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2 **Temperature-related intraspecific variability in the behavioral**
3 **manipulation of acanthocephalan parasites on their gammarid hosts**

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9
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11 acanthocephalan

Abstract. Understanding the effect of temperature on ecologically important species has become a major challenge in the context of global warming. However, the consequences of climate change cannot be accurately predicted without taking into consideration biotic interactions. In particular, parasitic infection constitutes a widespread biotic interaction, and parasites impact their hosts in multiple ways, eventually leading to consequences on communities and ecosystems. Here, we explored the effect of temperature on the anti-predator behavior of a keystone freshwater invertebrate, the amphipod *Gammarus fossarum*. Gammarids regularly harbor manipulative acanthocephalan parasites, which modify their anti-predator behavior in ways that potentially increase the probability of trophic transmission to their definitive hosts. We investigated the impact of temperature on gammarids infected by two acanthocephalan parasites, *Pomphorhynchus tereticollis* and *Polymorphus minutus*. Uninfected and naturally infected gammarids were acclimatized to different temperatures, and their behavior was measured. Our results show that the effect of infection on the phototaxis of gammarids increased with increasing temperature, with a stronger effect induced by *P. tereticollis*. In contrast, temperature had no effect on the alteration of refuge use or geotaxis observed in infected gammarids. Our results provide the first direct evidence that temperature can affect the extent of behavioral alteration brought about by certain parasite species. However, the consequences in terms of increased trophic transmission remain elusive, as the supposedly key anti-predatory behavior was not significantly affected by exposure of gammarids to different temperatures.

Introduction

Although parasites have been ignored for decades in the field of ecology, they are now widely recognized as important actors within ecosystems (Hudson *et al.*, 2006; Lefèvre *et al.*, 2009; Hatcher *et al.*, 2012). Parasitic organisms might represent close to half of all biodiversity according to certain estimations (Poulin and Morand, 2000; Dobson *et al.*, 2008), making parasitic infection one of the most common biotic interactions. Parasites have multiple impacts on their hosts that can have consequences on larger scales (Lefèvre *et al.*, 2009; Hatcher *et al.*, 2012). Differential pathogenic effects on several competing host species might for instance reverse the outcome of competition, or lead to the coexistence of species that would exclude each other in the absence of parasites (Hatcher *et al.*, 2012). Parasites with complex life-cycles that rely on trophic transmission are particularly likely to affect many components of the ecosystems, as they are embedded in large food webs and have the potential to affect several host species in succession. In particular, some of these parasites are able to induce phenotypic changes in their intermediate hosts, supposed to increase the probability of predation by their definitive hosts, thus increasing their own probability of transmission (Poulin and Thomas, 1999; Moore, 2002). Many of these effects lead to deep changes in communities and ecosystems, such that these parasites are sometimes referred to as ecosystem engineers (Thomas *et al.*, 1998, 1999; Sato *et al.*, 2012).

In the current context of global change, understanding how environmental conditions might alter ecologically important species has become a major challenge, notably due to the necessity to make accurate predictions about the consequences of such changes. Taking into consideration biotic interactions, and in particular parasites, is therefore particularly important, especially considering that climate change might induce unpredictable consequences on both inter- and intra-specific interactions (Tylianakis *et al.*, 2008; Rosenblatt and Schmitz, 2014). Temperature stands as a major parameter affecting many parasite-host

systems (Marcogliese, 2001; Harvell *et al.*, 2002; Barber *et al.*, 2016), and it is likely that the impact of global change on ecologically important species might depend on the parasites that they harbor. In particular, the nature of the cumulative effect of parasitism and temperature on hosts, whether additive or interactive, is hardly predictable (Labaude *et al.*, 2017).

Gammarids are widespread and abundant crustacean amphipods that are often seen as key species in freshwater ecosystems, where they constitute both important prey and predators for many species, modulating the composition of freshwater macroinvertebrates (Degani *et al.*, 1987; Friberg *et al.*, 1994; MacNeil *et al.*, 1997; Kelly *et al.*, 2002). They also play a major role in the maintenance of water quality through their roles as scavengers and shredders (MacNeil *et al.*, 1997), in particular through the consumption of dead leaves (Piscart *et al.*, 2009; Constable and Birkby, 2016). Gammarids are thus of significant ecological and economic importance as the quality of water is a key parameter in the emergence of water-borne diseases, ecosystem functioning, and maintenance of biodiversity (Klaphake *et al.*, 2001).

