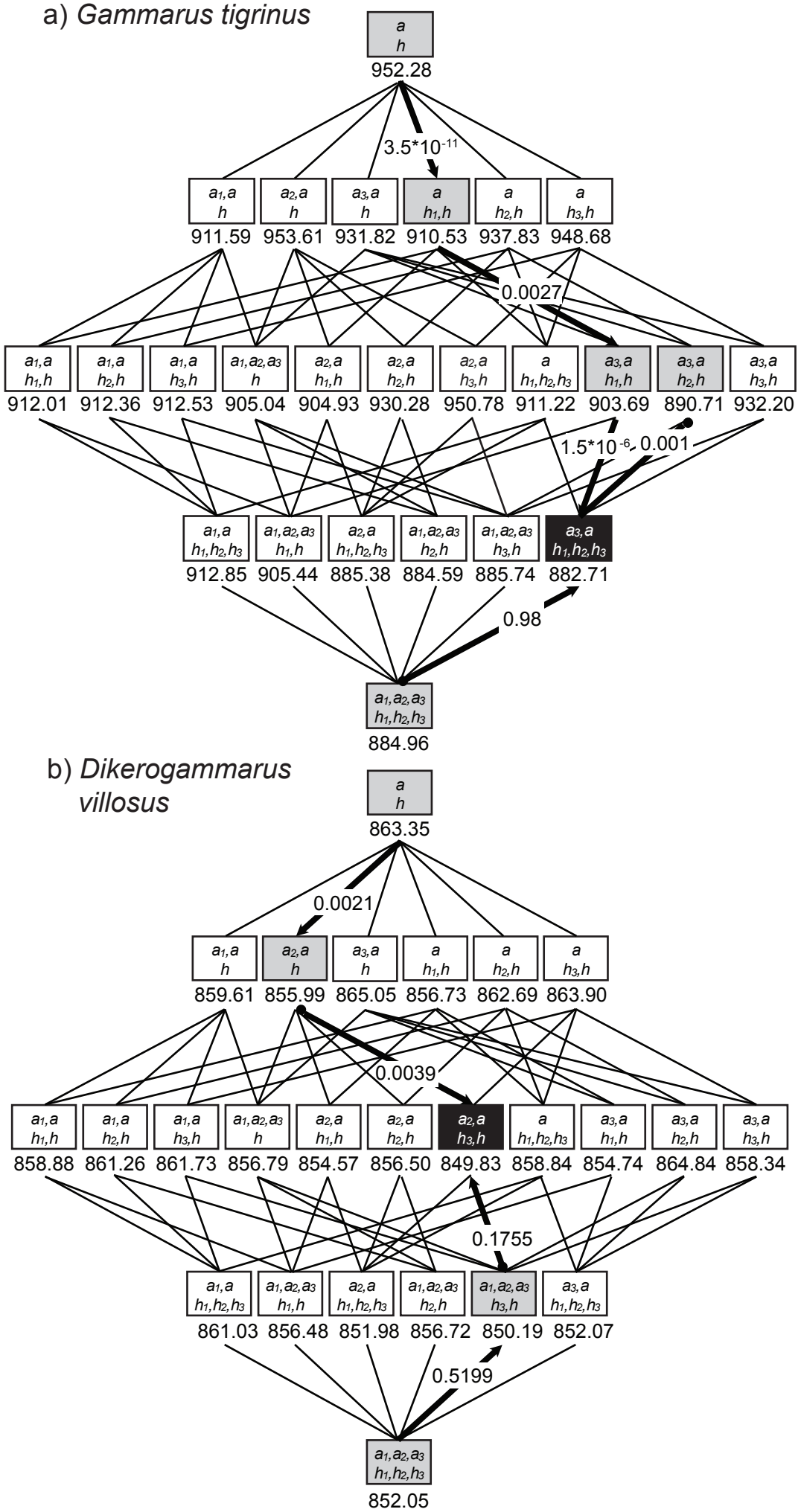

a) Gammarus tigrinus
Index of importance of the food types (%)
100
80
60
40
20
0
a
a
d
a
b
c
a
b
c
15
20
25
Water temperature (°C)
b) Dikerogammarus villosus
Index of importance of the food types (%)
100
80
60
40
20
0
a
a
c
a
b
c
a
a
c
15
20
25
Water temperature (°C)

