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Effect of the environment on the interaction between gammarids (Crustacea: Amphipoda) and their manipulative acanthocephalan parasites

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If I were asked to nominate my personal epitome of Darwinian adaptation, the ne plus ultra *of natural selection in all its merciless glory, I might hesitate between the spectacle of a cheetah outsprinting a jinking Tommie in a flurry of African dust, or the effortless streamlining of a dolphin, or the sculptured invisibility of a stick caterpillar, or a pitcher plant silently and insensibly drowning flies. But I think I'd finally come down on the side of a parasite manipulating the behavior of its host – subverting it to the benefit of the parasite in ways that arouse admiration for the subtlety, and horror at the ruthlessness, in equal measure.*

Richard Dawkins,
Host manipulation by parasites, 2012

Acknowledgements

May the English reader forgive me for using, just for this part, my beloved French.

Je devais avoir une dizaine d'années quand j'ai découvert, dans un magazine pour enfants, le métier d'éthologue : « personne qui étudie le comportement des animaux sauvages dans leur milieu naturel ». Dans mon esprit, j'imaginais une personne assise sur un arbre, un carnet à la main, passant ses journées à observer les animaux avec des jumelles. Une alternative alléchante à l'autre métier animalier par excellence quand on est jeune, vétérinaire. Quand est venu le moment des premiers choix d'orientation, l'idée était encore bien encrée avec tout de même un chiffre effrayant : « bac +8 ». On m'a fait remarquer que huit années, ce n'était pas grand-chose. J'ai répondu non sans ironie que c'était exactement la moitié de ma vie.

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Preface

"*Alien beings enter hosts and twist host behavior to sinister purposes…*"

It is no doubt that such words would perfectly fit in any science fiction novel. Yet, the sentence stands on the very first line of the introduction of Moore's pioneer book in the field of manipulative parasites, "*Parasites and the Behavior of Animals*" (Moore, 2002a). This ambiguity is not unfortunate. The book is written with perfect scientific accuracy, based on years of research, and stands as a reference for scientists in the field. Yet, Moore started her book in a quite unusual way. As the epigraph, she chose to put a quote that could not better illustrate the fascination raised by manipulative parasites. Although the quote itself highlights parasites' subtle strategies, the interest mostly comes from its author: Moore chose to quote Mr. Spock, a fictional character from *Star Trek*, as an introduction of her book.

Humans have long been fascinated by creatures capable of challenging their free will. No wonder that bloodthirsty vampires hypnotizing their victims, extraterrestrial beings entering humans to control their minds, or zombies craving to bite healthy people to transmit their deadly condition are such a common topic in entertainment books and movies. Many fictional stories are based on reality, and it is no doubt that manipulative parasites represent an unquenchable source of new story material.

The remarkable ability of manipulative parasites to disrupt the behavior of their hosts is at the origin of their fame, even among non-scientist people. Nowadays, countless videos and images flourish on the Internet, depicting the big stars in manipulation. Several books have been entirely devoted to manipulative parasites, the last one largely targeting a non-scientific public (McAuliffe, 2016). My own very first glimpse of the marvelous world of manipulative parasites goes back more than ten years ago, from a comic strip depicting the strange habit of a freakish parasite, the lancet liver fluke (Boulet *et al.*, 2005). Back in that time, I would never have imagined that manipulative parasites, these seemingly mysterious and exotic creatures, would become one of the most amazing topics I have ever studied.

Chapter I. Introduction

Parasitic organisms might constitute an approximately half of all biodiversity on Earth (Poulin & Morand, 2000; Dobson *et al.*, 2008). These organisms, ranging from viruses and bacteria to complex animals such as helminths or crustaceans, use other organisms as resources and habitats. Many parasites – qualified as heteroxenous – rely on several successive host species to develop and reproduce. It is usually accepted that these complex life-cycles result from the addition of a new host in originally simple life-cycles (Parker *et al.*, 2003, 2015a, 2015b). Such an inclusion of a new host has led to the appearance of a critical step in the life of parasites: the transmission between their different hosts.

Many parasites rely on a trophic transmission. They develop into successive larval stages in one or several intermediate host(s). They then need their last intermediate host to be eaten by their definitive host – the host in which parasites reach their maturity and reproduce – to achieve their transmission. The success of this strategy largely depends on the probability of predation between the two hosts.

Numerous parasite species, referred as manipulative parasites, have been shown to induce phenotypic changes in their intermediate hosts (reviewed in Hughes et al., 2012; Moore, 2002b). It is now widely recognized that some of these alterations, in particular regarding the appearance and behavior of their hosts, might have direct consequences on the probability of predation of parasites' intermediate hosts by their definitive hosts. First, parasites that reached the stage that is transmissible to their definitive hosts induce alterations in the phenotype of their intermediate hosts that are believed to disrupt their anti-predator strategy. For instance, infected hosts might be attracted to the odor of their predator, rather than being repulsed (e.g. Berdoy et al., 2000; Kaldonski et al., 2007), or present an increased conspicuity (e.g. Fuller et al., 2003; Loot et al., 2002). Accordingly, infected individuals often show an increased susceptibility to predation, illustrating the "trophic facilitation" induced by parasites (e.g. Knudsen et al., 2001; Lafferty and Morris, 1996; Perrot-Minnot et al., 2007). Second, the presence of manipulative parasites that did not yet reach the transmissible stage, and thus need their intermediate host to stay alive, might lead to the protection of their host. Indeed, an opposite alteration of anti-predatory behavior has been documented in some species, with evidence that hosts harboring such larval stages of parasites are less predated than uninfected individuals (e.g. Dianne et al., 2011; Hafer and Milinski, 2016; Hammerschmidt et al., 2009). Although it is still debated whether the changes induced by manipulative parasites are adaptive or not (Poulin, 1995; Cézilly *et al.*, 2010; Perrot-Minnot *et al.*, 2012), there is now increasing evidence showing that these parasites have numerous effects on their hosts, ultimately leading to changes in their probability of transmission.

The fact that some parasites can manipulate the phenotype of their hosts is a pretty recent discovery. Parasites in general were ignored for a long time, mostly because of their peculiar life-cycles that were difficult to apprehend, along with their small size that made them invisible without a microscope. Even after the discovery of parasites, the very notion that such primitive organisms,

deprived from a nervous system and considered as degenerative life forms unable to survive on their own, could alter the behavior of hosts, much larger and complex, probably required some degree of innovative thinking. However, the idea that some parasites might increase the conspicuity of their hosts to predators emerged in the 19th century. One of the first cases ever described is, not surprisingly, one of the most impressive examples of phenotype changes. The trematode *Leucochloridium paradoxum* is a bird parasite infecting snails as intermediate hosts. The sporocysts of the parasite develop in broodsacs in tentacles of a snail, altering their size, shape and coloration. In addition, the broodsacs can be seen conspicuously pulsating through the snail tentacles, making them look like caterpillars. In 1853, while the life cycle of the trematode was not yet understood, von Siebold meticulously described the sporocysts (Fig. 1) and hypothesized that their contractile moves could attract birds attention (von Siebold, 1853). Starting from the middle of the 20th century with the description of *Dicrocoelium dendriticum* (Mapes, 1951; Mapes & Krull, 1951; Krull & Mapes, 1952), other cases of manipulation by parasites were documented. *D. dendriticum* is a trematode, better known as the lancet liver fluke, that lives in the liver of ruminants. Its first intermediate host, a snail, gets infected by eating the eggs of the parasite. However, manipulation occurs in the second intermediate host, an ant that also gets infected by the consumption of larval stages of the parasites after they escaped the snail through its mucous. Infected ants were reported to crawl up to the top of blades of grass and firmly grab them with their mandibles, such an elevated position probably increasing their probability of unintentional grazing by ruminants.

The two trematodes, *L. paradoxum* and *D. dendriticum*, stand as historical cases in the discovery of manipulation by parasites, and remain the most famous cases along with *Toxoplasma gondii*, the parasite responsible of the human toxoplasmosis disease, known to turn rats innate aversion to cat odor into attraction (Berdoy *et al.*, 2000; Vyas *et al.*, 2007). Since their discovery, numerous and diverse other parasites have been documented as modifying the phenotype of their hosts, in a way that is believed to

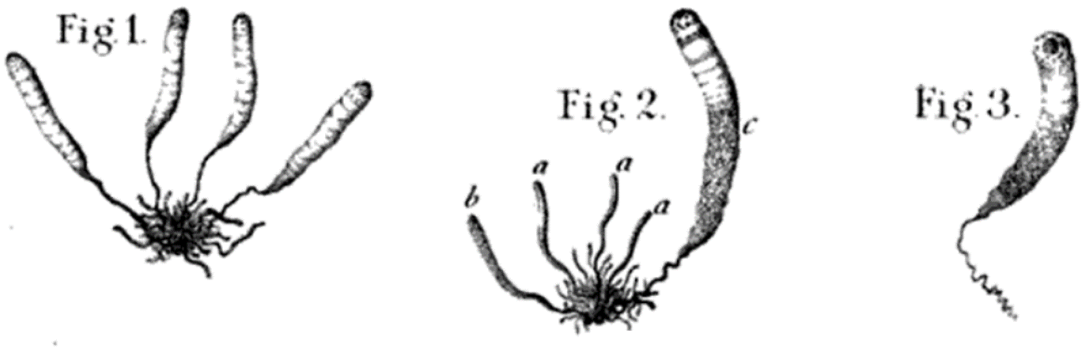

Figure 1. Illustration of the broodsacs of the trematode *Leucochloridium paradoxum* from one of the most ancient descriptions of the parasite, and the first known to suggest its manipulative ability (von Siebold, 1853).

increase their own probability of transmission, and thus reproduction (see Hughes et al., 2012; Moore, 2002b, 1995, for reviews).

Ever since, many articles depicting the way that some parasites might affect the behavior and the appearance of their hosts to their own benefit are loaded with clues highlighting the captivation raised by manipulative parasites, even among scientists. The vocabulary used in scientific publications testifies such fascination. Manipulative parasites are often referred as "puppeteer" or "puppet masters". The most famous cases of manipulation have their own nicknames: "lighthouse snails" for *L. paradoxum*, "zombie ants" for *D. dendriticum*, not to mention the "fatal attraction" or "morbid attraction" illustrating parasites that, just like *T. gondii*, change their hosts innate aversion for predators into attraction, or the "suicide-committing crickets" designing the deadly effect induced by nematomorph parasites on their insect hosts. The fame of manipulative parasites was also probably influenced by their major place in Dawkins' book, "*The Extended phenotype*" (1982), in which he developed the said "extended phenotype" concept. According to this concept, a gene not only has effects on the phenotype of individuals in which it belongs, but can also affect their environment, in particular, such as it is observed in manipulative parasites, the phenotype of other organisms.

Although the fascination raised by the subtle and morbid strategies of manipulative parasites has probably been at the origin of the scientific interest, it also had some perverse effects on the way that manipulative parasites were globally perceived. In her book that stands as a reference in the field of manipulative parasites, Janice Moore, a pioneer in this field, expressed her regrets that parasites, despite a growing interest from scientists, were still ignored in certain fields of biology, such as ecology, epidemiology or neurobiology (Moore, 2002a). She stressed that "such neglect is remarkable, for parasites alter the behavior of animals in ways that impinge upon most of the areas of interest to ecologists". Indeed, significant attention was devoted to the description of the diversity of host manipulation among parasites (Thomas *et al.*, 2011), and Moore complained that manipulative parasites "are often viewed as little more than cute tricks or one-of-a-kind novelties" (reported in McAuliffe, 2016).

Moore was one of the first scientists to realize that manipulative parasites, through the accomplishment of changing the phenotype of their hosts, could have deep ecological implications. Following the pioneer work made by Bethel and Holmes (e.g. Bethel and Holmes, 1977, 1974, 1973), she studied the effects of acanthocephalan parasites on their crustacean intermediate hosts (Moore, 1983, 1984). These parasites are known to induce several modifications in the behavior of their intermediate hosts, such as altered phototaxis and activity, ultimately leading to an increase of predation from their fish or bird definitive hosts (Moore, 2002a). Interviewed in 2012 (McAuliffe, 2016), Moore related that the discovery of organisms capable of taking their hosts from one habitat to another made her soon realize the ecological importance of parasites. In 1995, she wrote a review in which she chose, in an

emphasis, to highlight that “parasites can so greatly alter a host’s behavior that they change its ecological function” (Moore, 1995). In this very review, she also reached some important conclusions regarding ecological effects of manipulative parasites, such as alteration in animals’ distribution and abundance.

Parasites in general have long been kept outside the scope of ecology. For a long time, the study of parasites was restricted to parasitologists, mostly with medical considerations. In parallel, as stressed out by Moore in the introductory chapter of a recent book devoted to manipulative parasites (Hughes *et al.*, 2012), “ecology was mainly about birds and mammals, perhaps insects and plants, but certainly not about worms, much less things invisible without a microscope”. However, the ecological implications of parasites are now widely recognized, and their study is not anymore restricted to pure parasitologists, but is rather at the interface of several fields of science. Numerous articles pointed out that parasites can have wide consequences on their environment, and that environment can also affect them in many ways (e.g. Lefèvre et al., 2009; Loreau et al., 2004; Marcogliese, 2004). Scientific books have emerged covering the large topic of the interaction between parasites and their ecosystems (see for example Thomas et al., 2005a). Because of their peculiar habits, manipulative parasites also revealed to be particularly intricate in many components of their ecosystems, in particular within food webs (Lefèvre *et al.*, 2009), to the point that the ecological consequences of manipulative parasites stand as a major theme in one of the last books devoted to them (Hughes *et al.*, 2012).

Nowadays, understanding the interaction between the environment and systems of hosts and their manipulative parasites has become a major challenge. Indeed, there is an emerging awareness that apprehending the interaction between the environment and ecologically important species strongly requires to take into account their parasites. This is becoming particularly important in a context of global change. Indeed, the predictions of the consequences of environmental changes, such as rising temperatures or alteration in habitats, might completely depend on the consideration of manipulative parasites. On the other hand, understanding the impact of environment on manipulative parasites might also prove useful for a better understanding of the epidemiology of certain diseases. Finally, investigating the effect of environmental conditions might also provide new clues to understand the mechanisms, still not clearly identified, in which parasites can alter the phenotype of their hosts.

The purpose of my thesis is to better comprehend how environment might affect manipulative parasites and their impact on their hosts, using ecologically important species. Before introducing further the very subject of my thesis, the following pages stand as an introductory state-of-the-art, summarizing the ecological consequences of manipulative parasites, discussing about the potential impact of various environmental alterations linked to global change, and highlighting some problematics that need to be answered.

ARTICLE 1

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Host manipulation in the face of environmental changes: Ecological consequences

Sophie Labaude, Thierry Rigaud, Frank Cézilly

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Abstract

Several parasite species, particularly those having complex life-cycles, are known to induce phenotypic alterations in their hosts. Most often, such alterations appear to increase the fitness of the parasites at the expense of that of their hosts, a phenomenon known as "host manipulation". Host manipulation can have important consequences, ranging from host population dynamics to ecosystem engineering. So far, the importance of environmental changes for host manipulation has received little attention. However, because manipulative parasites are embedded in complex systems, with many interacting components, changes in the environment are likely to affect those systems in various ways. Here, after reviewing the ecological importance of manipulative parasites, we consider potential causes and consequences of changes in host manipulation by parasites driven by environmental modifications. We show that such consequences can extend to trophic networks and population dynamics within communities, and alter the ecological role of manipulative parasites such as their ecosystem engineering. We suggest that taking them into account could improve the accuracy of predictions regarding the effects of global change. We also propose several directions for future studies.

Highlights

- Environmental changes can affect ecosystems in various ways
- Manipulative parasites are known to play numerous roles within ecosystems
- However, the effects of environmental changes on manipulation has been overlooked
- We review those effects and their potential consequences on larger scales
- We conclude with suggestions on the direction of future studies

Keywords

Ecosystems, environment, global changes, host manipulation, host-parasite interactions

Introduction

Understanding the consequences of environmental changes has become a major challenge in recent years in many fields of science. Parasitology is among the most sensitive topics regarding the effects of global changes, since accurate predictions about the expansion of parasites and their hosts might be essential to take appropriate measures to prevent epidemic diseases. Moreover, an increasing number of reviews have highlighted the potential impact of climate change on parasitism (e.g. MacLeod and Poulin, 2012; Marcogliese, 2001; Morley and Lewis, 2014). As a result, the number of theoretical models providing simulations about the future geographical range of parasites and their vectors is increasing too. However, most predictive parasitological studies have been limited to vector-

borne diseases affecting either humans, livestock, or domestic animals (White *et al.*, 2003; Genchi *et al.*, 2009; Paaijmans *et al.*, 2010; Moore *et al.*, 2012; Mordecai *et al.*, 2013; Stensgaard *et al.*, 2013; Giles *et al.*, 2014; Sternberg & Thomas, 2014), with noticeable exceptions such as blood parasites in wild birds (Fuller *et al.*, 2012; Loiseau *et al.*, 2013).

Parasitic organisms altogether might represent close to half of all biodiversity (Poulin & Morand, 2000; Dobson *et al.*, 2008). Apart from causing diseases, there is increasing evidence that they can play pivotal roles in ecosystems (Thomas et al., 1997; Hatcher et al., 2012). In particular, many parasites are able to alter their hosts' phenotypes, with far-reaching consequences for, for instance, population dynamics or the persistence of species in ecosystems (Lefèvre *et al.*, 2009).

Parasites that are able to manipulate their hosts are very diverse, ranging from viruses (Ingwell *et al.*, 2012) and bacteria (Werren *et al.*, 2008) to many eukaryote organisms, including animals such as cestodes, trematodes, or acanthocephalans (Poulin & Thomas, 1999). The number of hosts susceptible to be manipulated by parasites is also wide, including both vertebrate and invertebrate species (Poulin & Thomas, 1999), and even plants (Ingwell *et al.*, 2012). Interestingly, the inventory of manipulative parasites also includes medically and veterinary important species that are already well studied (Hurd, 2003; Lagrue & Poulin, 2010), such as parasites causing malaria (Koella *et al.*, 1998), toxoplasmosis (Berdoy *et al.*, 2000), or rabies (Klein, 2003). However, even though the manipulative abilities of those parasites could have implications for epidemiology and pathology (Lagrue & Poulin, 2010), epidemiologic models tend to completely ignore them.

Similarly, despite the importance of host manipulation by parasites for ecosystems and health, the effects of environmental changes on their ecological roles are largely ignored. After emphasizing the ecological importance of manipulative parasites, we show here that environmental changes can interact with them in many different ways, leading to consequences that deserve more attention, especially in the area of conservation, in order to make accurate predictions regarding the effects of global change.

Ecological importance of host manipulation by parasites

Parasites are widely recognized to have numerous effects on communities and ecosystems, in particular through density-dependent pathogenic effects on their hosts (Hatcher *et al.*, 2012). For instance, differential host susceptibility and tolerance can reverse the outcome of competition, when the fitness of the superior competitor is more impaired by parasitic infection than that of other host species. The presence of parasites might then lead to the coexistence of several species that would otherwise exclude each other. Moreover, parasites influence the organization of communities and,

through that, play such an important role in the stability of ecosystems that they have been proposed to serve as a proxy of their quality (Hudson *et al.*, 2006). On the other hand, parasites can also have negative effects on biodiversity, such as causing local extinctions (McCallum & Dobson, 1995).