Gammarids serve as intermediate hosts for several species of acanthocephalan parasites that use either birds or fish as definitive hosts. These parasites affect their gammarid hosts in multiple ways. In particular, acanthocephalan parasites can manipulate the behavior of gammarids in a way that appears to increase their probability of being predated by their definitive hosts (Lagrue *et al.*, 2007; Perrot-Minnot *et al.*, 2007; Dianne *et al.*, 2011; but see Perrot-Minnot *et al.*, 2012). Along with other impacts, such as alteration of food consumption (Fielding *et al.*, 2003; Médoc *et al.*, 2011; Labaude *et al.*, 2017) or metabolic rate (Rumpus and Kennedy, 1974; Labaude *et al.*, 2015a), such modifications might affect the role of gammarids within ecosystems.

Temperature affects several traits in the association between acanthocephalan parasites and their gammarid hosts. Both the time of development of parasites (Olson and Pratt, 1971;

Tokeson and Holmes, 1982) and their success of establishment in their definitive fish host (Sheath *et al.*, 2016) depend on temperature. On the other hand, temperature also affects the metabolism (Pöckl and Humpesch, 1990), growth (Moenickes *et al.*, 2011), or consumption of gammarids (Foucreau *et al.*, 2016), with potential effects on their interaction with parasites. In addition, the cumulative effect of acanthocephalan parasites and temperature on gammarids feeding behavior is dependent on gammarids' aggregation context, with different consequences of the two parameters on the leave consumption of isolated individuals and individuals in groups (Labaude *et al.*, 2017).

Temperature may also lead to variation in the rapidity or intensity of parasite manipulation (i.e. how parasites manipulate host behaviour in link with predation probability), whether directly or due to effects on other parameters, although only indirect evidence exists so far (reviewed in Labaude *et al.*, 2015b). For instance, gammarids experimentally infected during winter were slower to display altered behaviors than gammarids infected in spring with the same parasite populations (Franceschi *et al.*, 2010). Such result might arise from a positive effect of temperature on host and parasite metabolism. Following this hypothesis, higher temperature experienced by infected gammarids might also lead to stronger manipulation, in the case where such manipulation depends on host or parasite metabolism. Moreover, gammarids survival decreases at high temperatures (Maazouzi *et al.*, 2011; Foucreau *et al.*, 2014), and parasites would benefit in increasing their manipulating effort to secure their transmission (Thomas *et al.*, 2002, 2011). Given the predicted increase of temperature in future years, improving our understanding of the consequences of temperature on host-parasite relationships is particularly relevant (Labaude *et al.*, 2017).

Here, we tested the effect of exposure to different temperatures on the behavioral changes induced by two acanthocephalan species, the fish parasite *Pomphorhynchus tereticollis* and the bird parasite *Polymorphus minutus*, on their gammarid host *Gammarus*

fossarum. These parasites manipulate different behaviors that are believed to be specific to the definitive host species (Tain *et al.*, 2006). While *P. tereticollis* induces an inversion of the photophobic behavior of its intermediate host (Tain *et al.*, 2006) and a decrease in its use of refuges (Perrot-Minnot *et al.*, 2007), infection by *P. minutus* leads to reversed geotaxis, with gammarid individuals staying closer to the water surface than uninfected ones (Bauer *et al.*, 2005). We relied on naturally infected gammarids with cystacanth parasites, the last larval stage that is infective for the definitive host and at which such changes occur in hosts, and investigated the effect of acclimatization at different temperatures on the behavior of uninfected and infected individuals.