a) *Gammarus tigrinus*

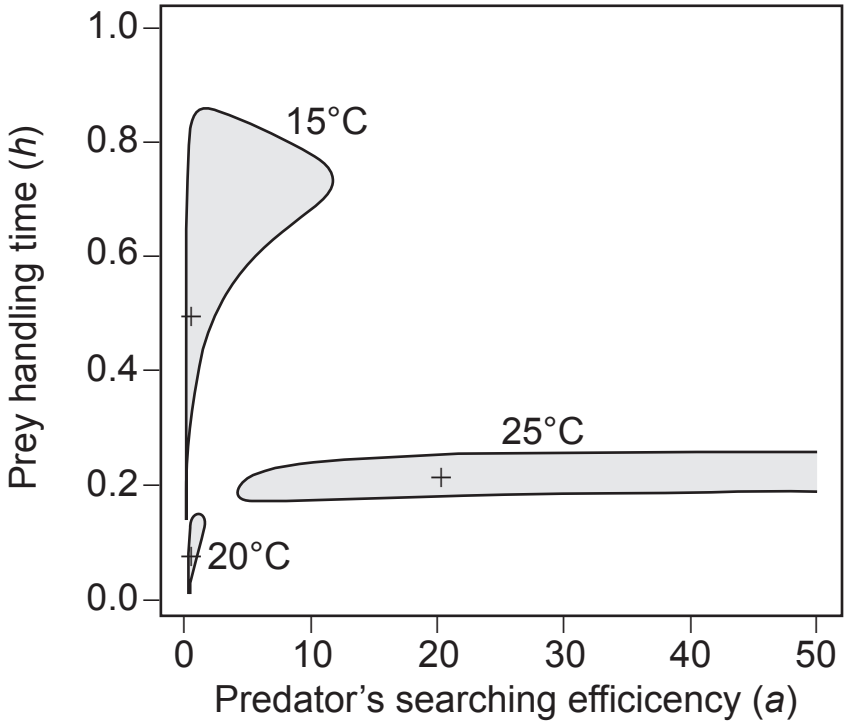


b) *Dikerogammarus villosus*




a) Gammarus tigrinus
a h
952.28
3.5*10-11
a1,a h
911.59
a2,a h
953.61
a3,a h
931.82
a h1,h
910.53
a h2,h
937.83
a h3,h
948.68
0.0027
a1,a h1,h
912.01
a1,a h2,h
912.36
a1,a h3,h
912.53
a1,a2,a3 h
905.04
a2,a h1,h
904.93
a2,a h2,h
930.28
a2,a h3,h
950.78
a h1,h2,h3
911.22
a3,a h1,h
903.69
a3,a h2,h
890.71
a3,a h3,h
932.20
1.5*10-6
0.001
a1,a h1,h2,h3
912.85
a1,a2,a3 h1,h
905.44
a2,a h1,h2,h3
885.38
a1,a2,a3 h2,h
884.59
a1,a2,a3 h3,h
885.74
a3,a h1,h2,h3
882.71
0.98
a1,a2,a3 h1,h2,h3
884.96
b) Dikerogammarus villosus
a h
863.35
0.0021
a1,a h
859.61
a2,a h
855.99
a3,a h
865.05
a h1,h
856.73
a h2,h
862.69
a h3,h
863.90
0.0039
a1,a h1,h
858.88
a1,a h2,h
861.26
a1,a h3,h
861.73
a1,a2,a3 h
856.79
a2,a h1,h
854.57
a2,a h2,h
856.50
a2,a h3,h
849.83
a h1,h2,h3
858.84
a3,a h1,h
854.74
a3,a h2,h
864.84
a3,a h3,h
858.34
0.1755
a1,a h1,h2,h3
861.03
a1,a2,a3 h1,h
856.48
a2,a h1,h2,h3
851.98
a1,a2,a3 h2,h
856.72
a1,a2,a3 h3,h
850.19
a3,a h1,h2,h3
852.07
0.5199
a1,a2,a3 h1,h2,h3
852.05

a) *Gammarus tigrinus*



b) *Dikerogammarus villosus*