An important aspect is that all parasites are embedded in large food webs. In particular, parasites with complex life-cycles have the potential to impact several host species in succession, making their global impact (see below) even more consequent. Some of those parasites are able to induce phenotypic modifications in their intermediate hosts, which are believed to be more than simple pathological effects. Through host manipulation, parasites are thought to enhance their own fitness, in particular by increasing their probability of transmission from one host to another, at the expense of that of their hosts (Thomas *et al.*, 2005b). Many theoretical as well as empirical studies have highlighted that this phenomenon, along with more classic pathogenic effects, can have profound ecological impacts on a large scale, ranging from host populations to ecosystems (Lefèvre *et al.*, 2009). Although manipulative parasites can affect ecosystems in diverse ways, three major effects can be distinguished: the impact of parasites on food webs, their influence on the population dynamics of host species, and their impact on habitats.

Impact on food webs

Trophically-transmitted parasites often manipulate their intermediate hosts in ways that increase their probability of being predated by definitive hosts. For instance, killifish (*Fundulus parvipinnis*) parasitized by the trematode *Euhaplorchis californiensis* are up to 31 times more susceptible to predation than uninfected individuals (Lafferty & Morris, 1996). The effect on the energy flow is even more substantial considering that the increased vulnerability to predation induced by parasites is often not restricted to suitable hosts (Kaldonski *et al.*, 2008; Seppälä *et al.*, 2008a), leading to a higher predation by other species, as illustrated by cockles (*Austrovenus stutchburyi*) being exploited as intermediate hosts by trematode parasites. Infected cockles typically remain lying on the sediment surface (Thomas & Poulin, 1998), where they are more conspicuous to birds that serve as a definitive host for trematodes. However doing so, infected cockles also become more vulnerable to predation by fish which constitute 'dead-end' predators for parasites (Mouritsen & Poulin, 2003).

Manipulative parasites can also create new trophic interactions. One of the most spectacular examples comes from nematomorph parasites (*Gordionus spp.*), which induce their terrestrial insect hosts into jumping in the water (a crucial stage in the life cycle of the parasite; Sato et al., 2011). Empirical evidence shows that manipulated insects represent a new and substantial energy intake for fish (Sato *et al.*, 2011), with the interesting consequence of decreasing fish predation on benthic

invertebrate communities, thus leading to subsequent decrease in algae biomass, and, ultimately, to a reorganization of the whole ecosystem (Sato *et al.*, 2012).

Another impact of parasites on food webs, though not necessarily restricted to manipulative ones, lies in the alteration of the functional role of their hosts. For instance, several acanthocephalan parasites are known to alter the feeding ecology of their intermediate hosts, decreasing predation rate in amphipods (Fielding *et al.*, 2003) or reducing the consumption of detritus in isopods (Hernandez & Sukhdeo, 2008). Such alterations can have substantial effects within ecosystems, especially when modified host species play important functional roles (Hernandez & Sukhdeo, 2008).

Impact on population dynamics

Host modifications induced by manipulative parasites are likely to alter hosts population dynamics and structure. For instance, the trematode *Gynaecotyla adunca* alters the vertical distribution of its snail host on sandbars (Curtis, 1987). Several gammarid species infected by acanthocephalan parasites present altered geotactic or phototactic preferences (Bauer *et al.*, 2000, 2005; Haine *et al.*, 2005), supposed to drive them to areas where they are more exposed to predators. By altering both the behavior and morphology of their hosts, parasites can then lead them to occupy new ecological niches (Ponton *et al.*, 2005; Miura *et al.*, 2006). Along with effects on individual distribution, other phenotypic alterations induced by manipulative parasites are likely to induce ecological segregation, through dividing the host population into two sub-units consisting of infected vs. uninfected individuals, each of them having its own properties (Lefèvre *et al.*, 2009).

Manipulative parasites are also likely to modify predator-prey dynamics. Evidence from mathematical modelling (Fenton and Rands 2006) suggests that manipulation can influence both predators' and prey's abundance, and induce oscillations in their population densities that are likely to have consequences on the dynamics of other species within the ecosystem. Accordingly, Lafferty and Kuris (2012) suggested that the parasite *Echinococcus granulosus* might be responsible for the persistence of moose and wolves on Isle Royale. Indeed, recordings suggest that infection with *E. granulosus* increases moose vulnerability to wolves (Joly & Messier, 2004). As suggested by another mathematical model (Hadeler & Freedman, 1989), the parasite might be essential for wolves to be able to feed on moose, and to persist in the ecosystem. The presence of the parasite and its interaction with moose and wolves might actually prevent the demographic explosion of moose populations, which would lead to over-grazing followed by starvation, as was observed before colonization by wolves (Lafferty & Kuris, 2012).

Similarly, manipulative parasites can drive competition between hosts. In the same way that non-manipulative parasites can affect closely-related host species with different susceptibility and tolerance to infection, host species can also present different susceptibility to manipulation. Hatcher et al. (2014) used a mathematical model to show that parasite manipulation can change the outcome of the competition between two hosts showing mutual predation, and determine whether the two host species can coexist or not. In addition, some studies have shown that parasites do not always manipulate closely-related host species to the same extent (Thomas *et al.*, 1995; Bauer *et al.*, 2000). For instance, amphipods *Gammarus pulex* infected by the acanthocephalan *Pomphorhynchus laevis* show reversed phototaxis, while that of infected *G. roeseli* remains unaltered (Bauer *et al.*, 2000).

Impact on habitats

By modifying the phenotypes of their hosts, manipulative parasites may create new habitats for other species, or change habitats' parameters, endorsing the role of ecosystem engineers (Thomas *et al.*, 1999). When infected by the parasite *Sacculina carcini*, the green crab, *Carcinus maenas*, stops molting (O'Brien & van Wyk, 1985). Its cuticle then becomes a permanent substrate on which an epibiont community can develop (Thomas *et al.*, 1999; Mouritsen & Jensen, 2006). Another illustration comes from cockles (see above) infected by trematode parasites. Parasitized individuals, which are unable to burrow in the sand, also become a substrate with new properties for epibionts to colonize. Thomas et al. (1998) showed that the presence of parasites can then facilitate the coexistence of two epibionts, anemones and limpets, by providing the limpets with a new substrate unsuitable for anemones due to their vulnerability to desiccation, thus preventing them from predating upon limpets. Moreover, Mouritsen and Poulin (2005) put forward that biodiversity is higher on mudflats when those parasites are present, an observation that could be explained by the cockles' impaired bioturbation potential.

How environmental changes can alter the roles of manipulative parasites

Parasite manipulation results from complex interactions between properties of parasites, properties of their hosts, and many biotic and abiotic environmental factors (Fig. 2). It appears therefore very plausible that any environmental change might affect not only manipulation itself, but also its consequences. Considering the effects of parasite manipulation on a large scale, those consequences might in turn induce new environmental changes or modify their intensity, thus altering the role of parasites within ecosystems. To emphasize the complexity behind all the interacting components of systems involving parasite manipulation, illustrated in the Figure 2, we provide here a few examples

about the outcome of the interaction between manipulative parasites and several environmental modifications of major concern.

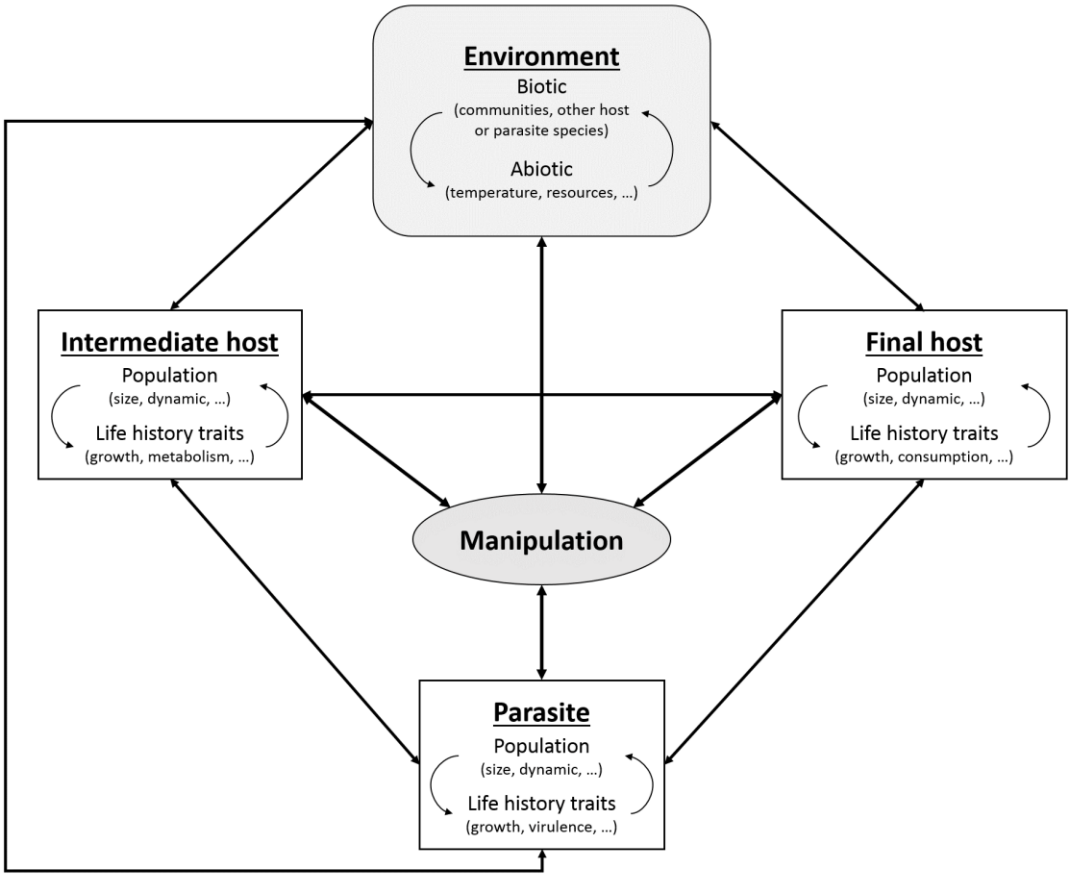

Figure 2. Schematic representation of all the interacting factors in a system involving parasite manipulation. The intensity of host manipulation induced by parasites is likely to be influenced by a variety of parameters concerning the parasites, their hosts and environmental properties. In return, manipulation can also have an impact on those parameters. Moreover, all components in the systems also interact with each other.

Climate change

Temperature is one of the most important abiotic factors affecting parasites' biology (see Morley and Lewis, 2014; Morley, 2011; Thomas and Blanford, 2003 and references therein). When focusing on parasite manipulation, it is important to take into account that modifications induced by environmental factors on the ways parasites alter their hosts are likely to be indirect. Indeed, the intensity of parasitic manipulation is dependent on many parameters intrinsic to the physiology, morphology or population dynamic of both hosts and parasites (reviewed in table 1). Any environmental factor affecting those parameters is then susceptible to also have effects on the extent of host modifications induced by parasites. Acanthocephalan parasites and their amphipod intermediate hosts constitute one of the most studied host-parasite systems in the world of parasite

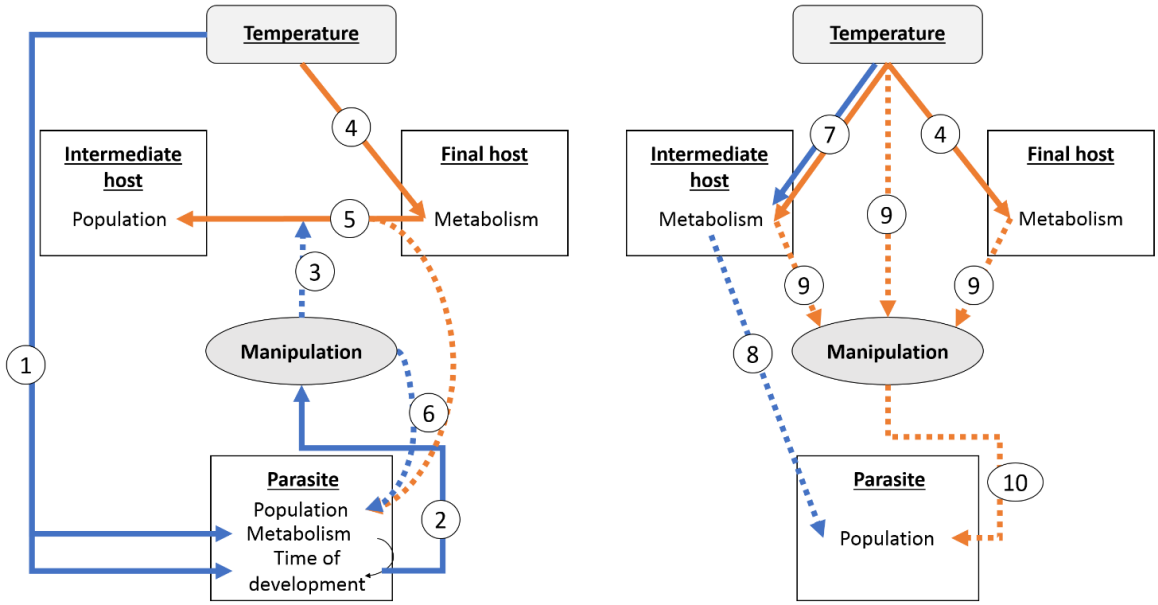

Figure 3. Examples of the impacts of temperature on a system of gammarid species infected by acanthocephalan parasites. Final host varies depending on parasite species (either a fish or a bird). Solid lines represent assumption supported by studies, while dotted lines are expectations that remain to be investigated. In this system, (1) the temperature widely influences the time of development or parasites within the intermediate hosts, which is likely to be driven by the metabolic rate of parasites (Tokeson & Holmes, 1982). Several studies suggested that (2) the time of development of parasites is linked to the intensity of their manipulation (Franceschi *et al.*, 2008, 2010a), which in turn might (3) influence the increase of predation rate between the final host and the intermediate host. (4) Temperature is also likely to influence the final host metabolism (Byström *et al.*, 2006), (5) influencing its predation rate (Byström *et al.*, 2006). Altogether, (6) modifications in manipulation and predation rates are likely to induce changes in parasites' population. Meanwhile, (7) temperature also affects the metabolism of gammarid hosts (Issartel *et al.*, 2005), inducing changes in their food consumption (Pellan *et al.*, 2015). (8) Given that infection depends on food consumption, the risk of infection might vary accordingly, affecting parasites' population. Although its direct effect has not been investigated yet, (9) temperature is also likely to alter the intensity of manipulation, for instance through its effect on hosts' metabolism and activity, and therefore (10) secondarily impact parasite population dynamic.

manipulation (Cézilly *et al.*, 2013). Various studies have shown that several traits in both hosts and parasites can be important to explain variation observed in the intensity of manipulation at the intraspecific level (Table 1). Interestingly, many of those traits appear to be sensitive to temperature, as well as to other environmental factors (Fig. 3). For instance, several studies suggest that the time taken by one parasite to develop in its intermediate host could subsequently affect the intensity of behavioral alterations in that host (Franceschi *et al.*, 2008, 2010a). As for many other parasites, the development time of acanthocephalans is largely influenced by temperature (Tokeson & Holmes, 1982), which thus can indirectly drive the intensity or timing of manipulation.

Table 1. Parameters affecting the intensity of parasite manipulation

Parameter	Host	Parasite	Trait modified	Reference
Parameters intrinsic to the parasite				
Age/stage of the parasite	Amphipod	Acanthocephalan	Phototaxis	Franceschi et al., 2010a, 2008
	Amphipod	Acanthocephalan	Refuge use	Dianne et al., 2011
	Isopod	Acanthocephalan	Mating behavior	Sparkes et al., 2006
	Insect	Protozoan	Host-seeking	Koella et al., 2002
	Insect	Nematomorph	Jumping into water	Sanchez et al., 2008
	Rodent	Nematode	Activity	Dolinsky et al., 1985
	Fish	Trematode	Aggressiveness	Mikheev et al., 2010
Parasite sibship	Amphipod	Acanthocephalan	Phototaxis	Franceschi et al., 2010a
Parasite population	Amphipod	Acanthocephalan	Phototaxis	Franceschi et al., 2010b; Labaude et al., 2015b
Genetic strain	Amphipod	Acanthocephalan	Phototaxis	Perrot-Minnot, 2004
Parasite sex	Isopod	Acanthocephalan	Coloration	Benesh et al., 2009b
Parasite size	Amphipod	Acanthocephalan	Phototaxis	Dianne et al., 2012
	Fish	Cestode	Demelanization	Ness and Foster, 1999
Parameters intrinsic to the host				
Host size	Isopod	Acanthocephalan	Coloration	Benesh et al., 2009b
Host weight	Amphipod	Acanthocephalan	Activity	Dianne et al., 2014
Host age	Fish	Trematode	Motionless	Poulin, 1993
Parameters relative to the infection				
Parasites total volume	Isopod	Acanthocephalan	Coloration	Benesh et al., 2009b
Parasite load	Amphipod	Acanthocephalan	Phototaxis	Franceschi et al., 2008
	Fish	Trematode	Motionless	Poulin, 1993
	Mollusca	Trematode	Burrowing ability	Mouritsen and Poulin, 2003
Multi-infection with different stages	Amphipod	Acanthocephalan	Phototaxis	Dianne et al., 2010
	Copepod	Cestode	Activity	Hafer and Milinski, 2015
Multi-infection with different parasite species	Amphipod	Acanthocephalan, microsporidia	Geotaxis	Haine et al., 2005
	Amphipod	Acanthocephalan	Vertical distribution	Cézilly et al., 2000
	Mollusca	Trematodes	Distribution	Miura and Chiba, 2007
	Mollusca	Trematodes	Shell size	Miura and Chiba, 2007

Climate-mediated physiological stress can have substantial effects on host immunity, thus increasing host susceptibility to infection (Cheng *et al.*, 2005; Dittmar *et al.*, 2013). Beyond an increase

in the number of infected hosts, the intensity of manipulation may also depend upon host immuno-competence (Adamo, 2002). Therefore, climate-mediated stress may lead to widely infected and manipulated populations. On the other hand, some manipulative parasites have been shown to suppress the immune response of their hosts (Cornet *et al.*, 2009a), a phenomenon that could increase host susceptibility to manipulation, but also to infection by other parasites (Cornet & Sorci, 2010). The cumulative effects of both parasite immune-suppression and climate-mediated stress have not been investigated yet, but the combination of the two phenomena may ultimately increase host mortality, with potential consequences for both host and parasite population dynamics.

Several manipulative parasites also present seasonal variations, not only in their prevalence, but also in the intensity of their manipulation. For instance, some acanthocephalan parasites induce a stronger change in refuge use by their isopod hosts during spring, compared to summer or fall (Benesh *et al.*, 2009a). Benesh et al. (2009a) suggested that seasonal variations in isopod behavioral alterations could result from a manipulation strategy adjusted to seasonal variation in the diet of definitive hosts. Regardless of whether seasonal modifications in manipulation are adaptive or not, temperature changes are very likely to alter such seasonality through their influence on both host and parasite ecology. For instance, a spatial overlap between intermediate and definitive hosts might appear only during a short period of time (Marcogliese, 2001). Under such circumstances, one would expect parasite's manipulative efforts to have been tuned by natural selection to coincide with this period, in order to maximize transmission. However, rapid changes in temperatures leading to modifications in the spatial distribution of both hosts and parasites may eventually result in the peak of manipulative efforts occurring at the wrong time.

Direct effects of temperature on host manipulation are poorly known. Considering that the behavior of uninfected individuals can be dependent upon temperature, and knowing that temperature affects both host and parasite metabolism (see for example Le Lann et al., 2014, where the behavior and physiology of both aphid hosts and their parasitoids are altered by temperature in different degrees), there is every reason to believe that temperature could affect the intensity of host modifications induced by parasites. In addition, parasite manipulation can directly involve behaviors related to temperature. For instance, Macnab and Barber (2012) showed that plerocercoid parasites induce a preference for warmer temperatures in their fish host, a result also found in snails infected by a trematode parasite (Bates *et al.*, 2011). As it is the case for many other altered host traits, such an attraction can lead to a spatial segregation between infected and uninfected individuals. However, as the ambient temperature reaches the temperature preferred by infected individuals, this dichotomy would disappear, along with its potential environmental effects.