Materials and Methods

Sampling and acclimatization

Gammarus fossarum individuals were collected in the Norges River (eastern France, 47°21'42.7"N 5°09'29.6"E, where gammarids are infected with *P. tereticollis*) in September 2015, and in the Vèze River (eastern France, 47°14'01.9"N 5°34'37.4"E, where gammarids are infected with *P. minutus*) in June 2016, using a kick sampling method with a hand net. The Norges River only contains *G. fossarum* species (Lagrue *et al.*, 2014; Labaude *et al.*, 2017). Although Lagrue *et al.* (2014) found that more than half of gammarids from the Vèze River belonged to the closely-related *G. pulex* species, more recent genetic analyses performed on 457 individuals sampled in the Vèze River in May 2015 showed that about 90% of them ($n = 410$) belonged to *G. fossarum* (S. Labaude, unpublished data). Given the small proportion of *G. pulex*, and since the two cryptic species are often considered as a single taxonomic unit (Karaman and Pinkster, 1977), all individuals were used in this study. The brightly colored cystacanth stages of these parasites are visible through the cuticle of gammarids, allowing a

preliminary selection of infected individuals directly in the field. Uninfected individuals were also captured.

Individuals from each population were randomly allocated to several groups that were acclimatized for 12 days in the laboratory at different temperatures. Individuals from the Norges River were acclimatized at three temperatures (10, 14, or 18°C), whereas individuals from the Vèze River were separated into only two groups (14 or 18°C), due to sampling limitations resulting from the low prevalence of infection with *P. minutus*. Temperatures were chosen to be in accordance with naturally fluctuating temperatures experienced by gammarids in their habitat (Pöckl *et al.*, 2003), and fell within the range of temperatures measured within the two rivers in 2015 (S. Labaude, personal data). To limit stress, individuals were maintained together in groups at each temperature, in an oxygenated mix of water collected in their river and dechlorinated, UV-treated tap water. They were fed *ad libitum* with conditioned elm leaves, and maintained under a 12:12 light:dark cycle regime. Due to different technical requirements associated with the behavioral tests, water temperature was controlled in two different ways. Because individuals had to be visible from above during behavioral tests (see below), individuals from the Norges River were maintained in water baths, following Labaude *et al.* (2017). Boxes containing individuals (and further test devices) were plunged into water that was constantly pumped through a temperature control device (TANK TK-1000 Chiller, Teco US). Individuals from the Vèze River, which were tested in vertical devices, were maintained and tested in refrigerators with transparent doors (Liebherr FKv 5443). These two systems allowed acclimatization and experiments for each population at all temperatures to occur concomitantly in the same room. The water temperature was controlled daily using digital thermometers.

Phototaxis and refuge use

Overall, 368 individuals (167 infected by *P. tereticollis* and 201 controls) from the Norges River were used for phototaxis tests and, among them, 176 individuals (76 infected and 100 controls) were randomly chosen to be tested for refuge use. The two sets of experiments were conducted during the same day.

After the acclimatization period to different temperatures, single individuals were introduced into horizontal glass tubes (22 cm long, 3.2 cm diameter) containing a dark zone (half of the tube being covered with black plastic to ensure complete opacity) and a light zone, following the design described in Perrot-Minnot (2004). Tubes were previously filled with aerated water. Water temperature was maintained during the course of the experiment with the same device as described for acclimatization. After 5 min of habituation in the tube, the position of every individual was recorded every 30 s during 5 min and scored as zero (dark zone) or one (light zone). Summed phototaxis scores for each individual ranged from zero (strongly photophobic, always in the dark zone) to 11 (strongly photophilic, always in the light zone).

To test for the use of refuges, single individuals were placed in boxes (10.5 × 16 cm) filled with 250 ml of oxygenated water with temperature controlled for each group as previously described. A refuge was available in each box, consisting of a saucer terracotta pot (8.5 cm of diameter) cut in half, with a 1-cm hole in the convex part (see Dianne *et al.*, 2014). After 5 min of habituation in the device following the introduction of gammarids, the position of each individual was recorded every 2 min during a 30-min observation period, and scored as zero (outside the refuge) or one (inside the refuge). Summed refuge scores ranged from zero (always outside the refuge) to 16 (always inside) for each individual.

For the two tests, a random number was assigned to each individual in order to ensure that the experimenter was unaware of the infection status of individuals.

Geotaxis

In total, 51 individuals infected by *P. minutus* and 59 uninfected individuals from the Vèze River were used for geotaxis tests.