In oceans, the rise of CO_2 not only induces an increase in temperature, but is also accompanied by a decrease of pH, a phenomenon known as ocean acidification (Feely et al., 2004). Ocean acidification induces deep biological negative consequences, such as decreased calcification rates in phytoplankton, corals and mollusks (Feely et al., 2004), but also alterations in metabolism, growth or survival in various invertebrate larvae (e.g. Bechmann et al., 2011). By analogy, similar negative effects have been suspected in parasites, particularly those with free-stage larvae (MacLeod & Poulin, 2012), and a recent study showed that exposure to experimentally acidified water reduces survival and longevity in cercariae and metacercariae of four species of marine trematodes (MacLeod & Poulin, 2015). However, another study showed that the immune response of the mussel, *Mytilus edulis*, was more affected by modifications in temperature than in pH, although both a high temperature and a decrease in pH changed the abundance and diversity of pathogens (Mackenzie *et al.*, 2014). Indirect effects of ocean acidification on parasite manipulation can be expected, through such negative effects on hosts and parasites (Figure 2), and could, like other stressors, destabilize trophic interactions (MacLeod & Poulin, 2012). However, the direct effect of ocean acidification on manipulation is unknown, and remains to be investigated.

Changes in community composition: biological invasions

The introduction of non-native species in new areas is often associated with the globalization of human transportation around the world, but also with alterations in habitat parameters, that make them suitable for non-native species. Biological invasions represent a major cause of biodiversity loss, and often induce profound changes in native communities' structure, leading to new environmental modifications (Molnar *et al.*, 2008). The invasion success of an exotic species in a new area relies on many factors, including properties of the new ecosystem as well as properties of the invading species. There is increasing evidence that parasites may play an important role in the successful establishment of invasive species (Dunn *et al.*, 2012). Interestingly, manipulative parasites have received much attention from scientists in relation to biological invasions.

There are many ways in which manipulative parasites can influence invasion success. First, following the "enemy release hypothesis", species might escape their parasites when invading a new area (Torchin *et al.*, 2002; Torchin & Mitchell, 2004). This phenomenon might, among other reasons, result from the fact that the invasion process is initiated by a small number of individuals, thus reducing the probability that they bring with them the whole community of parasite species from their native range. Moreover, manipulative parasites often present complex life-cycles, and are thus sensitive to the absence of any obligatory host in the new ecosystem. Torchin et al. (2005) found that while a native mud snail was infected by ten native trematode parasites, an introduced sympatric mud snail only

harbored one introduced trematode. This “enemy release” directly leads to “parasite manipulation release”, which is likely to have consequences. For instance, the predation facilitation induced by some parasites is supposed to negatively impact the population dynamics of their hosts. Conversely, an absence of parasites might then lead to an explosion of the host population (as suggested above in the case of moose and wolves).

Parasites can also have indirect effects by affecting the competitive interactions between native and invasive closely-related host species, through differential effects on each host species (Hudson and Greenman, 1998; see above). Mediated competition is often highlighted in the case of parasites causing a higher mortality due to pathogenic effects in one of the competitive host species (Dunn *et al.*, 2012). Apart from pathogenic effects, host mortality can also be driven by the consequences of manipulation, especially when parasites alter the behavior of their intermediate hosts in ways that increase their probability of being predated by definitive hosts. In many French rivers, the native amphipod *G. pulex* has to face competition from its closely-related invader, *G. roeseli* (Karaman & Pinkster, 1977a). Although both species can be infected by the acanthocephalan *P. laevis*, only the native species shows a reversed phototactic behavior when infected (Bauer *et al.*, 2000). The same result has been found in the Irish native amphipod *G. duebeni celticus*, whose phototaxis is altered by the acanthocephalan *Polymorphus minutus*, while that of its invasive rival *G. tigrinus* is not (MacNeil *et al.*, 2003a). In both cases, only the native species has to face an increase in predation by fish when infected, which is likely to facilitate the invasion by the congeneric rival species (Lagrue *et al.*, 2007). However, other altered behaviors may influence the competition between native and exotic rivals. For instance, the Irish amphipod *G. d. celticus* is being replaced by the introduced *G. pulex*, which induces numerous changes in freshwater macroinvertebrate communities (Kelly *et al.*, 2006). Dick et al. (2010) reported that *G. pulex* harboring the acanthocephalan *Echinorhynchus truttae* have a higher predatory rate, consuming significantly more preys than uninfected individuals. Together with a higher parasitic prevalence compared to the native species (Dick *et al.*, 2010), this functional response could give a competitive advantage to the invasive species. Conversely, Sargent et al. (2014) found that parasites *Microphallus spp.* reduce the foraging behavior of the invasive crayfish species *Orconectes rusticus*, potentially affecting its invasion success.

Competition between native and exotic species can be more direct, particularly when predation occurs between them. Manipulative parasites have the potential to drive the outcome of such a competition, as has been shown by Hatcher et al. (2014) (see above). The replacement of Irish *G. d. celticus* amphipods by *G. pulex* (see above) can be partly explained by mutual predation biased in favor of the invader. However, infection with the acanthocephalan *E. truttae* reduces the predatory impact of the exotic species, thus potentially slowing down the invasion process (MacNeil *et al.*,

2003b). This example also highlights the complexity of the impact of parasites: being infected can be both a disadvantage (lowered ability to predate upon the competitor species) and an advantage for the invasive species (modification of the functional response, see above). In the field, the impact of parasites on the competitive abilities of their hosts can be deduced from spatial variation in co-occurrence. For instance, the amphipod *Crangonyx pseudogracilis* co-occurs with *G. pulex* more frequently when the latter is parasitized by *P. minutus*, a phenomenon that can be explained by a reduced predation rate on *C. pseudogracilis* by parasitized *G. pulex* (MacNeil & Dick, 2011).

Another aspect of biological invasions concerns the introduction of new parasites within an ecosystem. In particular, invasive species can bring new parasites with them, which are also likely to interfere with the invasion process. Bacela-Spychalska et al. (2014) reported that the microsporidian *Cucumispora dikerogammari*, which dispersed together with its invasive host *Dikerogammarus villosus*, is likely to decrease its host's predatory pressure on communities through altered behavior. Moreover, the arrival of new parasite species, which might be able to affect both invasive and local host species, may increase the size of the infra-community of parasites. Many hosts would then harbor several parasites with different interests in terms of transmission, either because they target different species as final hosts or because they differ in developmental stage, and, hence, infectivity to final hosts. One of the consequences of such multi-infections, apart from increased immunological and energetic costs for the host, would be a modification of the parasite-induced alterations following a competition for manipulation inside the host ("sabotage" hypothesis, Hafer and Milinski, 2015), and thus a modification of the effects of manipulation on population dynamics (see table 1 for examples).

Finally, even though most of the studies concerning the impact of parasites in invasions focused on the effects on invasive and native host species, it is important to keep in mind that many non-host species interact with them. Consequences might first emerge at the scale of the whole ecosystem if invasive species or their native competitors are key species, as is the case of many gammarid species (Kelly *et al.*, 2002). In addition, in the case of invasions driven by parasites through their effects on predation facilitation, other predator species might benefit from the arrival of invasive hosts, as a new source of food. As illustrated by the case of nematomorph parasites (see above), the introduction of new food resources in food webs can have large consequences on many parameters of an ecosystem.

Pollution

Human activities are responsible for the release of more and more pollutants in the environment, especially in freshwater ecosystems (Loos *et al.*, 2009). Toxic chemicals could influence parasite

manipulation in various ways, although the interaction between pollution and parasite manipulation itself has received very little attention from scientists (Thomas *et al.*, 2011). As discussed earlier with the effects of climate, pollution can, in the same way, impact host or parasite traits, which could in turn have consequences on the extent of manipulation. Moreover, pollutants often constitute a stress for hosts, impacting their immuno-competence (Lafferty & Kuris, 1999). Thus, one direct consequence would be a higher prevalence of parasites due to an increase in hosts susceptibility to infection (Khan, 1990). In addition, many studies showed that infection by parasites increases hosts susceptibility to pollutants in terms of mortality (Brown & Pascoe, 1989; Gismondi *et al.*, 2012a, 2012b; Khalil *et al.*, 2014).

Chemical substances can also directly interfere with behavioral changes induced by manipulative parasites. Although the mechanisms through which parasites manipulate their hosts are not yet fully understood, the potential role of neuromodulators has been pointed out in several cases (Adamo, 2002; Perrot-Minnot & Cézilly, 2013). It is then very likely that certain pollutants, especially pharmaceuticals, might interfere with those mechanisms. For instance, gammarids infected by manipulative fish acanthocephalans present an increase in brain serotonin immunoreactivity (Tain *et al.*, 2006). In addition, the experimental injection of serotonin in uninfected gammarids led to several behavioral alterations that are quite similar to those induced by acanthocephalan fish parasites (Tain *et al.*, 2006; Perrot-Minnot *et al.*, 2014). Interestingly, fluoxetine, a reuptake inhibitor of serotonin that is widely prescribed as an anti-depressant, can be found in many natural streams (Kolpin *et al.*, 2002). Guler and Ford (2010) found that exposure to both serotonin and fluoxetine altered phototaxis and geotaxis in marine amphipods, two traits often modified by acanthocephalan parasites. In addition, De Lange et al. (2006) showed that even low concentrations of fluoxetine could affect the activity of freshwater amphipods. Although, to our knowledge, the combined effects of manipulative parasites and drug releases have not been investigated, it is very likely that either the intensity of manipulation (due to cumulative effects) or its outcome in terms of increased susceptibility to predation (due to a homogenization of both infected and uninfected hosts behavior), might be altered.

Behavioral alterations induced by parasites rely on hosts' sensory and locomotor systems, which can also be altered by chemical compounds. For instance, host ability to detect chemical cues signaling the presence of a predator and to respond to them can be disrupted by some manipulative parasites. While rats normally display a natural aversion for cat odor, individuals infected by *Toxoplasma gondii* show no aversion, and sometimes attraction, to odors of certain cats (Berdoy *et al.*, 2000; Kaushik *et al.*, 2014). Amphipods *G. pulex* infected by the acanthocephalan *P. laevis* are also attracted to predator odor (Baldauf et al., 2007; Kaldonski et al., 2007; Perrot-Minnot et al., 2007). Pollutants are very diverse and can have many negative effects, including disruption of hosts' sensory

systems, such as chemoreceptive performances (Tierney & Atema, 1986; Blaxter & Hallers-Tjabbes, 1992), and might then interfere with manipulation based on the detection and reaction to chemical cues coming from predators. Moreover, those disruptions are likely to have consequences on the physiology and behavior of both uninfected and infected individuals (Scott & Sloman, 2004; Zala & Penn, 2004). Once again, the interaction between those effects and the alterations induced by manipulative parasites remain to be investigated.

Despite the lack of studies about the effects of pollutants, parasites have received substantial attention from scientists in relation to their ability to accumulate heavy metals such as cadmium and lead. Although the phenomenon is not restricted to manipulative parasites, it has been particularly well documented in adult acanthocephalans (Sures *et al.*, 1999) infecting diverse vertebrate hosts, such as rats (Scheef *et al.*, 2000) or fish (Sures & Taraschewski, 1995). In such host species, harboring parasites might be an advantage in polluted environments, because of the ability of parasites to detoxify host tissues (Thomas *et al.*, 2000a). Larval acanthocephalan parasites, on the other hand, can affect the antitoxic response of their intermediate hosts to heavy metals (Gismondi *et al.*, 2012a), often inducing a higher mortality (Brown & Pascoe, 1989). However, this pattern may actually depend on the sex of the host. Indeed, Gismondi et al. (2012b) found that, unlike females, infected male gammarids had both lower cadmium concentrations, and lower mortality compared to uninfected males. In this case, being infected might be, overall, beneficial, despite the increased probability of being predated.

Habitat and resources modifications

Environmental modifications can lead to other types of habitat alterations that are also likely to alter the interaction between hosts and their manipulative parasites. Importantly, habitat alterations might induce changes in the geographical distribution of species, including parasites' hosts and vectors (reviewed in Lafferty and Kuris, 1999).

Apart from effects on hosts' communities, the configuration of hosts' habitats, especially in rivers, can directly impact parasite manipulation or its outcome. For instance, *G. pulex* individuals manipulated by the acanthocephalan *P. laevis* were found to be significantly more predated than uninfected individuals only when refuges were available (Kaldonski *et al.*, 2007). One of the consequences of environmental changes could be a modification in the availability of refuges, notably due to modifications of water levels due to global warming. A decrease in refuge availability is then likely to make manipulation of gammarids ineffective. The alteration of phototaxis in amphipods infected with an acanthocephalan has also been shown to depend on light properties (Benesh *et al.*,

2005; Perrot-Minnot *et al.*, 2012). Considering that phototaxis is one of the most strongly altered behaviors in infected gammarids (Perrot-Minnot *et al.*, 2014), we can expect the light regime in the environment to play a role in the outcome of manipulation. In particular, eutrophication of freshwater bodies induces modifications of light penetration into the water (Van Duin *et al.*, 2001). The same phenomenon is also likely to alter underwater vision, and, hence, reduce the predatory success of final hosts. Thus, if parasite manipulation relies on visual cues to increase the susceptibility of infected hosts to predation, its efficiency might be altered following perturbations of the light regime (but see Perrot-Minnot et al., 2012).

Eutrophication, as well as modifications in any food resources, are also likely to alter host and parasite communities (see Marcogliese, 2001). Host life history traits, such as size or immune capacities, also depend on their diet. On the other hand, host resources are essential for parasites to develop, and many studies found that fewer parasites would develop if their hosts are starving (Pulkkinen & Ebert, 2004; Logan *et al.*, 2005; Seppälä *et al.*, 2008b). In contrary, an increase in host resources might reduce the competition between parasites within hosts, and allow the co-existence of multiple parasites (Dianne et al., 2012; Labaude et al., 2015b), leading to modifications in manipulation intensity (see table 1). Substantial host resources might also lead to the development of larger parasites, and Dianne et al. (2012) highlighted that larger larval acanthocephalans induce deeper modifications in phototactic preferences of their gammarid hosts. The distribution of hosts' resources can also influence the trophic transmission of parasites. For instance, Luong et al. (2014) found that the availability of alternative food resources for final hosts decreased their infection by trophically-transmitted parasites, as a consequence of reduced predation upon intermediate hosts. In this case, manipulation might, once again, become ineffective. Although their direct effects on manipulation remain to be studied, resources might thus play a role in the interaction between manipulative parasites and their hosts.

Conclusions and future directions

The examples provided here highlight the importance of the interaction between environmental changes and manipulative parasites. However, most of the studies cited here considered this interaction in single specific contexts. Although simplifications are essential to disentangle the roles of each component, it has to be kept in mind that many of the factors discussed above might occur simultaneously. For instance, ecosystems often face several anthropic disturbances in concert, while only few studies considered such combined effects (e.g. Alonso et al., 2010). On the other hand, a single factor is also likely to affect several protagonists of ecosystems. For instance, we highlighted

earlier that fluoxetine might increase predation on exposed prey, by inducing behavioral modifications that are close to those induced by manipulative parasites. However, this increased predatory rate might be balanced by impaired predation success in fish predators exposed to fluoxetine (Gaworecki & Klaine, 2008). We suggest that future studies should adopt a more integrative approach, taking into account multiple components of the systems as well as their interactions. For this, long term studies and field studies might be appropriate tools to bring a better understanding of the complexity underlying the role of manipulative parasites in a changing world. For example, as proposed earlier in this review, we suggest to investigate the combined effects of both parasite-mediated and climate-mediated stresses on the immune system, in order to understand effects on parasite manipulation, and investigate the combined effects of manipulative parasites and contaminant releases on the host's susceptibility to predation. We also propose to explore the effect of global change on several components of systems involving manipulative parasites. For instance, although testing the effect of an increase of temperature on host manipulation is needed, its consequences cannot be understood without also testing the effect of temperature on transmission success, since both the intermediate (manipulated) and the final hosts (predator of the intermediate host) will experience the increase in temperature.

Most of the environmental changes considered here are quite recent, such that adaptive modifications might not be visible yet, leading to a higher consideration from scientists for direct ecological consequences rather than evolutionary ones. However, the intensity or the timing of manipulation are likely to evolve in response to global change. For instance, hosts might suffer from a higher mortality induced by many stressors, such as higher temperatures and pollution. Thomas et al. (2002a) suggested that parasites might benefit from adjusting their exploitation strategy depending on the probability of near death of their host. If expected life-span is reduced for every individual host, we might expect an overall better success for parasites which are able to manipulate their hosts sooner and in more efficient ways, allowing a higher probability of transmission to the next host before the death of their intermediate host. Similarly, Lebarbenchon et al. (2008) suggested that parasite strains with different levels of virulence might be selected when environmental conditions affect the survival of infective stages. In the case of manipulative parasites, higher manipulative efforts might be expected as a compensation for the loss of infective stages in those environments. However, the adaptation of manipulative parasites to rapid environmental changes is questionable, as it relies on parameters which have been poorly studied. For example, only a few studies are available on both host and parasite genetic variation (review in Thomas et al., 2011), the raw material for evolutionary adaptation. Therefore, investigations on genetic variation and reaction norms among contrasted

environments are necessary to know if responses of manipulative parasites to environmental changes (i) are possible and (ii) result from selection or phenotypic plasticity.

On the other hand, we might expect manipulation to decrease in response to other environmental disturbances. As discussed earlier, harboring parasites accumulating heavy metals could be advantageous for their definitive hosts in a polluted environment, due to the parasites ability to detoxify the host. Those predator hosts might then benefit from feeding specifically on infected preys, whether manipulated or not. Therefore, it could be worth investigating the consequences of benefits associated with detoxification on the manipulation phenomenon to answer the following questions: Are predation behaviors of definitive hosts different between polluted and clean environments? Could manipulation be counter-selected in polluted environments, provided that contamination show some stability in time?

Finally, manipulative parasites deserve more attention in applied sciences. Despite their numerous roles, epidemiologic models keep ignoring their impact on the spread of infectious diseases. In the field of conservation biology, they are also largely overlooked. However, their impact on the success of biological invasions proves that introduced species should be considered along with their parasites in order to make accurate predictions on their probability of establishment success. Thus, apart from invasion problematics, manipulative parasites are also likely to drive the success of reintroductions, for example. In the case of population reinforcement with individuals coming from different geographic locations, the question would arise whether or not those individuals should be relocated with their own parasites, and whether local manipulative parasites are likely to alter those individuals in a similar ways, thus not disturbing the role of reintroduced animals in the ecosystem. Manipulative parasites, although they could be a burden in conservation biology, are also likely to become helpful tools. In a recent paper, Tompkins and Veltman (2015) showed that *T. gondii* could be used to improve vertebrate pest control. This parasite induces several behavioral modifications in its rat host, among which a decreased neophobia and an increased activity (Webster, 1994; Webster *et al.*, 1994). Rats constitute a highly invasive species in New Zealand, and a substantial threat for indigenous species. Trapping is often used to control rats' populations, but the natural neophobia of rats renders them hard to capture. Tompkins and Veltman (2015) reported that infection by *T. gondii* widely increases the trapability of rats, and that infection would reduce the trapping efforts required to maintain rat population under a threshold for conservation benefit. We follow them in considering that other manipulative parasite species might be of interest for ecosystems and population management.

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Chapter II. Material and methods – Generalities

1. Biological models

1.1. Acanthocephalan parasites: a relevant model to study parasite manipulation

As early as the end of the 17th century, one of the most famous scientists of its time, Antonie van Leeuwenhoek (1693), drew the picture of the spiny proboscis of a worm found in the intestine of an eel (Fig. 4). This illustration stands as the first known picture of an Acanthocephala (Crompton & Nickol, 1985), and also highlights one of the most remarkable morphological characteristics of this group, at the origin of their common names as “thorny-headed worms” or “spiny-headed worms”. The unique invaginable and retractile proboscis covered with recurved hooks, used by adults to attach to the definitive host’s intestinal wall, along with other morphological characteristics, made them particularly difficult to classify among other taxa, to the point where Acanthocephala were considered for a long time as a whole independent phylum.

Figure 4. Illustration from the 17th century of the proboscis of an acanthocephalan parasite (van Leeuwenhoek, 1693).

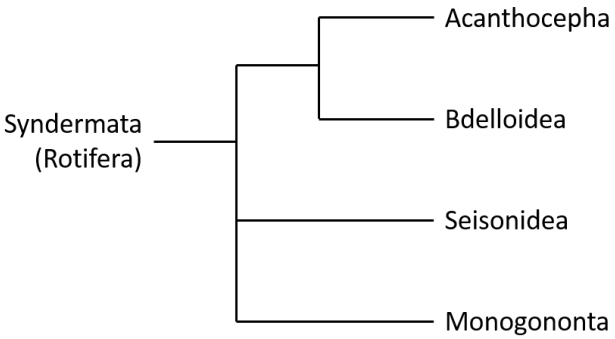

Figure 5. Simplified phylogenetic tree of the Syndermata according to recent studies.

It is now widely recognized that Acanthocephala share a monophyletic origin with Bdelloidea, Seisonidea and Monogononta, forming altogether the group of the Syndermata (sometimes still referred as Rotifera, see Fig. 5). However, the place of Acanthocephala within this group is still debated. Recent molecular analyses suggest that Acanthocephala are probably the sister group of

Bdelloidea (García-Varela *et al.*, 2000; Weber *et al.*, 2013; Sielaff *et al.*, 2016), although the results can differ depending on the part of the genome considered (Sielaff *et al.*, 2016).

The Acanthocephala constitute quite a small group, with around 1100 species currently recognized. Because they do not usually infect humans (except for accidental infections, e.g. Schmidt, 1971; Tada *et al.*, 1983; Ikeh *et al.*, 1992), or cause a special threat to domestic animals or livestock, they have often been considered as a minor group of interest by parasitologists (Kennedy, 2006). Nevertheless, several books were devoted exclusively to the Acanthocephala (e.g. Crompton, 1970; Crompton & Nickol, 1985; Kennedy, 2006).

Paradoxically, the interest of the Acanthocephala might, at least in part, resides in the relative morphological and ecological uniformity within the group (see for instance Taraschewski, 2000). All species are endoparasites, with comparable life-cycles. They usually use arthropods as intermediate hosts that get infected by consuming the eggs containing the acanthor larvae. The larvae grow into two different stages within the intermediate host: the acanthella and the cystacanth stages, only the latter being infectious for the definitive host. The transmission to the definitive host is usually trophic. Many vertebrate species are known to serve as a definitive host for one or several species of acanthocephalan parasites (e.g. birds, mammals, fish, amphibians, reptiles, see Kennedy, 2006). Thus, despite the relatively small number of species known to date, Acanthocephala might actually represent a common and widespread group of parasites (Kennedy, 2006). Acanthocephala are dioecious, and male and female individuals present a sexual dimorphism, such as a longer body size for females compared to males (Parshad & Crompton, 1982). After reaching sexual maturity within the digestive tract of their definitive host, adults reproduce through internal fertilization and eggs are released into the digestive tract of their host. Contrary to many other parasites, no other forms of reproduction, such as hermaphroditism, parthenogenesis or asexual multiplication have been reported in Acanthocephala (Parshad & Crompton, 1982).

Their most remarkable particularity, however, is their ability to alter the behavior of their intermediate hosts, in a way that is believed to increase their probability of being transmitted to the definitive host. Although parasite manipulation is a phenomenon found in a wide range of other organisms, including viruses (Ingwell *et al.*, 2012), bacteria (Werren *et al.*, 2008) and many animal species such as cestodes (Sánchez *et al.*, 2007), trematodes (Reisinger *et al.*, 2015) or nematodes (McCurdy *et al.*, 1999), the Acanthocephala excel in this domain, with all the acanthocephalan species studied so far being known to be manipulative. Thus, manipulation is believed to be derived from an ancestral trait in the Acanthocephala (Moore & Gotelli, 1990). However, their manipulation is sometimes subtle, and some acanthocephalan parasites of the same intermediate host species can

induce different behavioral modifications that are believed to be specific to the definitive host species. For instance, two acanthocephalan species having fish as a definitive host were shown to induce an increase in the photophily of their intermediate amphipod host, but not in its geotactic preferences (Tain *et al.*, 2006). The opposite was observed for a bird parasite, which induced modifications in the geotaxis of the same amphipod species, but did not change its phototaxis (Bauer *et al.*, 2005; Tain *et al.*, 2006). Subtlety goes further as there are some evidence that parasites could adopt strategies to avoid non-host predators (Seppälä *et al.*, 2008a; Médoc & Beisel, 2011), for instance through circadian variations in manipulation avoiding their peak periods of activity (Lagrue *et al.*, 2007). Finally, it is now widely accepted that the manipulation of acanthocephalan intermediate hosts can occur at the non-infective acanthella stage. Several studies pointed out a reversed manipulation at this stage, leading to the "protection" of the host through reinforced anti-predatory behaviors (Dianne *et al.*, 2011, 2014). Although the adaptive value of manipulation is still highly debated (Poulin, 1995; Cézilly *et al.*, 2010), including in acanthocephalan parasites (Perrot-Minnot *et al.*, 2012), these peculiar strategies suggest that manipulation could have evolved in response to its benefits for the parasites.

Apart from all their specificities, including their high propensity to manipulate their hosts in various ways, acanthocephalan parasites are easily found in the wild, and the study of the behavioral alterations that they induce in their host does not require complicated techniques or materials. Thus, the Acanthocephala represent a group of historical importance in the study of manipulation (Thomas *et al.*, 2005b), and continue to be largely used as an important model in this domain.

1.2. Gammarids and acanthocephalans: impacts on a key freshwater species

Among the numerous species that are intermediate hosts for acanthocephalans, amphipods are among the most studied model regarding the modifications induced by parasites on their behavior or on other traits (see table 2). Gammarid amphipods constitute an important crustacean group that is present in a wide range of aquatic habitats (MacNeil *et al.*, 1997; Piscart *et al.*, 2009), and widespread in European rivers (Karaman & Pinkster, 1977a). They are considered as key species in freshwater ecosystems, mainly because of their important role within food webs. First, they live in dense populations and often represent the dominant macro-invertebrate species in terms of biomass (MacNeil *et al.*, 1997). They are thus an important trophic resource as a prey for many species (Degani *et al.*, 1987; Friberg *et al.*, 1994). Second, gammarids are themselves predators for many invertebrate species (MacNeil *et al.*, 1997; Kelly *et al.*, 2002), and their impact is important enough to modulate the composition of freshwater communities of invertebrates (Kelly *et al.*, 2006). However, gammarids are

opportunistic feeders, and their regime is not restricted to living preys. In fact, they are considered as important scavengers and shredders in freshwater ecosystems (MacNeil *et al.*, 1997; Felten *et al.*, 2008), playing a central role in the decomposition of organic matter (Piscart *et al.*, 2009; Constable & Birkby, 2016) and, consequently, in maintaining water of high quality (Maltby *et al.*, 2002).

Gammarid amphipods are the intermediate hosts of several acanthocephalan parasites that are known to induce multiple effects on their hosts, such as altered behavior, immune system, or metabolic rate (table 2). These modifications can also lead to alterations in the ecological role of gammarids, and have consequences on the ecosystems. For instance, acanthocephalan parasites have been shown to play a substantial role in the success of invasion by gammarids species. Many invasive gammarid species have been reported in European rivers, often replacing native gammarid species (Karaman & Pinkster, 1977a; Bollache *et al.*, 2004; Grabowski *et al.*, 2006; Devin & Beisel, 2008). Native gammarid species often show a higher susceptibility to manipulation than their invasive counterparts (Bauer *et al.*, 2000), leading to a higher probability of being predated, thus facilitating the invasion. In addition, the impact of acanthocephalan parasites on the functional role of their hosts can drive the competition between native and invasive species. For instance, Dick *et al.* (2010) reported that invasive *Gammarus pulex* had a higher predatory rate when they were infected by *Echinorhynchus truttae*. Considering the higher parasitic prevalence in this invasive species compared to its native rival *Gammarus duebeni celticus* (MacNeil *et al.*, 2003c; Dick *et al.*, 2010), this functional response could give a competitive advantage to the invasive species. This impact of acanthocephalan parasites on gammarids invasion success might have consequences on the rest of the ecosystem. Indeed, Piscart *et al.* (2011) highlighted that the trophic role of *G. pulex*, especially concerning inorganic matter, leaves and wood, declined in the presence of another species, *Echinogammarus berilloni*. Constable & Birkby (2016) showed that the presence of the invasive *Dikerogammarus haemobaphes* also reduced leaves consumption by *G. pulex*. Moreover, invasive species, despite also belonging to the Gammaridae family, may not have the same functional role as native species, as pointed out by many studies (Bollache *et al.*, 2008; Piscart *et al.*, 2011; Médoc *et al.*, 2015; Constable & Birkby, 2016).

Of course, one of the most obvious ways in which acanthocephalan parasites might alter the role of gammarids within ecosystems is by modulating their probability of being predated. Indeed, as expected under the hypothesis that manipulation might increase the transmission between intermediate and definitive hosts in trophically-transmitted species, there is clear evidence that gammarids infected by parasites at the cystacanth stage have a higher probability of being predated by definitive hosts (Hindsbo, 1972; Bethel & Holmes, 1977; Kaldonski *et al.*, 2007; Perrot-Minnot *et al.*, 2007; Dianne *et al.*, 2011; Jacquin *et al.*, 2014). Conversely, acanthella stages also modulate predation probability by inducing an increase of gammarids anti-predator behaviors (Dianne *et al.*, 2011).

Table 2. Modifications induced by acanthocephalan parasites on their amphipod intermediate hosts.

Trait modified	Parasite species	Host species	References
A. Changes in amphipods behavior			
Phototaxis	*Pomphorhynchus laevis*	*Gammarus pulex*	Kennedy *et al.*, 1978; Brown & Thompson, 1986; Bauer *et al.*, 2000; Cézilly *et al.*, 2000; Perrot-Minnot, 2004; Tain *et al.*, 2006, 2007, Franceschi *et al.*, 2008, 2010a, 2010b; Dianne *et al.*, 2012; Durieux *et al.*, 2012; Perrot-Minnot *et al.*, 2014
	Pomphorhynchus laevis	*Gammarus fossarum*	Perrot-Minnot *et al.*, 2014
	Pomphorhynchus laevis	*Echinogammarus stammeri*	Maynard *et al.*, 1998
	Pomphorhynchus tereticollis	*Gammarus pulex*	Perrot-Minnot, 2004; Tain *et al.*, 2006; Perrot-Minnot *et al.*, 2012
	Polymorphus minutus	*Gammarus lacustris*	Hindsbo, 1972
	Polymorphus paradoxus	*Gammarus lacustris*	Bethel & Holmes, 1973
	Polymorphus marilis	*Gammarus lacustris*	Bethel & Holmes, 1973
	Corynosoma constrictum	*Hyalella ezteca*	Bethel & Holmes, 1973
	Plagiorhynchus allisonae	*Transorchestia chiliensis*	Lagrue *et al.*, 2016
Geotaxis	*Pomphorhynchus laevis*	*Gammarus pulex*	Perrot-Minnot *et al.*, 2014
	Pomphorhynchus laevis	*Gammarus fossarum*	Perrot-Minnot *et al.*, 2014
	Polymorphus minutus	*Gammarus pulex*	Marriott *et al.*, 1989; Cézilly *et al.*, 2000; Bauer *et al.*, 2005
	Polymorphus minutus	*Gammarus roeseli*	Bauer *et al.*, 2005; Haine *et al.*, 2005; Médoc *et al.*, 2006
	Polymorphus minutus	*Echinogammarus berilloni*	Jacquin *et al.*, 2014
Refuge use	*Pomphorhynchus laevis*	*Gammarus pulex*	Kaldonski *et al.*, 2007; Perrot-Minnot *et al.*, 2014; Labaude *et al.*, 2015a (this thesis)
	Pomphorhynchus laevis	*Gammarus fossarum*	Perrot-Minnot *et al.*, 2014
	Pomphorhynchus tereticollis	*Gammarus pulex*	Perrot-Minnot *et al.*, 2007
	Leptorhynchiodes thecatus	*Hyalella azteca*	Stone & Moore, 2014
Aggregation	*Pomphorhynchus laevis*	*Gammarus pulex*	Durieux *et al.*, 2012

Table 2. (continued)

Trait modified	Parasite species	Host species	References
Aggregation	*Pomphorhynchus laevis*	*Gammarus fossarum*	Perrot-Minnot *et al.*, 2014
	Corynosoma sp.	*Gammarus pseudolimnaeus*	Lewis *et al.*, 2012
Activity	*Pomphorhynchus laevis*	*Gammarus pulex*	Perrot-Minnot *et al.*, 2014
	Pomphorhynchus laevis	*Gammarus fossarum*	Perrot-Minnot *et al.*, 2014
	Pomphorhynchus laevis	*Echinogammarus stammeri*	Maynard *et al.*, 1998; Dezfuli *et al.*, 2003
	Polymorphus minutus	*Gammarus pulex*	Thünken *et al.*, 2010
	Polymorphus minutus	*Echinogammarus berilloni*	Jacquin *et al.*, 2014
	Leptorhynchiodes thecatus	*Hyalella azteca*	Stone & Moore, 2014
Reaction to predator odor	*Pomphorhynchus laevis*	*Gammarus pulex*	Baldauf *et al.*, 2007; Kaldonski *et al.*, 2007
	Pomphorhynchus tereticollis	*Gammarus pulex*	Perrot-Minnot *et al.*, 2007
	Echinorhynchus borealis	*Pallasea quadrispinosa*	Benesh *et al.*, 2008
Clinging behavior	*Polymorphus minutus*	*Gammarus pulex*	Bauer *et al.*, 2005
	Polymorphus minutus	*Gammarus roeseli*	Bauer *et al.*, 2005
	Polymorphus paradoxus	*Gammarus lacustris*	Helluy & Holmes, 1990
Drifting behavior	*Pomphorhynchus laevis*	*Gammarus pulex*	McCahon *et al.*, 1991; Lagrue *et al.*, 2007
	Pomphorhynchus laevis	*Echinogammarus stammeri*	Maynard *et al.*, 1998; Wellnitz *et al.*, 2003
Microhabitat	*Echinorhynchus truttae*	*Gammarus pulex*	MacNeil *et al.*, 2003c
Reaction to disturbance	*Polymorphus minutus*	*Gammarus pulex*	Marriott *et al.*, 1989
B. Alterations of amphipods reproduction			
Females fecundity	*Pomphorhynchus laevis*	*Gammarus pulex*	Bollache *et al.*, 2002
	Pomphorhynchus laevis	*Gammarus roeseli*	Haine *et al.*, 2005
	Polymorphus minutus	*Gammarus pulex*	Bollache *et al.*, 2002
	Polymorphus minutus	*Gammarus roeseli*	Haine *et al.*, 2005
	Polymorphus minutus	*Echinogammarus tibaldii*	Dezfuli *et al.*, 2008b
Males pairing success	*Pomphorhynchus laevis*	*Gammarus pulex*	Bollache *et al.*, 2001
	Polymorphus minutus	*Gammarus pulex*	Bollache *et al.*, 2001

Table 2. (continued)

Trait modified	Parasite species	Host species	References
Males pairing success	*Polymorphus paradoxus*	*Gammarus lacustris*	Zohar & Holmes, 1998
	Polymorphus marilis	*Gammarus lacustris*	Zohar & Holmes, 1998
C. Alterations in the feeding of amphipods			
Predation	*Polymorphus minutus*	*Gammarus pulex*	MacNeil & Dick, 2011
	Polymorphus minutus	*Gammarus roeseli*	Médoc *et al.*, 2011a
	Echinorhynchus truttae	*Gammarus pulex*	Fielding *et al.*, 2003; MacNeil *et al.*, 2003b; Dick *et al.*, 2010
Food consumption	*Pomphorhynchus laevis*	*Gammarus pulex*	McCahon *et al.*, 1988; Pascoe *et al.*, 1995
	Pomphorhynchus tereticollis	*Gammarus fossarum*	Labaude *et al.*, 2016 (this thesis)
	Polymorphus minutus	*Gammarus roeseli*	Médoc *et al.*, 2011a
D. Modifications of host physiology and metabolism			
Immune system	*Pomphorhynchus laevis*	*Gammarus pulex*	Rigaud & Moret, 2003; Cornet *et al.*, 2009a, 2009b; Cornet & Sorci, 2010; Cornet, 2011
	Pomphorhynchus laevis	*Gammarus roeseli*	Rigaud & Moret, 2003
	Polymorphus minutus	*Gammarus pulex*	Rigaud & Moret, 2003
	Polymorphus minutus	*Gammarus roeseli*	Rigaud & Moret, 2003
Lipid content	*Pomphorhynchus laevis*	*Gammarus pulex*	Plaistow *et al.*, 2001
Glycogen	*Pomphorhynchus laevis*	*Gammarus pulex*	Plaistow *et al.*, 2001
Respiration	*Pomphorhynchus laevis*	*Gammarus pulex*	Rumpus & Kennedy, 1974; Labaude *et al.*, 2015a (this thesis)
Haemocyanin	*Pomphorhynchus laevis*	*Gammarus pulex*	Bentley & Hurd, 1993
Color	*Polymorphus minutus*	*Gammarus lacustris*	Hindsbo, 1972
Response to toxicants	*Polymorphus minutus*	*Gammarus roeseli*	Gismondi *et al.*, 2012a, 2012c
E. Neurological alterations			
brain serotonergic activity	*Pomphorhynchus laevis*	*Gammarus pulex*	Tain *et al.*, 2006, 2007
	Pomphorhynchus tereticollis	*Gammarus pulex*	Tain *et al.*, 2006
	Polymorphus paradoxus	*Gammarus lacustris*	Maynard et al., 1996

Although the mechanisms on which relies behavioral manipulation of gammarids by acanthocephalan parasites are not clearly identified yet, there is evidence that manipulation could be a multi-dimensional phenomenon, that might result from the dysregulation of a limited number of key neuromodulators in gammarids (Cézilly & Perrot-Minnot, 2010). This hypothesis was supported by the fact that serotonin injections were found to mimic the effect of acanthocephalan parasites, inducing changes in multiple behaviors (Perrot-Minnot *et al.*, 2014), while parasites were shown to induce changes in the brain serotonergic activity of their gammarid hosts (Tain *et al.*, 2006). Nevertheless, the mechanisms are likely to differ between certain acanthocephalan species. In particular, the alteration of geotaxis induced by the bird parasite *P. minutus* seems to not rely on the serotonin pathway, but rather could be linked with anaerobic metabolism (Tain *et al.*, 2006; Perrot-Minnot *et al.*, 2015).

1.3. Studied species

Several acanthocephalan species using gammarids as intermediate hosts can be found in European rivers (see Fig. 6 for examples). Among them, three species were studied during this work: the fish species *P. laevis* and *P. tereticollis*, and to a minor extent the bird species *P. minutus*. Fish parasites are known to make their intermediate host more photophilic and less willing to use refuges (see table 2 for references), possibly leading gammarids toward places that are more exposed to predation by fish. Accordingly, the predation of gammarids infected by *P. laevis* cystacanths was found to be higher than that of control gammarids only when refuges where available (Kaldonski *et al.*, 2007). *P. minutus* is rather known to induce changes in the geotaxis of gammarids, with infected gammarids swimming closer to the surface (see table 2 for references), presumably where they are the more susceptible to predation by birds.