Geotaxis, the response of individuals to gravity, was estimated as the average vertical position of individuals in the water column. After the acclimatization period, single individuals were introduced in 500 ml-graduated measuring cylinders (35 cm high, 6 cm diameter) filled with aerated water. Cylinders were vertically divided into five zones of equal height. A plastic net was placed along the inside wall of each cylinder, providing gammarids a substrate on which they could cling, as available on river banks. This was important since both the swimming and clinging behaviors can be altered by the parasite (Bauer *et al.*, 2005). Each cylinder was placed in a refrigerator at the relevant temperature. The light was presented only horizontally, through the glass door, thus preventing any confounding vertical phototactic reaction. After 2 min of habituation in the cylinder, the position of each gammarid was recorded every 30 s for 5 min, and a score was given according to the zone within the water column (from one for the bottom to five for the top). Summed geotaxis scores ranged from 11 (always at the bottom) to 55 (always at the top) for each individual.

Measurements and dissections

At the end of the experiment, the sex of each individual was determined using the size and shape of its first and second pairs of gnathopods, which are sexually dimorphic in amphipods (Hume *et al.*, 2005). All individuals were measured (height of the fourth coxal plate) using a Nikon ZMZ1500 dissecting microscope and Lucia G 4.81 software, and dissected. The developmental stage (acanthella or cystacanth) and the species of parasites

found within gammarids were determined based on morphological identification. As manipulation of the parasite depends on both acanthocephalan species and developmental stage, only individuals harboring *P. tereticollis* (for phototaxis and refuge use tests) and *P. minutus* (for geotaxis tests) parasites at the cystacanth stage were kept (hereafter referred as “parasitized” individuals). Individuals harboring other acanthocephalan species (a few *Pomphorhynchus laevis* and *Echinorhynchus truttae* were found), or acanthella stages were discarded. Gammarids in which no parasite could be found were considered as “control” individuals.

Data analyses

None of the three scores (phototaxis scores, refuge scores, and geotaxis scores) met normality and homoscedasticity conditions, even after data transformation. We therefore used non-parametric statistics. In both populations, the size of gammarids did not differ between parasitized and control individuals (data not shown), and was thus not considered in subsequent analyses. Comparisons between males and females for each infection status and at each temperature showed that there was no difference in the scores of individuals, for the three behaviors tested (data not shown). Therefore, sex was not considered in subsequent analyses.

First, the effect of temperature on each score was assessed using Kruskal-Wallis tests. The effect size of the differences between each temperature was then calculated for each score and for each infection status (control or parasitized) using Cliff’s delta (Cliff, 1996). Cliff’s delta was also used to compare the scores between control and parasitized individuals, at each temperature. Cliff’s delta is a scale-less parameter, ranging from -1 to 1, that is robust to non-normally distributed data. It is used to represent the size of the effect, in this case the

difference between two groups, as well as the direction of this difference. Moreover, its confidence intervals can be used to assess the significance between these differences, replacing classical statistic tests or post hoc tests. Medians and 95% confidence intervals of the Cliff's delta were calculated using the R-package 'orddom' (version 3.1). Other statistical analyses were performed using R version 3.1.1 software (R Foundation for Statistical Computing).

Results

Temperature significantly affected the amount of time spent in light ("phototaxis score") of *G. fossarum* individuals infected by *P. tereticollis* (Kruskal-Wallis, $\chi^2 = 22.86$, d.f. = 2, $P < 0.0001$, Fig. 1A), and control individuals (Kruskal-Wallis, $\chi^2 = 23.30$, d.f. = 2, $P < 0.0001$, Fig. 1A). The phototaxis score was significantly higher at 14°C and 18°C compared to 10°C, in both parasitized and control individuals (Fig. 2A). However, while there was a clear trend for phototaxis score to increase with temperature between 14°C and 18°C for parasitized individuals, that of control individuals did not differ significantly between these two temperatures (Fig. 2A), with even a trend to decrease. As a result, the difference of behavior between control and parasitized individuals was significant at 14°C and 18°C, with a stronger effect at 18°C (Fig. 2B).