Despite their different definitive hosts, the three species have comparable life cycles (Crompton & Nickol, 1985; Kennedy, 2006). Adults reproduce within the intestine of the definitive hosts. The eggs containing the acanthor larvae are released in the river along with the vertebrate feces. Gammarids get infected by consuming the eggs. After passing through the gammarid intestinal wall, acanthocephalan larvae establish in the haemocoel and grow in two distinct larval stages: the acanthella and the cystacanth stages. At this later stage, the parasite becomes infective for its definitive host and behavioral manipulation enhancing its probability of trophic transmission is observed. After the predation of the gammarid by the definitive host, the contact with the bile induces parasites' proboscis to evert (Kennedy *et al.*, 1978; Tain *et al.*, 2006), allowing the parasite to attach in

the intestine wall of its host. Facultative paratenic hosts, such as small-sized fish, were also reported to occur in the cycle of fish parasites (Médoc *et al.*, 2011a; Emde *et al.*, 2012).

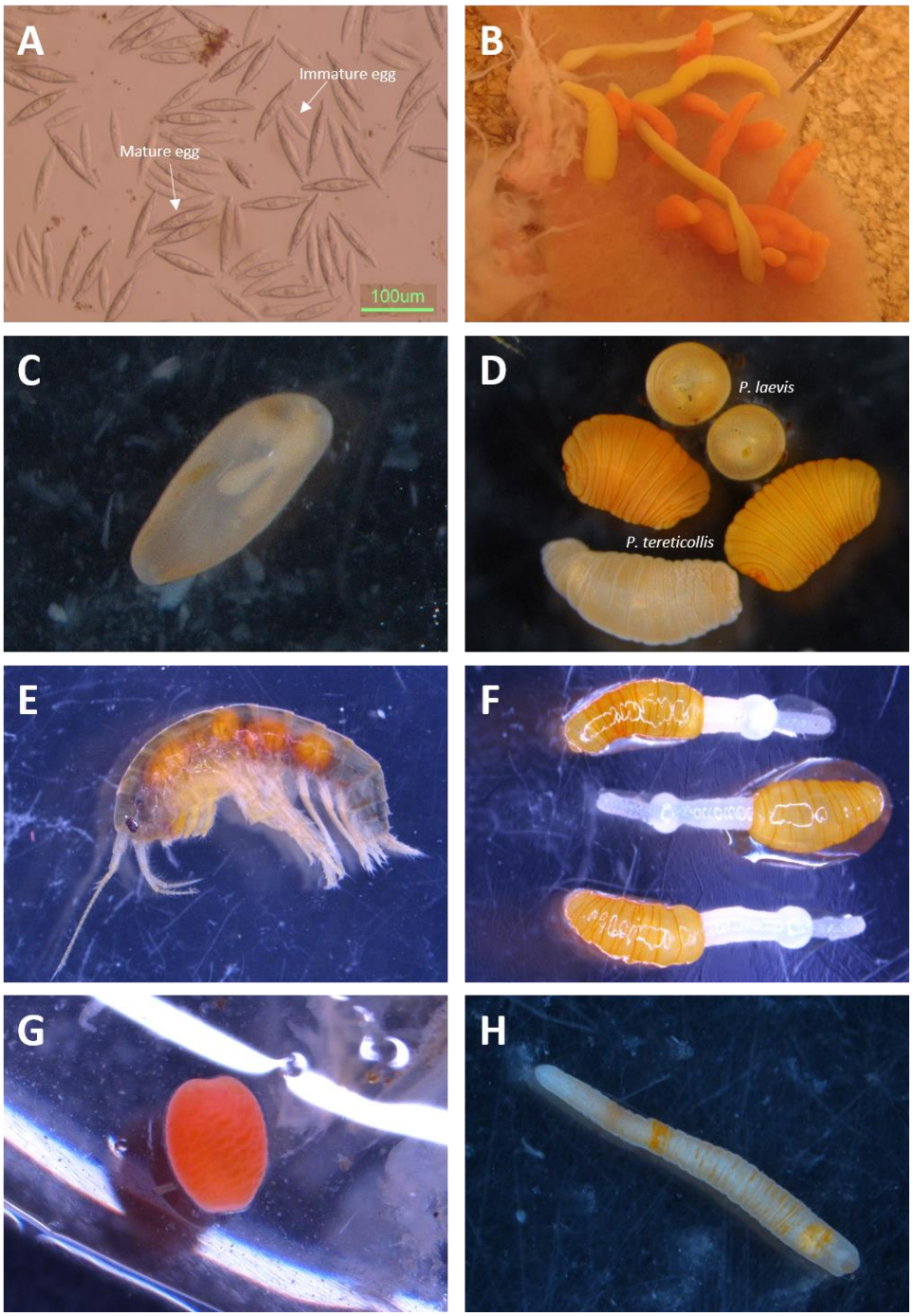

Figure 6. Photos of different species of acanthocephalan parasites, at different developmental stages. (A) Mature and immature eggs of *P. laevis*. (B) Adults *Pomphorhynchus sp.* attached on the intestine wall of a fish. (C) Acanthella stage of *P. laevis*. (D) Cystacanth stages of *P. laevis* and *P. tereticollis*. (E) Gammarid infected with several *P. tereticollis* cystacanths. (F) *P. tereticollis* cystacanths with everted proboscis. (G) Cystacanth stage of *P. minutus*. (H) Cystacanth stage of *E. truttae*.

2. Main methods

2.1. Selection of gammarid individuals

Although adult gammarids can be maintained for several months in the laboratory, rearing individuals is particularly difficult, and our current techniques and equipment were not sufficient to allow the survival of offspring. Thus, all individuals studied during this work were sampled in the wild. The “kick-sampling” method was used (Hynes, 1954), which consists in scratching the bottom of the river to dislodge hidden individuals. Gammarids were then captured with a hand net.

Several gammarid populations in Burgundy have been widely studied for years (e.g. Franceschi *et al.*, 2010a; Lagrue *et al.*, 2014), and populations were selected according to several characteristics required by each experiment, such as the absence or presence of acanthocephalan parasites, or the species of gammarid required. Moreover, using populations that are already well studied can prove useful to link the results with other characteristics of the population.

Several species of gammarids can be found in the rivers of eastern France. Some species, such as *G. roeseli*, present particular morphological characteristics (Karaman & Pinkster, 1977b), and can thus be distinguished from other species with visual identification. However, many rivers contain individuals from both *G. pulex* and *G. fossarum* closely-related species (Lagrue *et al.*, 2014). Although some minor morphological differences have been reported between these two cryptic species (Karaman & Pinkster, 1977a; Mayer *et al.*, 2012), *G. pulex* and *G. fossarum* cannot be reliably distinguished by visual inspection, even under a microscope, such that they have often been considered as a single taxonomic unit (Karaman & Pinkster, 1977a). However, several studies highlighted differences in these species, including in the way that they are affected by acanthocephalan parasites (Westram *et al.*, 2011). Thus, gammarids that we used were genetically characterized. Populations were primarily selected after the work of Lagrue *et al.* (2014), who calculated the proportion of each species in many eastern France rivers. Moreover, we conducted further genetic analyses on the chosen populations, either to confirm that most, if not all, individuals belonged to one species, or to determine the species of every single gammarid used from rivers presenting high proportions of both species (see below for detailed protocol).

Because infection success is higher in males than in females (Franceschi *et al.*, 2008), studies based on experimental infestations were conducted on males only. However, sex appears to have

generally no effect on the extent of behavioral modifications (Bauer *et al.*, 2000, 2005; Cézilly *et al.*, 2000). Males and females form amplexus, a mate-guarding behavior where males carry females beneath their ventral surface prior to copulation (Sutcliffe, 1992). Males could easily be selected by separating the couples. When both males and females were considered in the experiment, individuals were systematically sexed at the end, based on the size and shape of their first and second pairs of gnathopods, which present a sexual dimorphism in amphipods (Hume *et al.*, 2005). Individuals were also systematically measured (see below) and dissected to assess their infection status (number, developmental stage and species of parasites).

Genetic identification of gammarids

Gammarids DNA was extracted from fresh individuals or from individuals preserved in 100% ethanol. For each individual, a two millimeters long piece of body was mashed into a tube containing 60 µl of sodium hydroxide (NaOH). After a two minutes heat treatment at 100°C, tubes were immediately put on ice and 540 µl of Tris-HCl buffer (100 nM) were added. Extracted DNA was diluted 10 times before amplification.

Polymerase chain reactions (PCR) were used to amplify the DNA of the cytochrome c oxidase subunit I (COI), using the two universal primers LCO1490 (sequence 5'-3': GGTCAACAAATCATAAAGATATTGG) and HCO2198 (sequence 5'-3': TAAACTTCAGGGTGACCAAAAAATCA; Folmer *et al.*, 1994). PCR were performed in a final volume of 50 µl containing 5 µl of DNA extract, 200 nM of each primer, 200 µM of dNTPs and 0.5 Units of Taq DNA polymerase with 1X Taq buffer. Thermal cycling started with an initial denaturation at 95°C (three minutes), followed with 35 cycles of 95°C (20 s), 40°C (45 s), 65°C (60 s) and a final incubation of two minutes at 65°C. Amplified DNA was then digested using RFLP method (Restriction Fragment Length Polymorphism; Lagrue *et al.*, 2014), in a total volume of 20 µl containing 10 µl of PCR extracts and 10 Units of Vspl (Asel) restriction enzyme with 2X O Buffer. This enzyme recognizes AT^TAAT sites. Digestion proceeded overnight at 37°C, and the reaction was stopped by a final incubation at 65°C for 20 minutes. An electrophoresis of 1 µl of the resulting fragments, mixed with 5 µl of Bromophenol blue, was performed in an agarose gel (2 %). After 30 minutes of migration (100 V), the profiles of restricted fragments products were visualized with ethidium bromide on a UV bench. Due to differences in DNA sequences, *G. pulex* DNA is typically restricted into two fragments, while only one is visible for *G. fossarum*, allowing the distinction between the two species.

In total, the species of 1071 gammarids from three different rivers was determined during this work. Results are presented in table 3. Contrary to Lagrue *et al.* (2014), our samplings were not made

in order to take into account all possible habitats, and might thus not be completely representative of what is found in the rivers. However, it is interesting to note that the proportions of each species were substantially different than those observed by Lagrue *et al.* (2014), coming from samplings made prior to 2013. Moreover, the relative proportion of each species also differed in our observations between the different samplings. It is thus possible that the relative importance of each species is naturally fluctuant along time.

Table 3. Proportion of *G. pulex* and *G. fossarum* among the populations of gammarids from several rivers.

River	GPS coordinates	Sampling date	Sample size	Proportions observed	Proportions found by Lagrue *et al.* (2014)
Suzon	47°24'14.45"N, 4°53'1.46"E	December 2013	247	98 % *G. pulex*, 2% *G. fossarum*	70.5 % *G. pulex*, 29.5 % *G. fossarum*
		April 2014	88	95.5 % *G. pulex*, 4.5 % *G. fossarum*	
Vèze	47°14'1.42"N, 5°34'37.69"E	August 2014	243	14.4 % *G. pulex*, 85.6 % *G. fossarum*	56.1 % *G. pulex*, 43.9 % *G. fossarum*
		May 2015	457	10.3 % *G. pulex*, 89.7 % *G. fossarum*	
Norges	47°21'41.38"N, 5°9'30.16"E	May 2015	36	100 % *G. fossarum*	100 % *G. fossarum*

Body characteristics of G. pulex *and* G. fossarum

It is widely considered that the height of gammarids fourth coxal plate is a reliable proxy for their size (Bollache *et al.*, 2000), and this parameter is largely considered in studies. One of the main reasons is that the coxal plate measurement leads to less observational errors than that of the total size of the individual, which depends on how curved is the gammarid during its measurement. However, the correlation between the two parameters might differ between species. Moreover, our experiments sometimes required to estimate the correlation between linear measurements and the dry weight of gammarids. Thus, we characterized the three parameters and their relationships for the two gammarid species *G. pulex* and *G. fossarum*, from individuals belonging to the main populations that were used in this work.

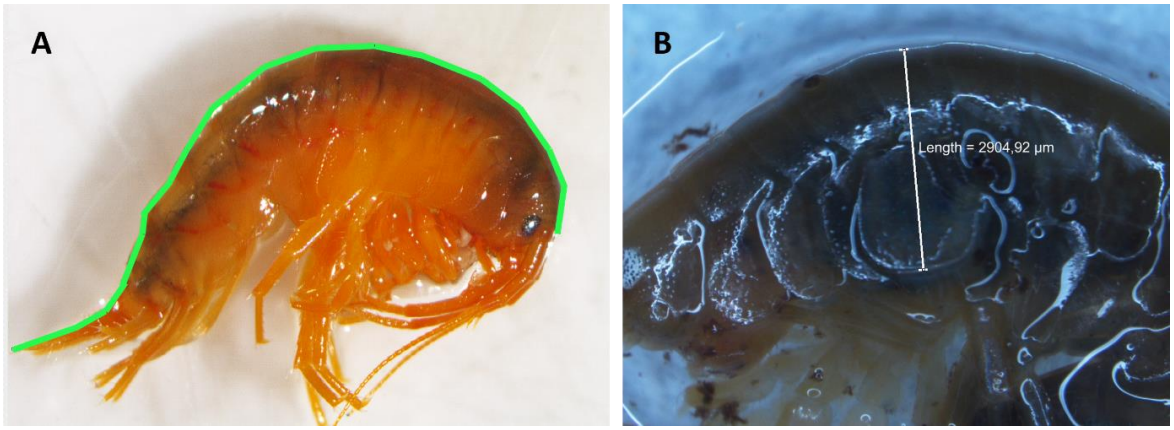

Figure 7. Measurement of gammarids. (A) Measurement of the total body length of gammarids in their natural curved position (green line) and (B) measurement of the fourth coxal plate (white line).

G. pulex individuals (68 males and 65 females) were collected on November 2014 from the Suzon River (eastern France, 47°24'14.45"N, 4°53'1.46"E), and *G. fossarum* individuals (68 males and 69 females) were collected on December 2015 from the Norges River (eastern France, 47°21'41.38"N, 5°9'30.16"E). Individuals were killed in ethanol and measured using a microscope and Lucia G 4.81 software. The height of the fourth coxal plate was measured, as well as the total length of the gammarids, from the tip of the rostrum to the end of the telson, in their natural curved position (see fig. 7). Individuals were then dried in a 50°C chamber during 64 hours, and immediately weighed to the nearest tens of milligram.

Table 4. Body dimensions of *G. pulex* and *G. fossarum* gammarids (means ± standard deviation).

		Body length (mm)	Fourth coxal plate (mm)	Dry weight (mg)
G. pulex	Males	15.04 (± 1.64)	2.54 (± 0.30)	8.30 (± 1.83)
	Females	10.78 (± 0.76)	2.05 (± 0.17)	3.56 (± 0.63)
G. fossarum	Males	11.52 (± 0.97)	1.96 (± 0.18)	4.29 (± 0.81)
	Females	8.94 (± 0.83)	1.63 (± 0.18)	2.88 (± 0.70)

Although our sampling technique was not sufficient to characterize the dimensions of average gammarids in each population (this requires samplings of all age classes in all possible micro-habitats to avoid sampling biases), we found a sexual dimorphism that is already well documented in gammarids (Hume *et al.*, 2005), with males on average larger than females (table 4, Fig. 8). Moreover, *G. pulex* individuals were globally larger than *G. fossarum* individuals, in the two populations that we investigated (table 4, Fig. 8).

Table 5. Linear relationships between the different body dimensions of *G. pulex* and *G. fossarum* gammarids.

		R^2	Slope	Intercept	P-value
Dry weight (mg) according to body length (mm)					
G. pulex	Males	0.75	0.97	-6.26	< 0.0001
	Females	0.37	0.50	-1.86	< 0.0001
G. fossarum	Males	0.65	0.68	-3.55	< 0.0001
	Females	0.52	0.62	-2.63	< 0.0001
Dry weight (mg) according to the fourth coxal plate (mm)					
G. pulex	Males	0.68	5.08	-4.62	< 0.0001
	Females	0.28	1.98	-0.49	< 0.0001
G. fossarum	Males	0.49	3.20	-1.97	< 0.0001
	Females	0.45	2.62	-1.38	< 0.0001
Body length (mm) according to the fourth coxal plate (mm)					
G. pulex	Males	0.61	4.33	4.03	< 0.0001
	Females	0.38	2.78	5.06	< 0.0001
G. fossarum	Males	0.49	3.80	4.09	< 0.0001
	Females	0.43	2.99	4.08	< 0.0001

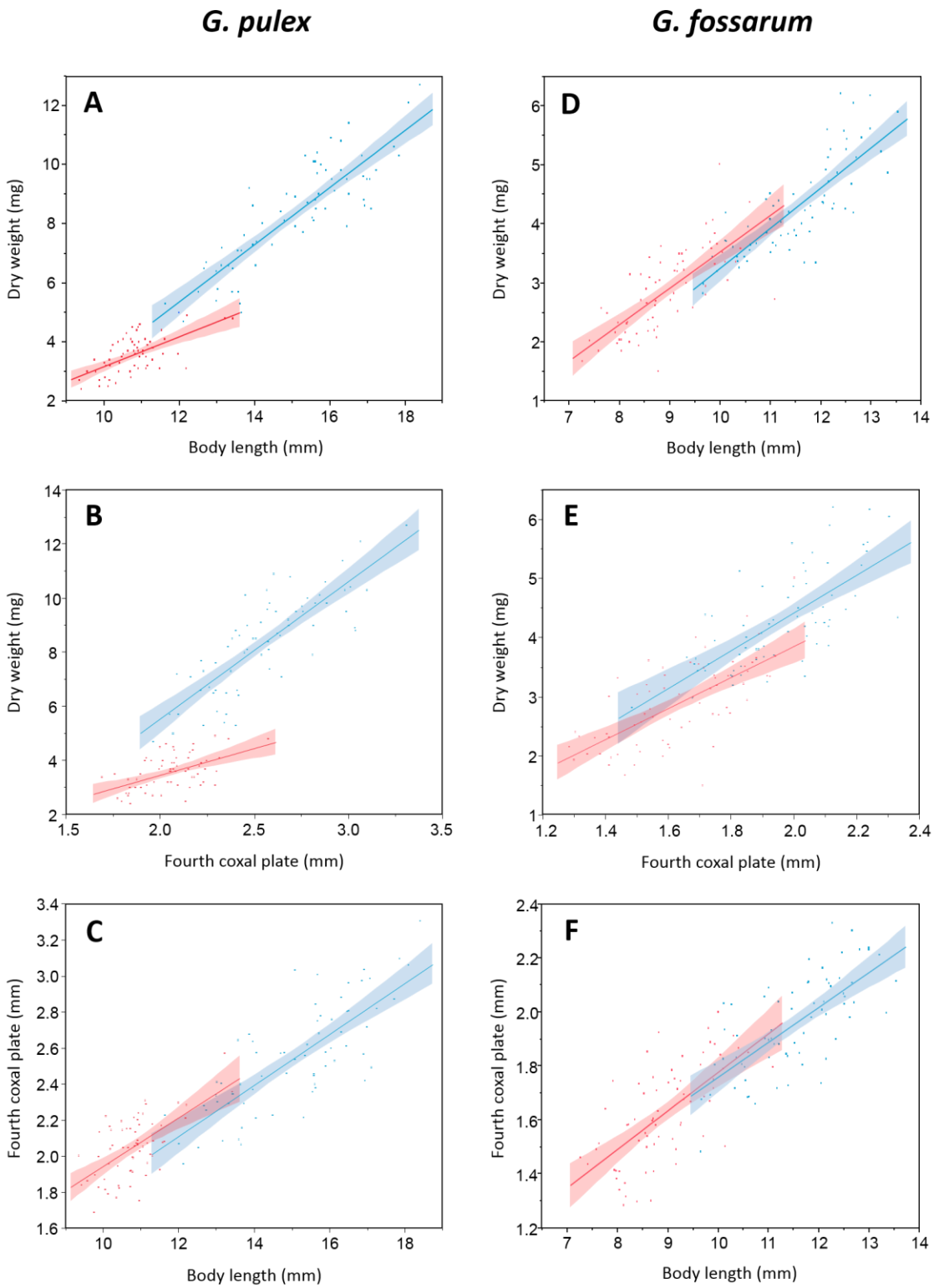

Figure 8. Linear relationships with 95 % confidence intervals between the different body dimensions (dry weight, total body length and length of the four coxal plate) for *G. pulex* and *G. fossarum* gammarids. The red lines and dots correspond to females and the blue lines and dots correspond to males.

The three traits measured were all significantly correlated between each other, although there were differences in the correlation between males and females for *G. pulex* individuals (Table 5). All the relationships, including between dry weight and linear measures, were linear. These results confirmed that the height of the fourth coxal plate is a reliable proxy for the size of gammarids in both species, and that it can also be used as a proxy for the weight of *G. fossarum* individuals.

2.2. Gammarids infections

Experimental infestation vs naturally-infected gammarids

Gammarids infected by acanthocephalan parasites can be obtained by two different ways: either naturally-infected gammarids can be directly sampled in the field, or uninfected gammarids can be experimentally infected in the laboratory. The techniques selected in this work depended on the requirements of each experiment, since both types of infection have pros and cons. Among the critics against the use of naturally infected gammarids, there was a concern that the difference in behavior observed between infected and control individuals could have preceded infection, such that the difference was due to a higher propensity of certain individuals to get infected compared to others. However, studies based on experimental infestations also highlighted such behavioral differences (Franceschi *et al.*, 2008), thus discarding this hypothesis. The main concern about the use of naturally infected individuals remains in the uncertainty of the past of the infection. Indeed, this technique does not allow to control the environmental conditions experienced by the gammarids and their parasites, such as the temperature or the resources. Moreover, it is not possible either to control for the age of the parasite, and there might be a high variability due to infestation by parasites from many clutches (Franceschi *et al.*, 2010b). On the other size, experimental infestations allow to control the environmental conditions during the development of the parasite, and gammarids are infected on the same date with parasite eggs coming from a restricted number of mothers. However, the choice of using experimental infestation or naturally infected gammarids also derived from different technical requirements. Indeed, naturally infected gammarids, especially with the cystacanth stage of the parasite, are easy of obtain. Parasites are visible through the translucent cuticle of gammarids (Fig. 6E), allowing the selection of infected gammarids directly in the field. Moreover, it is possible to distinguish between species through gammarids cuticle, due to differences in color and size (Fig. 6D), although dissections at the end of the experiments were systematically used to confirm the species. On the contrary, experimental infestations are time consuming. The technique (described bellow) requires the collection of parasite eggs from wild fish, the genetic identification of their species and the

infestation process per se. Then, gammarids have to be maintained for two or three months for the parasites to develop. Apart from the technical requirements of such a maintenance, there exists a risk of uncontrolled mortality of gammarids before the achievement of parasites development, as well as a risk of low success of infestation. Indeed, the success of infestation is highly variable, depending, among other parameters, on the population of parasites and the population of gammarids used (Franceschi *et al.*, 2010a). In this work, both techniques were used depending on the hypothesis that was tested in each experiment.

Identification of the parasite species – genetic analyses

Fish from eastern France rivers are regularly infected by several species of acanthocephalan parasites. Because *Pomphorhynchus* adults present low dimorphism between species (Perrot-Minnot, 2004), it is hardly possible to select parasites based on visual identification. Thus, eggs were collected from several adult parasites, and their species was identified using genetic analyses conducted on tissues from each mother.

For each individual, a piece of parasite tissue was mashed in a 1.5 ml tube with 500 µl of CTAB extraction buffer pre-warmed at 60°C. After the addition of 10 µl of Proteinase K (20 mg/ml), tubes were incubated overnight at 60°C. Tubes were brought back to ambient temperature, and 2.5 µl of RNase A were added. After 30 minutes at 37°C, 500 µl of phenol chloroform:isoamyl (1:1) were added and the tubes were gently mixed by inversion. Tubes were centrifuged at 15°C for 8 minutes at 12000 rpm (revolutions per minute). The supernatant was extracted and mixed by inversion with 500 µl of chloroforme:isoamyl alcohol (24:1). After 15 minutes of centrifugation (15°C, 10000 rpm), the supernatant was transferred into 500 µl of cold isopropanol, and the mix was stored overnight at -20°C for DNA precipitation. DNA was centrifuged for 30 minutes (4°C, 15000 rpm) and pellets were rinsed twice with 70% ethanol. After air drying for two hours, DNA was suspended in 100 µl of ultrapure sterile water.

Polymerase chain reactions (PCR) were used to amplify ITS rDNA of acanthocephalans. Primers BD1f (sequence 5'-3': GTCGTAACAAGGTTTCCGTA) and AC/ITS1r (sequence 5'-3': TTGCGAGCCAAGTGATTCAC) were used to generate DNA sequences that differ in the number of base pairs between acanthocephalan species, allowing the differentiation of species after DNA migration. PCR were performed in a final volume of 10 µl containing 2 µl of DNA extract, 200 nM of each primer, 200 µM of dNTPs and 0.25 Units of Taq DNA polymerase with 1X Taq buffer. Thermal cycling started with a two minutes initial denaturation at 94°C, followed with 39 cycles of 94°C (20 s), 50°C (45 s), 65°C (45 s) and a final incubation of five minutes at 65°C. An electrophoresis of 1 µl of the PCR products

mixed with 2 µl of Bromophenol blue was performed in an agarose gel (3 %). After one hour of migration (135 V), the DNA profiles were visualized with ethidium bromide on a UV bench. The size of PCR products was compared with a DNA size standard (100 bp ladder) and with positive controls (DNA from the two main species, *P. tereticollis* and *P. laevis*), and species were determined.

Experimental infestation – procedure

Parasite eggs were extracted from several acanthocephalan females, selected on their longer size compared to males (Parshad & Crompton, 1982), from the intestine of wild chubs (*Leuciscus cephalus*). Eggs were preserved in water at 5°C during the genetic identification of their species (see above). Only eggs from *P. laevis* species were used for experimental infestations. Eggs were examined under a microscope to assess their maturity, and only clutches containing a high proportion of mature eggs were used for infestation. Mature eggs, which consist of several envelopes containing the acanthor larvae, were distinguished from immature eggs based on their size, their shape, and the typical presence of a visible central mass (Fig. 6A, Parshad & Crompton, 1982). Selected clutches were mixed together for the infestation to avoid infection failure due to poorly-infective clutches (Franceschi *et al.*, 2010b), and to provide a sufficient number of eggs. The final eggs concentration within the mix was estimated under a microscope.

Gammarids were starved by pairs in glass dishes (6 cm diameter) for 24 hours before the infestation, to ensure that they would consume the food on which parasite eggs were deposited. A quantity of egg suspension corresponding to 100 eggs per gammarid (i.e. 200 eggs per glass dish) was deposited on a piece of elm leaf (one centimeter square). This quantity was chosen after the work of Franceschi *et al.* (2008), and corresponds to the best compromise to ensure a sufficient infection success and a limited risk of multi-infected gammarids. One infected piece of leaf was placed in each glass dish. Control individuals received the same treatment as experimentally infected individuals, except that no parasite eggs were deposited on leaves. Gammarids were allowed to feed on leaves for 48 hours. After this time, all gammarids were put in individual clean glass dishes and randomly distributed among the different treatments.

2.3. Maintenance of individuals

Gammarids were maintained in the laboratory under controlled conditions. Circadian rhythms were artificially preserved with a 12:12 light:dark cycle. The maintenance of gammarids depended on the experiment: individuals that were experimentally infected (and their uninfected controls) were kept in

separate glass dishes, while gammarids could be maintained in groups or individually for experiments involving naturally infected gammarids. Water, which was changed regularly, consisted of an oxygenated mix of water from the river of origin of gammarids and dechlorinated, UV-treated tap water. The temperature was maintained using different devices described for each experiment. Individuals were fed *ad libitum* using dead elm leaves. Leaves were dried for a week before being autoclaved at 120°C for 20 minutes. Leaves were then conditioned for at least one week in oxygenated water before their use. Gammarids that were maintained in individual glass dishes were regularly checked to follow their survival.

2.4. Behavioral tests

Different behavioral tests were used during this work that depended on the species of parasite considered, and thus on its expected impact on its host behavior (see table 2). Individuals infected with fish parasites (*P. laevis* or *P. tereticollis*) at the cystacanth stage were tested for the time spent inside a refuge or for their phototaxis. To test for refuge use, gammarids were individually placed in boxes (10.5 × 16 cm) filled with 250 mL of water, with a refuge at one extremity consisting of a saucer terracotta pot (8.5 cm of diameter) cut in half and opened with a one centimeter hole in the convex part (Fig. 9A). To test for phototaxis, single gammarids were placed in horizontal glass-tubes (22 cm long, 3.2 cm of diameter) containing a light zone and a dark zone (half of the tube being covered with

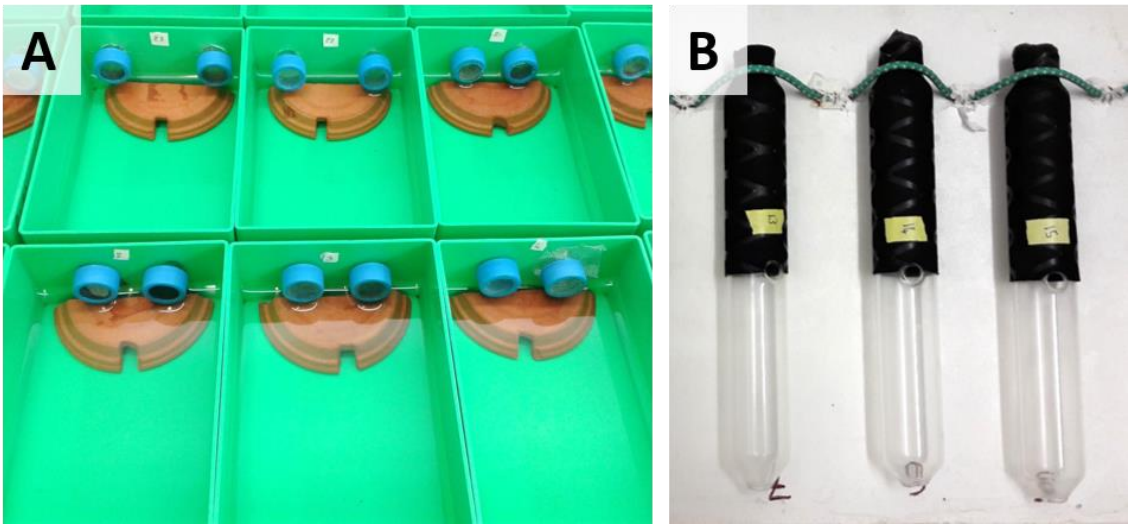

Figure 9. Experimental devices used to test for (A) the use of refuges and (B) the phototaxis of uninfected and infected gammarids.

black plastic to ensure complete opacity (Fig. 9B). After a short period of acclimatization, the position of each gammarid (inside or outside the refuge; in the dark or in the light zone) was recorded by scan sampling at determined time intervals. A score was given at each period of time according to the position of the individuals, and scores were summed at the end of the experiment. Control individuals were systematically tested concomitantly with infected individuals. A particular attention was paid to ensure blind recordings, with test devices numbered independently from individual identification.

Other tests were conducted depending on the hypothesis that was tested, and their protocols are described for each experiment.

2.5. Thesis overview

Numerous environmental conditions are susceptible to affect the interaction between gammarids and their acanthocephalan parasites, as suggested by my review (see Article 1). Here, the effect of two parameters were investigated: host food resources and temperature. These two parameters are highly variable in European rivers, both at spatial and temporal scales. Moreover, temperature and the availability of resources are among the parameters that are particularly affected by global change.

First, the effect of the quality and quantity of host food resources were investigated using an experimental infection (Chapter III). Gammarids were maintained into two different experimental conditions of resources, with either a rich or a poor diet, during the development of their parasites. The effect of such variation in diet was measured on several infection parameters and on the impact of parasites on their hosts in terms of metabolism and behavior. Originally, this experiment aimed at testing the effects of both host resources and temperature. Thus, although the results presented here only concern gammarids maintained at 17°C during the development of their parasites, the exact same experiment was also conducted in parallel at 7°C. However, the development of parasites was so slow at this temperature that none of the parasites reached the cystacanth stage after nine months of experiment, such that their hosts died before any behavioral test could be conducted.

The drastic effect of temperature observed on parasite development in the first experiment comforted the choice to study further this parameter. Thus, the following chapters largely focus on the impact of temperature. First, the effect of temperature was investigated on the alteration of gammarids behavior induced by two different acanthocephalan species (Chapter IV.1). For this experiment, naturally infected gammarids were used, and three behaviors were tested after acclimatization of gammarids at different temperatures in the laboratory. Second, experimental infections were used to study the impact of two temperatures during the development of parasites

(Chapter IV.2). Similarly as the first experiment, several infection parameters were measured and the behavior of gammarids was tested. Finally, to improve our understanding of the effect of temperature on parasite development, in terms of rapidity and intensity, the effect of temperature on the immune system of uninfected gammarids was investigated (Chapter IV.3).

The multiple effects of environmental conditions found on the interaction between gammarids and their acanthocephalan parasites are likely to have consequences on the role of gammarids within their ecosystems. Such ecological consequences are investigated in the Chapter V. First, infected and uninfected gammarids might present different thermic preferences, such that the actual effect of temperature might also be affected by their microhabitat choice. This was verified in a preliminary experiment (Chapter V.1.). Second, the shredder role of gammarids is known to be of great importance in their rivers, and was shown to be affected both by parasites and temperature. The cumulative effect of these two parameters was thus investigated, with consumption tests conducted on naturally infected gammarids acclimatized at three different temperatures in the laboratory (Chapter V.2). Third, because manipulative parasites are mostly known to alter the probability of predation of their intermediate hosts by their definitive hosts, it is likely that the role of gammarids as preys might also depend on a cumulative effect of temperature and parasites. Although only preliminary experiments could be conducted in the frame of this thesis, this aspect is discussed (Chapter V.3).

The purpose of the last chapter of my thesis (Chapter VI) is to draw general conclusions from my work, discuss some consequences that were not addressed in particular discussions of each chapter, and provide perspectives for future experiments to further investigate the impact of environmental conditions on the relationship between manipulative parasites and their hosts.

Chapter III. Impact of resources

ARTICLE 2

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Influence of host nutritional condition on post-infection traits in the association between the manipulative acanthocephalan Pomphorhynchus laevis *and the amphipod* Gammarus pulex

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Abstract

Background

Several parasites with complex life-cycle induce phenotypic alterations in their intermediate hosts. According to the host manipulation hypothesis, such phenotypic alterations are supposed to increase the fitness of the parasite at the expense of that of its intermediate hosts through increasing the probability of transmission to next hosts. Although the phenomenon has received a large attention, the proximate factors modulating the occurrence and intensity of host manipulation remain poorly known. It has been however suggested that the amount of energy reserves in the intermediate host might be a key parameter, although its precise influence on the intensity of manipulation remains unclear. Dietary depletion in the host may also lead to compromise with other parasite traits, such as probability of establishing or growth or virulence.

Methods

Here, we address the question through performing experimental infections of the freshwater amphipod *Gammarus pulex* with two different populations of the acanthocephalan fish parasite *Pomphorhynchus laevis*, and manipulation of host nutritional condition. Following exposure, gammarids were given either a “standard” diet (consisting of elm leaves and chironomid larvae) or a “deprived” food treatment (deprived in proteins), and infection parameters were recorded. Once parasites reached the stage at which they become infective to their definitive host, refuge use (a behavioral trait presumably implied in trophic transmission) was assessed, and metabolic rate was measured.

Results

Infected gammarids exposed to the deprived food treatment showed a lower metabolic rate, indicative of a lower body condition, compared to those exposed to the standard food treatment. Parasite size was smaller, and, depending on the population of origin of the parasites, intensity of infection was lower or mortality was higher in deprived hosts. However, food treatment had no effect on either the timing or intensity of behavioral modifications.

Conclusions

Overall, while our results suggest that acanthocephalan parasites develop better in hosts in good condition, no evidence was found for an influence of host nutritional condition on host manipulation by parasites.

Keywords

Deprivation; Energetic constraints; Food resources; *Gammarus pulex*; Parasite manipulation; *Pomphorhynchus laevis*

Background

Many parasites with complex life cycle are known to alter the phenotype of their hosts (Poulin & Thomas, 1999; Moore, 2002a). In particular, trophically-transmitted parasites often induce phenotype modifications in their intermediate hosts that appear to make them more vulnerable to predation by definitive host species, thus possibly increasing their probability of completing their life cycle (Thomas *et al.*, 2005b; but see Kaldonski *et al.*, 2009; Perrot-Minnot *et al.*, 2012). This phenomenon, known as "parasite manipulation", has been shown to play diverse and important roles, such as altering host population ecology (Ponton *et al.*, 2005), affecting food webs in ecosystems (Sato *et al.*, 2011), or driving disease dynamics (Koella *et al.*, 1998). However, and despite numerous examples of behavioral alterations in many different host-parasite associations (Moore, 2002a), this phenomenon is not yet fully understood (Klein, 2005; Cézilly *et al.*, 2010; Poulin, 2010).

In particular, the proximate factors that modulate the occurrence and intensity of host manipulation remain poorly known. It has been however suggested that the amount of host's energy reserves could play a key role, although its precise influence remains unclear. On the one hand, it has been predicted that parasites should adjust their exploitation strategy to the physiological condition of their hosts, possibly leading to an increase or acceleration of behavioral changes in hosts in poor condition (Thomas *et al.*, 2002a). This is because the risk for a parasite to die before trophic transmission occurs should be higher in hosts in poor nutritional condition (Poulin, 2003; Benesh & Valtonen, 2007a). On the other hand, it has been suggested that displaying a modified behavior is costly for hosts, such that only hosts in good body condition should be able to show altered behavior (Thomas *et al.*, 2011). More recently, Maure *et al.* (2013) suggested that parasites have been selected to leave enough resources to their hosts to allow them to express manipulated behaviors (the "host energetic resource constraint hypothesis", hereafter HERC hypothesis).

So far, only a few studies have addressed the importance of energy resources in the interaction between manipulative parasites and their hosts. However, some evidence exists for an energetic cost of harboring a manipulative parasite. For instance, Lettini & Sukhdeo (2010) showed that isopods infected by acanthocephalan parasites allocated about 21% of their energy production to parasite growth, at the expense of their own reproduction (but see Shik *et al.*, 2011). Other studies have

provided some evidence for reduced growth resulting from competition for host resources in manipulative parasites co-occurring in a single host (Dianne *et al.*, 2012), or have revealed negative associations between the speed of parasite development and the intensity of manipulation (Franceschi *et al.*, 2010b), or between host survival and parasite fecundity (Maure *et al.*, 2011). All those studies tend to suggest that host energetic reserves are a limited resource for the parasite. In addition, it has been shown that host resources could be modified by the presence of a parasite. In particular, glycogen content was increased in the amphipod *G. pulex* (Plaistow *et al.*, 2001) and in the isopod *Caecidotea intermedius* (Caddigan *et al.*, 2014) infected by acanthocephalan parasites, compared to uninfected individuals, while additional modifications in lipid and glucose contents was also observed in the amphipod *Gammarus insensibilis* infected by a trematode parasite (Ponton *et al.*, 2005).