Temperature had no effect on the amount of time spent inside refuges ("refuge score") for both control individuals (Kruskal-Wallis, $\chi^2 = 0.57$, d.f. = 2, $P = 0.75$, Fig. 1B) and infected ones (Kruskal-Wallis, $\chi^2 = 1.83$, d.f. = 2, $P = 0.40$, Fig. 1B). The refuge score of individuals infected by *P. tereticollis* was lower than that of controls (Fig. 1B), illustrating a lower trend to use refuges, although this difference was significant only at 18°C (Fig. 2B).

The non-significant differences were probably due to high inter-individual variation relative to the small sample size.

Correlations between phototaxis scores and refuge scores were all non-significant. The only trend observed was a negative correlation in parasitized individuals at 18°C (Table 1), with the more photophilic animals spending also more time out of refuges.

The geotaxis score of *G. fossarum* individuals infected by *P. minutus* was significantly higher than the score of control individuals (Fig. 3 and Fig 2B). Geotaxis scores were not influenced by temperature, with no difference between the two temperatures tested for both parasitized and control individuals (Fig. 3 and Fig 2).

Discussion

The temperature at which *Gammarus fossarum* were exposed for two weeks affected the intensity of phototaxis of *G. fossarum*, particularly in those infected with *Pomphorhynchus tereticollis* parasites. However, it had no impact on the time spent by gammarids inside refuges, independently of infection. No effect of temperature was found on gammarids geotaxis, whether infected or not by *Polymorphus minutus* parasites.

The differences in phototaxis observed between infected and control individuals across the three temperature tested here suggest that parasite manipulation was less efficient at low temperatures. Although both infected and uninfected animals were photophobic at low temperature, a gradual reversal of photophobia was observed in infected individuals at higher temperature, while this phenomenon stabilized in control individuals at the highest temperature. The increase in the manipulation of gammarids by *P. tereticollis* might be due to a physiological effect of temperature. Indeed, the metabolism of ectotherm species increases

274 with temperature (Gillooly *et al.*, 2001), including in acanthocephalan parasites which
275 develop faster at high temperature (Olson and Pratt, 1971; Tokeson and Holmes, 1982). Thus,
276 if the manipulation of host behavior is influenced by the physiology of parasites, it is possible
277 that increased temperatures led to more pronounced manipulation. However, such a
278 phenomenon hardly does not explain the increased phototaxis also observed in uninfected
279 individuals between 10°C and 14°C. The differences in phototactic behavior observed at
280 different temperatures might instead stem from the mechanisms underlying this behavior.
281 Serotonin plays a major role in the expression of phototactic behavior in gammarids:
282 *Pomphorhynchus* parasites increase the brain serotonergic activity of gammarids (Tain *et*
283 *al.*, 2006, 2007), and experimental injection of serotonin in uninfected individuals mimics the
284 behavioral changes induced by parasites (Perrot-Minnot *et al.*, 2014). The mechanisms by
285 which acanthocephalans induce modifications in gammarids serotonergic activity are
286 unknown (Lafferty and Shaw, 2013). However, serotonin production increases with increased
287 temperatures in invertebrates (Stefano and Catapane, 1977; Stefano *et al.*, 1977), as well as
288 many visual neural processes in invertebrates (Lim-Kessler *et al.*, 2008). These changes are
289 associated with modifications of other neuromodulators (Stefano and Catapane, 1977; Stefano
290 *et al.*, 1977), such that the consequences of these modifications can hardly be predictable.
291 However, an increase in serotonin levels could explain the increase of phototaxis between the
292 lowest temperature and the highest temperatures in our study, including in uninfected
293 gammarids. It is possible that in infected individuals, the level of serotonin due to increased
294 temperature and parasitic infection combine in the gammarid's brain to produce a higher
295 photophilic response. Serotonin levels were not measured here. Therefore, since the
296 invertebrate nervous system has a number of mechanisms designed to maintain homeostatic
297 function despite the temperature-driven changes (Lim-Kessler *et al.*, 2008) further studies are

needed to explore the mechanisms of the link between temperature, acanthocephalan parasites and photophily in gammarids, in order to verify this hypothesis.

Although variations in serotonin levels explain variations in the phototaxis behavior of gammarids, they have no significant effect on the gammarids' ability to use refuges (Perrot-Minnot *et al.*, 2014). This may explain the absence of significant effect of temperature on this trait in our study, and the lack of correlation between these two behaviors. Similarly, the manipulation of *G. fossarum* by *P. minutus*, in terms of geotaxis, was not affected by temperature, although the lowest temperature was not tested. However, the alteration of geotaxis may not rely on the same mechanisms as the ones involved in the alteration of phototaxis. Indeed, Tain *et al.* (2006) showed that the geotaxis of gammarids was not affected by experimental injections of serotonin, and that infection by *P. minutus* did not induce modifications in the serotonergic activity of gammarids. A recent study suggested a role of anaerobic metabolism and hypoxia in the manipulation induced by *P. minutus*: uninfected *Gammarus roeseli* displayed a negative geotaxis under hypoxia, while an injection of lactate and succinate in uninfected gammarids also mimicked the parasite-induced reversion of geotaxis (Perrot-Minnot *et al.*, 2015). It would be interesting to test the effect of temperature variations large enough to induce higher changes in the amount of oxygen dissolved in the water. If this were the mechanism, we would expect the geotaxis of both infected and uninfected individuals to increase at high temperatures.

Overall, our study suggests that temperature might be responsible for variations in the efficiency of the manipulation of certain behaviors of gammarids by acanthocephalans. In particular, the global increase of temperature might induce a higher efficiency of manipulation by some parasites, while other aspects of manipulation, or behavioral changes in other species, might not be affected. Some of our results are in line with those obtained by Benesh *et al.* (2009) in isopods. They found that, whereas the intensity of phenotypic changes induced

by the acanthocephalan *Acanthocephalus lucii* varied between seasons, experimental exposure to different temperatures did not change the intensity of manipulation of host behavior. Our study shows that some components of parasite-induced behavioral changes may be affected by temperature variation. Although the metabolism of acanthocephalan parasites is highly dependent on temperature, thus resulting in longer developmental time during cold periods (Olson and Pratt, 1971; Tokeson and Holmes, 1982), variations in the rapidity of their transmission to the next host might also arise from differences in their manipulation and partly explain the seasonal distribution documented in some acanthocephalan parasites (Van Cleave, 1916; Muzzall and Rabalais, 1975; Brown, 1989).

Parasites have numerous effects on communities and ecosystems, especially through the manipulation of the behaviour of their hosts (see Lefèvre *et al.*, 2009; Labaude *et al.*, 2015b for reviews). Consequently, modifications in manipulation following changes in temperature are also likely to have consequences at larger scales. In particular, acanthocephalan parasites are responsible for many modifications in the ecology of gammarids, including their feeding behaviour or microhabitat choice (Labaude *et al.*, 2017; MacNeil *et al.*, 2003). More to the point, an increase in the predation facilitation induced by parasites in response to increasing temperature might affect food webs, through alteration in the interaction between gammarids and their predators, as well as the dynamics of the populations of parasites, hosts and their predators. However, further studies are needed to determine the effect of temperature on the ecological outcome of manipulation, *i.e.* its effects in terms of predation facilitation induced by parasites. Indeed, although infected gammarids are more predated by the definitive hosts of parasites than uninfected ones (Bethel and Holmes, 1977; Perrot-Minnot *et al.*, 2007; Jacquin *et al.*, 2014), only certain behavioral changes have been demonstrated to be linked to this predation enhancement. More precisely, in gammarids, the presence of refuges and the intensity of their use was directly linked to