Although the potential influence of host resources on the interaction between hosts and manipulative parasites has been emphasized, no study so far has directly addressed the question. Here, we experimentally tested the HERC hypothesis (Maure *et al.*, 2013) using one of the most studied systems in parasite manipulation, the acanthocephalan parasite *Pomphorhynchus laevis* and its intermediate host, the freshwater crustacean amphipod *Gammarus pulex* (Cézilly *et al.*, 2013). This parasite reproduces in different fish species and grows in its intermediate gammarid host, inducing, once the infective larval stage (cystacanth stage) has been reached, numerous behavioral alterations, such as reversed reaction to light (Cézilly *et al.*, 2000), decreased conspecific attraction (Durieux *et al.*, 2012) or reduced refuge use (Kaldonski *et al.*, 2007), the latter being linked with the probability of predation by definitive fish hosts (Kaldonski *et al.*, 2007; Perrot-Minnot *et al.*, 2007; Dianne *et al.*, 2011).

We relied on experimental infections of *G. pulex* collected in the wild to address the influence of host nutritional condition on the intensity of manipulation and classical infection parameters such as prevalence and intensity. To test for the effect of resources, we provided gammarids with either a standard or a deprived food treatment during parasite development. We then followed infection parameters (survival of gammarids, infection prevalence and intensity, developmental stage of parasites), assessed metabolism, and performed behavioral tests on both infected and control gammarids. We measured a single behavior only, the rate of refuge use, because, although *P. laevis* induces an infection syndrome in its host (i.e. a series of symptoms that appear to result from some major physiological disruption in the infected host; Perrot-Minnot *et al.*, 2014), this is the one which is most directly involved in parasite trophic transmission (Perrot-Minnot *et al.*, 2007; Dianne *et al.*, 2011). According to the HERC hypothesis, lowered host condition should result in a lower exploitation by parasites, possibly an increase in host mortality, and a change in the intensity of behavioral alterations, either in the sense of a decrease, indicative of an unaffordable high cost of performing altered

behaviors (*sensu* Thomas *et al.*, 2011), or in that of an increase, indicative of a minimization of the risk of premature host death (*sensu* Thomas *et al.*, 2002a).

Methods

Sampling

Uninfected gammarids were collected in a small tributary of the Suzon River (Burgundy, eastern France; 47° 24'12.6"N, 4°52'58.2"E), in October and December 2013. Only males were kept, because parasites fail to develop in females more often than in males (Franceschi *et al.*, 2008), whereas sex appears to have generally no effect on the extent of behavioral modifications (Bauer *et al.*, 2000, 2005; Cézilly *et al.*, 2000). Genetic analysis (see Lagrue *et al.*, 2014), performed on one third of the individuals (n = 330), showed that about 97% belonged to the species *Gammarus pulex*, with the remaining 3% belonging to the closely-related *G. fossarum*. Gammarids were acclimated in the laboratory for two days before experimental infections, in a room maintained at 10°C, which corresponds to the temperature of their natural habitat, and under a 12:12 light:dark cycle.

Naturally infected chubs, *Leuciscus cephalus*, were caught in the Vouge River (Burgundy, eastern France, 47°9'34.36'' N 5°9'2.50'' E) in October, and in the Vair River (Vosges, eastern France, 48°11'44.3"N 5°53'57.3"E) in December 2013. Adult parasites were taken from the intestines of the fish, and characterized by genetic analyses with the method described in Franceschi *et al.* (2008). Only parasite eggs from the species *P. laevis*, collected from 10 females sampled in five fish for the Vouge population, and in 13 females sampled in two fish for the Vair population, were mixed for each population and used for experimental infections.

Infections were therefore made using hosts and parasites that did not co-evolved. However, we have previously used gammarids from Suzon river for experimental infections, so the system is now highly characterized (Franceschi *et al.*, 2008, 2010a, 2010b; Dianne *et al.*, 2012; Perrot-Minnot *et al.*, 2014). Notably, infection in gammarids from the Suzon river reflects the infection characteristics of all other gammarid populations tested so far, but they are more sensitive to acanthocephalan infections (Franceschi *et al.*, 2010a), allowing to optimize the experimental infection rate. In addition, Perrot-Minnot *et al.* (2014) showed that syndromes induced by experimental infection using these hosts and parasites from the Vouge river are highly correlated with those of a natural infections.

Experimental infections and treatments

Experimental infections were performed following the procedure detailed in Franceschi *et al.* (2008). Overall, 374 and 301 individuals were exposed to parasite eggs from the Vair population and the Vouge

population, respectively (hereafter referred as “Vair-infected” and “Vouge-infected” individuals). Three hundred control individuals were maintained under the same conditions without eggs. After 48 hours of exposure, gammarids were placed in individual crystallizers, and randomly divided into two groups with different food treatments. Food treatments were chosen according to the natural food regime of gammarids and spatial variation in food availability observed in the field. Indeed, several studies have reported that, if given a choice, gammarids will feed on both leaf materials (shredder regime) and preys (predator regime), while cannibalism is often observed when only leaves are provided (MacNeil *et al.*, 1997; Kelly *et al.*, 2002). In temperate streams or rivers, the quality and quantity of food resources are highly dependent on environmental factors and, therefore, vary between rivers (Moss, 2010). Even along the upstream-downstream gradients, both the proportion of leaf detritus and prey availability can vary (e.g. Rosi-marshall & Wallace, 2002; Eedy & Giberson, 2007). Thus, individuals from the “standard food treatment” were fed weekly, alternatively with conditioned elm leaves and dead chironomid larvae (which provide a high source of proteins, Policar *et al.*, 2012). Individuals from the “deprived food treatment” received only elm leaves, once every two weeks. All individuals were maintained in the same room at 17°C ± 0.5 with a 12:12 light:dark cycle. Water was changed once every two weeks, using an oxygenated mix of water from the Suzon River and dechlorinated, UV-treated tap water.

Monitoring

All gammarids were checked on a daily basis. Gammarids found dead were immediately measured and dissected under a binocular microscope to determine the intensity of infection. Although this population of *G. pulex* is not infected by *P. laevis,* individuals can be infected with another acanthocephalan parasite species, *Echinorhynchus truttae*. Such infected individuals (n = 9) were removed from the experiment. Six weeks after infection, all gammarids were checked once a week under a binocular microscope to determine whether they were actually parasitized by *P. laevis*, and to monitor the date of the switch between the acanthella stage (ovoid shape, translucent orange color) and the cystacanth one (spherical and more pronounced opaque color, Dezfuli *et al.*, 1991). The width of cystacanth larvae from 77 gammarids infected by the Vouge population was measured as a proxy for larval size (n = 160 parasites), in order to determine the effect of food treatment on cystacanth size.

Behavioral measurements of refuge use

Behavior was recorded three times (hereafter referred as “rounds”) on all infected individuals: one day, 10 days, and 20 days after the cystacanth stage was detected. Behavior of control individuals was

tested similarly three times. Gammarids were individually placed in boxes (10.5 x 16 cm) filled with 250 mL of water, and labelled with a number, giving no clue about the group treatment to which the gammarids were belonging, and, thus, allowing blind recording. Boxes were containing a refuge at one extremity, consisting of a saucer terracotta pot (8.5 cm of diameter) cut in half, with a one centimeter hole in the convex part (see Dianne *et al.*, 2014). A period of 10 minutes of acclimatization was allowed following the introduction of gammarids. Then, the position of each gammarid was recorded every three minutes during 90 minutes, and scores were given for every observation (one if the individual was inside the refuge, zero if it was outside), such that summed scores at the end of each round could range from zero (always outside the refuge) to 31 (always inside).

Metabolic rate

Metabolic rate was estimated for each gammarid from its oxygen consumption, measured three days after the second round of behavior measurements (about 13 day-old cystacanths). We used SDR SensorDish® Reader (PreSens, Germany), a non-invasive device based on fluorescence (Köster *et al.*, 2008), following the protocol presented in Perrot-Minnot *et al.* (2014). As oxygen consumption is known to vary with body mass (Glazier, 2010), individuals were weighed immediately after the measure, following a quick drying on soft tissue.

Statistical analysis

We used a nominal logistic regression to investigate which parameters had an effect on prevalence (i.e. the proportion of gammarids harboring at least one parasite among those exposed to the infection), and a generalized linear model with a quasi-Poisson distribution and a log link-function to analyze infection intensity among infected individuals. A linear model was used to analyze the effects of food treatment, intensity, and host body size on the size of cystacanth larvae, with individual host identity as a random factor. The speed of parasites development was analyzed using chi-square tests.

Survival analysis started on the 39th day after exposure, corresponding to the time when parasites had become large enough to be detected upon dissection. This allowed us to distinguish between actually infected individuals and individuals exposed to infection but not successfully infected. Thus, subsequent statistical analyses (survival as well as metabolic rate and behavior) do not include individuals exposed to infection in which no parasite developed. Cox regressions were used to analyze host survival. First, we took into account all individuals to investigate the effect of infection status (control or infected with each population of parasites) and food treatment (standard vs. deprived). In a second step, we considered only infected individuals to analyze the relative influences

of infection intensity (either one, two, or more than two parasites, see Franceschi *et al.*, 2008), population of origin of the parasites, and food treatment.

Metabolic rate, expressed in milligram of O_2 consumed per minute, was log-transformed to meet normality, and analyzed with ANOVAs. We first investigated among all individuals the effect of their mass, food treatment, infection status and all interactions. Then, the same procedure was used considering only infected individuals, to explore the effect of the population of origin of the parasites.

Scores of refuge use were analyzed as repeated measures using the nparLD function, a R software package for nonparametric analyses of right-censured longitudinal data, allowing the decrease in sample size with time due to individuals' death (Noguchi *et al.*, 2012). Among Vair-infected individuals, no gammarid from the deprived food treatment survived until the third behavioral round. Therefore, the effect of food and infection status (control, Vair-infected or Vouge-infected) along time (rounds of measurements: one day, 10 days and 20 days after parasites reached cystacanth stage) were analyzed considering only the first and second rounds of behavioral measurements. Another analysis was conducted using the three behavioral rounds, but considering only individuals from the standard food treatment, allowing us to both analyze changes in behavior over a longer period of time and assess the effect of the population of origin of parasites. For each analysis, 'ANOVA-type statistics' were performed, followed by post-hoc 'pair-comparisons' (see Noguchi *et al.*, 2012, for details).

Spearman tests were used in order to check for the presence of potential trade-offs between metabolic rate or mortality rate, and the intensity of behavioral scores. To that end, we used scores from the second round of behavioral tests, since metabolic rate was measured soon after (three days).

Statistical analyses were performed using JMP version 10.0.0 software (SAS Institute, Cary, NC, U.S.A.) and R version 3.1.1 software (R Foundation for Statistical Computing). For each analysis described above, all factors and their second order interactions were first entered in the models. Except for non-parametric analyses where this procedure was not possible, we then compared the Akaike Information Criterion (AIC) among all of the possible models, and presented that one minimizing the AIC.

Results

Infection parameters

The overall nominal logistic regression (Chi2 = 62.29, d.f. = 4, P <0.0001) showed that the success of infection (prevalence) varied widely between populations of parasites (Likelihood Ratio Chi-square, LR-Chi2 = 54.73, d.f. = 1, P <0.0001), ranging from 43.13 % for gammarids exposed to parasites from the Vair, to 70.85 % for gammarids exposed to Vouge parasites. The size of gammarids had a significant

positive effect on the probability of infection (LR- Chi2 = 6.23, d.f. = 1, P = 0.01), whereas food treatment had none (LR- Chi2 = 0.86, d.f. = 1, P = 0.35).

Among individuals harboring parasites, a GLM showed that parasite intensity was significantly influenced by the interaction between food treatment and parasite population (Table 6, Fig. 10). Infection intensity was significantly higher in gammarids infected with parasites from the Vouge population. In this population, the deprived food treatment induced no change in the intensity of infection (Chi2 = 1.65, p = 0.20), whereas in Vair-infected gammarids infection intensity was lower under the deprived food treatment (Chi2= 4.33, p = 0.04; Fig. 10).

Table 6. Effect of parasite population, food treatment and host size on the infection intensity. Generalized linear model analyzing the effect of parasite population, food treatment and gammarid size on the infection intensity (number of parasites harbored by gammarid hosts). A quasi-Poisson error term and a log link-function were used. The model presented here minimized the AIC criterion.

Source of variation	d.f.	LR- Chi2	P
Parasite population	1	52.48	<0.0001
Food treatment	1	1.22	0.27
Gammarids size	1	0.02	0.89
Parasite population x Food treatment	1	8.06	0.004
Whole model: Chi2 = 57.86, d.f. = 4, P <0.0001			

The width of cystacanth larvae followed a normal distribution. The model minimizing the AIC contained food treatment, parasite intensity and their interaction. The size of cystacanth larvae decreased with infection intensity ($F_{1,\ 53.65}$ = 8.50, P = 0.005). Parasites from the deprived food treatment tended to reach a smaller size than those from the standard food treatment (Fig. 11; $F_{1,\ 45.12}$ = 2.73, P = 0.11), whereas the interaction between food treatment and infection intensity was not significant ($F_{1,\ 53.65}$ = 0.10, P = 0.75), indicating that infection intensity and food treatment had additive effects on parasite size.

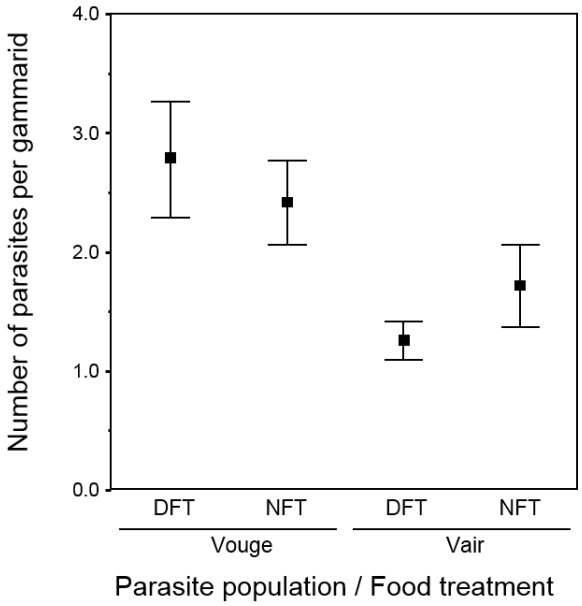

Figure 10. Parasite intensity within a host. Number of parasites per host according to the population of origin of parasites (Vouge and Vair) and the food-treatment received by the host (DFT or SFT, respectively deprived food treatment and standard food treatment). Dots represent means and error bars indicate 95% confidence interval.

Parasites were remarkably homogeneous in their development time, with all cystacanths appearing between the 10^{th} and the 11^{th} week after the infection, regardless of the population considered. In addition, there was no effect of food treatment on the speed of development (Chi-square test: Chi2 = 0.42, d.f. = 3, P = 0.94).

Host survival

Cox regression (Chi2 = 100.37, d.f. = 5, P <0.0001) considering all individuals showed that survival was significantly influenced by food treatment (LR- Chi2 = 16.02, d.f. = 1, P <0.0001), infection status (infected with each of the two parasite populations, or control individuals; LR- Chi2 = 67.04, d.f. = 2, P <0.0001) and their interaction (LR- Chi2 = 13.76, d.f. = 2, P = 0.001).

Overall, control individuals survived better than infected ones, irrespective of the food treatment (Fig. 12a). Vouge parasites were slightly less lethal than Vair parasites in gammarids exposed to the standard food treatment (Fig. 12a), but not in those exposed to the deprived food treatment.

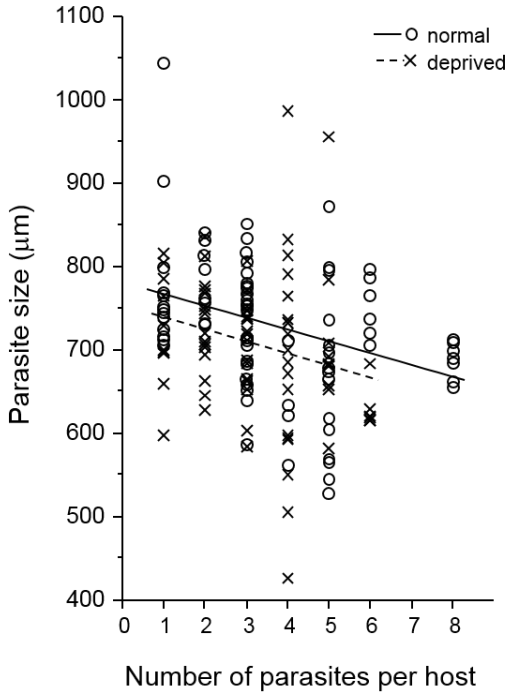

Figure 11. Parasites size at infective stage. Size of cystacanth larvae, according to infection intensity in each host, and food treatment. Each dot represents the width a larvae (µm). Circles and dotted line stand for the deprived food treatment, while crosses and full line represent the standard food treatment.

The deprived food treatment induced a significant decrease in the survival of control individuals, dropping to about half of that of individuals receiving the standard food treatment (Odd-Ratio from pairwise comparison, OR = 0.46, $CI_{95\%}$ = [0.34, 0.61], P <0.0001, Fig. 12a). This effect was also significant, but to a lower extent, in individuals exposed to Vouge parasites (OR = 0.75, $CI_{95\%}$ = [0.57, 0.99], P = 0.045, Fig. 12a), while no effect of food treatment was observed on the survival of individuals exposed to Vair parasites (P = 0.96, Fig. 12a).

Among infected individuals, a second Cox regression model (Chi2 = 15.46, d.f. = 4, P = 0.004) confirmed that host survival was higher in individuals infected with Vouge parasites compared to those infected with Vair parasites (LR- Chi2 = 7.67, d.f. = 1, P = 0.006). In addition, survival of individuals exposed to the standard food treatment was significantly higher than that of individuals exposed to the deprived food treatment (LR- Chi2 = 4.50, d.f. = 1, P = 0.03). Finally, the number of parasites had a significant influence on survival (LR- Chi2 = 7.93, d.f. = 2, P = 0.02, Fig. 12b), with a slightly better survival for gammarids harboring a single parasite.