348 differences in predation rate of gammarids infected by *Pomphorhynchus* parasites vs.
349 uninfected ones (Kaldonski *et al.*, 2007; Dianne *et al.*, 2011, respectively), while the intensity
350 of change in phototaxis was not (Perrot-Minnot *et al.*, 2012). Similarly, the reversed geotaxis
351 behavior was responsible for an increased predation in gammarids infected by *Polymorphus*
352 parasites (Jacquin *et al.*, 2014). Here, it is worth noting that the only behavior affected by
353 temperature is the one for which no causal link with predation enhancement could be
354 established. However, many other parameters might interact in the success of predation. In
355 particular, the behavior of gammarids depends on predation risk (Médoc *et al.*, 2009; Durieux
356 *et al.*, 2012; Lewis *et al.*, 2012), while temperature might also alter the behavior of predators
357 themselves (Brett, 1971; Elliott, 1976). In addition, temperature is likely to affect other
358 parameters, such as the reproduction of parasites (Barber *et al.*, 2016), leading to
359 modifications in the population dynamics of both hosts and parasites that are hardly
360 predictable. Finally, we did not control the environmental conditions during the development
361 of parasites, and this can lead to other sources of variability. For instance, acanthocephalans
362 develop faster at high temperatures (Olson and Pratt, 1971; Tokeson and Holmes, 1982), and
363 the efficiency of their manipulation increases with time after they reached the cystacanth stage
364 (Franceschi *et al.*, 2008; Labaude *et al.*, 2015a). Thus, it cannot be discounted that, in our
365 experiments involving naturally-infected gammarids, those maintained at 18°C harbored
366 parasites that already reached their highest manipulative intensity, while the manipulative
367 intensity of gammarids kept at lower temperatures was still increasing. In addition, gammarid
368 metabolism increases with temperature to a certain extent (Roux and Roux, 1967; Pöckl and
369 Humpesch, 1990; Issartel *et al.*, 2005), along with their mortality (Maazouzi *et al.*, 2011;
370 Foucreau *et al.*, 2014). As parasites completely depend on the survival of their hosts before
371 transmission, they would benefit in increasing their manipulating effort when the life
372 expectancy of their hosts decreases to secure their transmission (Thomas *et al.*, 2002, 2011).

Consistent with this hypothesis, Poulin (1993) found that the intensity of behavioral changes induced by trematode parasites was greater when their intermediate fish hosts were older. However, contradicting with this hypothesis, no significant changes in refuge use were observed in *G. pulex* infected with *P. laevis* under different survival conditions (Labaude *et al.*, 2015a). Future studies might therefore benefit from investigating the effect of temperature using experimental infestations, to control for environmental conditions during the development of the parasites.

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607

Tables

Table 1. Correlations (Spearman's rho) between phototaxis and refuge use scores in control and parasitized *G. fossarum*, at each temperature (10, 14, and 18°C).

Infection status	Temperature	Spearman's rho	<i>P</i>
Control	10°C	-0.167	0.344
	14°C	-0.310	0.124
	18°C	-0.053	0.743
Parasitized	10°C	-0.017	0.936
	14°C	-0.186	0.432
	18°C	-0.416	0.020 ¹

¹ non-significant after Bonferroni correction

Figure Legends

Figure 1. Individual *Gammarus fossarum* behavior scores for (A) phototaxis tests and (B) refuge use tests, according to their infection status (P or C, respectively parasitized by *P. tereticollis* or control) and temperature (10, 14, or 18°C). Thick lines represent medians, boxes represent upper and lower quartiles, and dotted lines represent upper and lower deciles. Sample sizes are indicated at the bottom of the graph.

Figure 2. Effect sizes (Cliff's delta, d) of the behavioral differences (A) between temperatures, for each test and each infection status (parasitized and control), and (B) between parasitized and control *G. fossarum* individuals, for each test (phototaxis, refuge use, and geotaxis) at each temperature (10, 14, and 18°C). Cliff's delta effect sizes are represented with their 95% confidence intervals, and their values are given. Values below zero (dotted line) indicate that the behavioral score was higher for the group specified on the left, while values above zero indicate higher scores for the group mentioned on the right. The difference is significant when the bar does not overlap zero. For instance, the first row indicates that phototaxis scores of control individuals were significantly higher at 14°C compared to 10°C.

Figure 3. Individual *Gammarus fossarum* geotaxis scores according to their infection status (P or C, respectively parasitized by *P. minutus* at the cystacanth stage, or control) and temperature (14 or 18°C). Thick lines represent medians, boxes represent upper and lower quartiles, and dotted lines represent upper and lower deciles. Sample sizes are indicated at the bottom of the graph.

Figure 1

A. Comparisons between temperatures

B. Comparisons between control and parasitized individuals

Figure 2

Figure 3