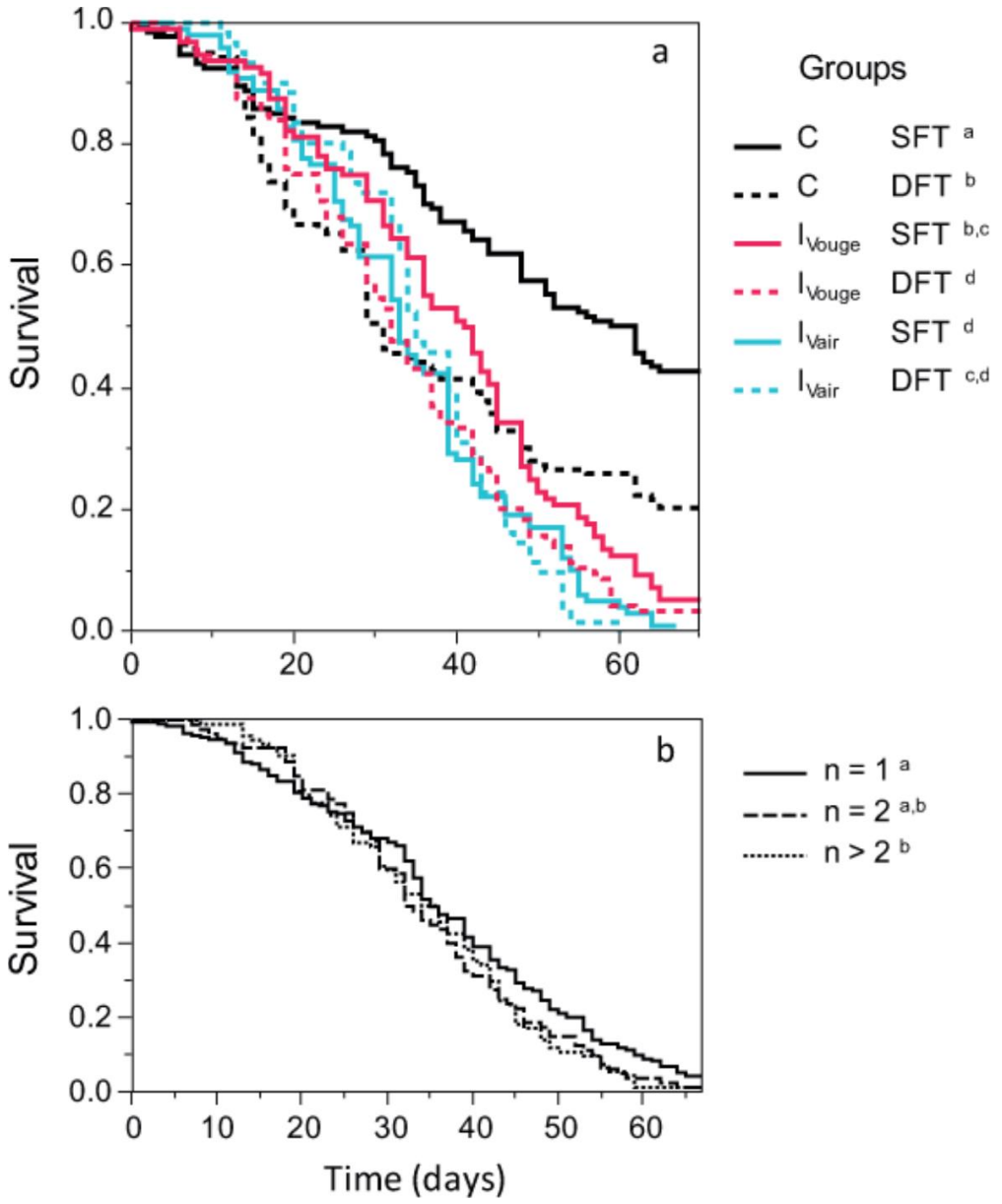

Figure 12. Hosts survival according to infection status and food treatment. Survival curves a) for all gammarids of the experiment according to their status (Control C, or infected by parasites from the Vouge or from the Vair rivers, respectively I_{Vouge} and I_{Vair}), and food treatment (standard food treatment SFT or deprived food treatment DFT); and b) for infected gammarids according to the number of parasites they harbor (n = one, two or more than two parasites per host). Time 0 was considered as the day from when we were able to determine whether gammarids did actually harbor a parasite or not. Letters in the legend indicate significant differences between groups, with similar letters indicating no difference (odd-ratios from pairwise comparisons, $p < 0.05$).

Metabolic rate

Oxygen consumption was significantly higher in infected individuals compared to control ones (ANOVA: $F_{-1,135}$ = 11.10, P = 0.001; Fig. 13), and was lower in gammarids from the deprived food treatment compared to those from the standard food treatment (ANOVA: $F_{-1,135}$ = 17.37, P <0.0001). Body mass of gammarids and all interactions were not significant and were removed from the model. Among infected individuals, a separate ANOVA indicated that the effect of food treatment was conserved (ANOVA: $F_{-1,55}$ = 9.97, P = 0.003), whereas there was no effect of the population of origin of parasites (ANOVA: $F_{-1,55}$ = 0.26, P = 0.61).

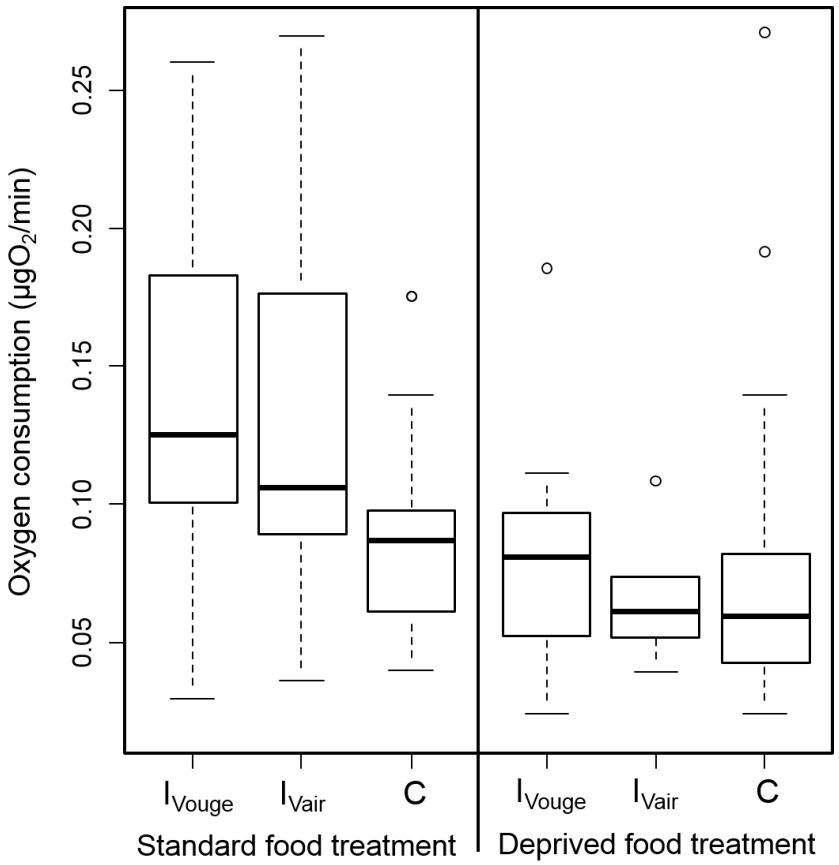

Figure 13. Hosts metabolism according to infection status and food treatment. Metabolic rate, expressed as oxygen consumption, of *Gammarus pulex* infected by parasites *Pomphorhynchus laevis* either from the Vouge (I_{Vouge}) or the Vair populations (I_{Vair}), or uninfected (control, C). Thick lines represent the medians, boxes represent the upper and lower quartiles, dotted lines represent the upper and lower deciles, and dots are outliers.

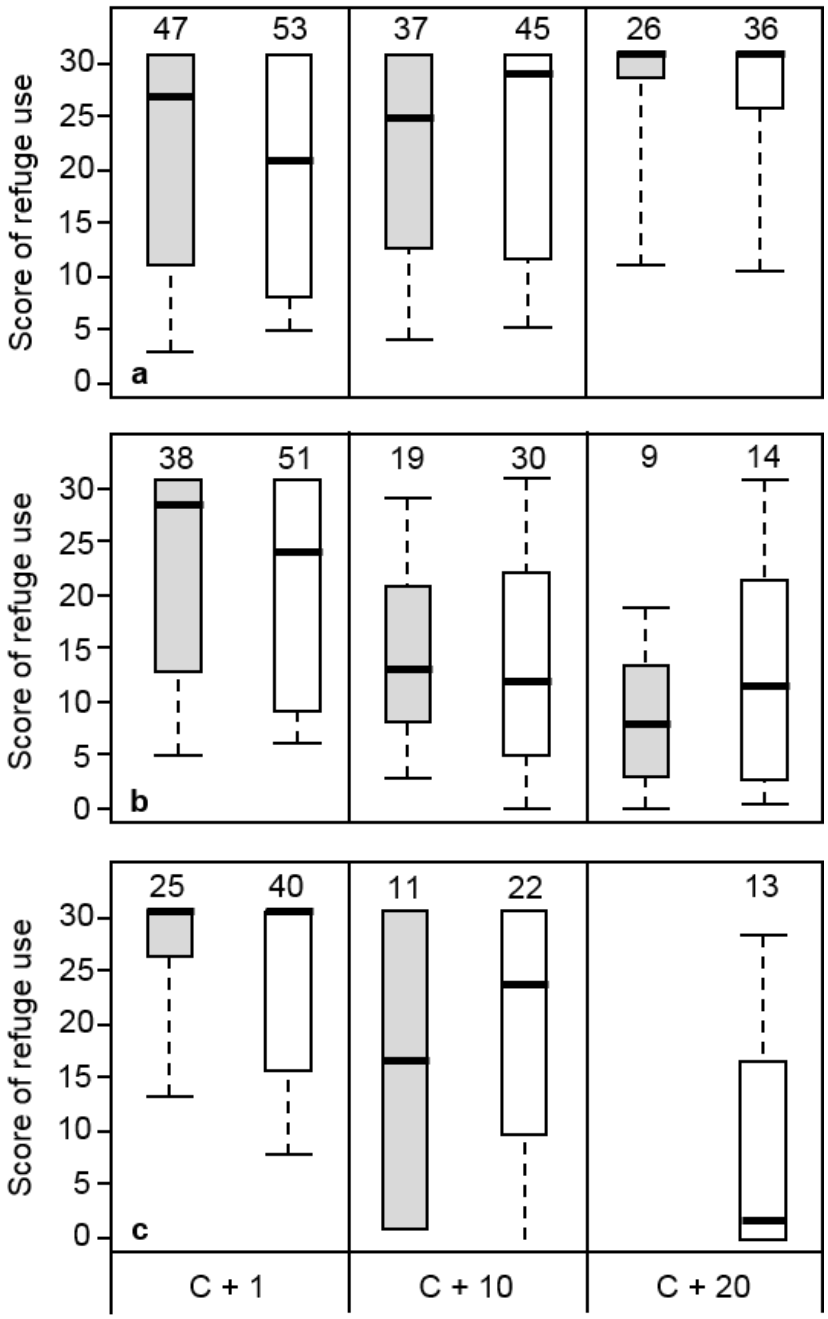

Figure 14. Host behavior according to infection status and food treatment. Scores of refuge use for a) control individuals; b) individuals infected by parasites from the Vouge population; and c) individuals infected by parasites from the Vair population. Grey plots represent groups who received the deprived food treatment and white plots stand for the standard food treatment. Scores are given for each of the three rounds: one day (C+1), 10 days (C+10) and 20 days (C+20) after detection of cystacanth stages. Sample sizes are given above each plot. Thick lines represent the medians, the boxes represent the upper and lower quartiles and dotted lines represent the upper and lower deciles.

Refuge use

In the model based on the two first behavioral rounds, parasite intensity and food treatment had no effect on refuge use (results not presented) and were removed from the analysis. The remaining model showed that infection status, time (behavioral rounds) and the interaction between these two factors significantly influenced refuge use (Table 7, Fig. 14). Post-hoc pair-comparisons revealed that refuge use decreased between the first and the second round for gammarids infected with both *P. laevis* populations (Fig. 14b, c), whereas it remained stable in control individuals (Fig. 14a, pair-comparisons 1 and 2 in Table 7). The intensity of refuge use also differed between the two infected groups (Table 7, pair-comparison 3), with a more pronounced decrease in refuge use in gammarids infected by parasites from the Vouge river compared to those infected by parasites from the Vair river (Fig. 14b, c).

Table 7. Behavioral scores during the two firsts rounds for all individuals. Results of the model from the nparLD R package, testing for the effects of status (Control, Vouge- and Vair-infected) and rounds of measurements on the scores of refuge use. Here, all individuals are considered regardless of their food treatment (not significant) but only the first two rounds are considered.

Factor	Statistic	d.f.	P
ANOVA TEST			
Status	5.50	1.95	0.004
Round	21.13	1	<0.0001
Status x round	9.54	1.64	0.0002
PAIR-COMPARISONS			
1) Vair-infected and Control individuals			
Status	1.44	1	0.23
Round	4.53	1	0.03
Status x round	10.97	1	0.0009
2) Vouge-infected and Control individuals			
Status	4.90	1	0.03
Round	13.56	1	0.0002
Status x round	29.14	1	<0.0001
3) Vair-infected and Vouge-infected individuals			
Status	10.13	1	0.001
Round	29.95	1	<0.0001
Status x round	0.15	1	0.70

The analysis of refuge use over the three series of behavioral tests, which was possible only for individuals from the standard food treatment (white labelling on Fig. 14), confirmed the strong interaction between infection status and rounds (Table 8). This interaction was due, as before, to the decrease vs. stability in refuge use between infected and control groups, respectively (Fig. 14, pair-comparisons 1 and 2 in Table 8). Refuge use also differed through time in infected individuals (Table 8, pair-comparison 3), with gammarids infected by parasites from the Vair river (Fig. 14c) decreasing their use of refuge during the third round, compared to gammarids infected with Vouge parasites (Fig. 14b).

Finally, all correlations between metabolic rate, survival and behavioral scores were non-significant (see Table 9).

Table 8. Behavioral scores during the three rounds for individuals from the standard food treatment. Results of the model from the nparLD R package testing for the effects of status (Control, Vouge- and Vair-infected) and rounds of measurement on the scores of refuge use. Here, only individuals from the standard food treatment are considered and the analysis was conducted on the three behavioral rounds.

Factor	Statistic	d.f.	P
ANOVA TEST			
Status	6.35	1.95	0.002
Round	6.69	1.90	0.002
Status x round	13.05	3.41	<0.0001
PAIR-COMPARISONS			
1) Vair-infected and Control individuals			
Status	6.99	1	0.008
Round	2.99	1.97	0.051
Status x round	24.85	1.97	<0.0001
2) Vouge-infected and Control individuals			
Status	12.97	1	0.0003
Round	1.28	1.73	0.27
Status x round	13.53	1.73	<0.0001
3) Vair-infected and Vouge-infected individuals			
Status	0.63	1	0.43
Round	17.52	1.9	<0.0001
Status x round	3.90	1.9	0.02

Discussion

Our results show that the experimental deprivation of host resources had significant effects on both host metabolism and survival. However, no consequence on the timing or the intensity of behavioral manipulation was observed.

Effects of reduced host resources

Experimental deprivation of host resources, through a decrease in quality and quantity, led to several significant modifications in both parasites and hosts. First, food treatment had a significant effect on host metabolism. In accordance with Hervant *et al.* (1997), the deprived diet induced a reduction in metabolic rate. This trend was conserved in infected individuals, while infection imposed an additional metabolic cost. Such an increase in metabolism has previously been reported in a crab parasitized by another acanthocephalan species (Haye & Ojeda, 1998, but see Rumpus & Kennedy, 1974) for contradictory result). Second, the deprived food treatment induced a rise in the mortality rate of gammarids. Although this rise was observed for both control and infected individuals, the effect of food deprivation was higher in the former. Those two main changes in hosts suggest that the deprived diet was, as expected, responsible for a general decrease in host body condition. In addition to those changes, the deprived diet induced a negative effect on parasites from one of the two populations, in terms of intensity of infection, while other parameters of infection (i.e. prevalence and timing of development) remained unaltered.

Table 9. Correlations between metabolism, behavior and survival. Spearman correlations between metabolic rate and behavior scores, and between survival and behavior scores (second behavioral round), for each infection status (individuals infected with the Vouge or the Vair population of parasites, and control individuals). When grouping the two infected groups, correlations were still not significant.

Factor	rho	n	P
Metabolic rate vs behavior			
Vouge-infected	-0.034	31	0.86
Vair-infected	0.015	23	0.95
Controls	-0.13	78	0.27
Survival vs behavior			
Vouge-infected	0.008	52	0.95
Vair-infected	-0.29	36	0.09
Controls	0.071	93	0.50

However, and contrary to our expectations and the predictions made by the HERC hypothesis (Thomas *et al.*, 2002a, 2011; Maure *et al.*, 2013), the deprived diet, while affecting host body condition, did not affect the intensity nor the timing of parasite manipulation, independently of the population of origin of the parasites. Several explanations can be proposed to explain why food treatment did not affect the behavior of infected hosts. First, contrary to what has been suggested (Thomas *et al.*, 2002a, 2011), behavioral alterations induced by parasites may not be a plastic, condition-dependent trait. Indeed, there was no correlation between either individual host survival or metabolic rate, and the intensity of behavioral manipulation, giving no evidence for any change in host exploitation strategy by parasites in terms of manipulation, following increased probability of host mortality.

Second, differences induced by the two food treatments may not have been important enough to induce significant plastic changes. This is however unlikely because host metabolism, host survival and parasite intensity were all affected by the deprived food treatment. It is unlikely that such differences were due to a lower food consumption by infected hosts compared to uninfected ones, as Fielding *et al.* (2003) showed that *Gammarus pulex* infected with another acanthocephalan parasite had similar feeding rates than controls, when they were fed with either leaves or dead chironomids. Third, resources may have been always sufficient to perform manipulation, such that food treatment would not influence host behavior, particularly if the energetic cost of refuge use is low. However, the weaker effect of food treatment on infected host survival compared to controls suggests that parasites exploited more resources when they were available, thus leaving a minimum to their hosts, although those extra resources were not invested in host manipulation. Alternatively, host manipulation as a whole could be a phenomenon requiring less energy than previously thought, such that resources available would not be a significant parameter among those leading to the variations observed in the intensity of parasite manipulation (see Thomas *et al.*, 2005b).

The higher exploitation of resources observed in hosts fed with the standard diet implies that parasites may have allocated this extra-energy to other fitness traits. Parasites could first reach a higher success of infection. In this study, however, experimental infections were conducted before we manipulated food resources, such that hosts did not differ in body condition before the infection. It is then not surprising that no difference was observed in prevalence between treatments. In contrast, food deprivation had a negative effect on parasites intensity in one of the two populations. Beckage & Riddiford (1983) also found that, in the lepidopteran species *Manduca sexta*, a lower number of hymenopteran parasites *Apanteles congregatus* would develop in hosts deprived from food. In the same way, other studies have shown that fewer parasites would develop if their hosts are starving (Pulkkinen & Ebert, 2004; Logan *et al.*, 2005; Seppälä *et al.*, 2008b). Therefore, additional resources in the host may allow the coexistence of multiple parasites, probably reducing the competition that occur among *P. laevis* sharing the same individual hosts (Dianne *et al.*, 2012). Ultimately, this could be

advantageous for the parasite because this would increase the probability of simultaneous transmission of several individuals, therefore increasing the probability of finding a mating partner in the definitive host (Brown *et al.*, 2001a).

An increase in the size of the parasites could also be a result of increased resources, leading to future beneficial effects, such as a better chance of establishment, and higher survival and fecundity in the definitive host (Poulin *et al.*, 2003; Steinauer & Nickol, 2003; Fredensborg & Poulin, 2005; Seppälä *et al.*, 2008b). Several studies have shown that parasite size increases with host size (Dezfuli *et al.*, 2001; Benesh & Valtonen, 2007a; Benesh *et al.*, 2009b), supporting a positive effect of higher levels of resources. Here, infection intensity significantly impacted the size of cystacanth larvae, confirming an effect of intra-host competition (Dianne *et al.*, 2012). Food treatment was however retained in the statistical model minimizing the AIC value, suggesting that this factor explains a part of the observed variance in parasite size, with cystacanth larvae being slightly smaller in the deprived food treatment.

Effects of parasite population

Our study also provides further evidence for the implication of the population of origin of parasites on the variability observed in behavioral manipulation (see Thomas *et al.*, 2011 for a review), as well as on other parameters of infection (Poulin, 2006). Among all the parameters considered in this study, only time to reach the cystacanth stage and the change induced in the metabolic rate of hosts were independent of the parasite population. Consistent with Franceschi *et al.* (2010a), we found that parasites prevalence was different between the two populations of parasites studied here. In addition, the deprived food resources induced a decrease in intensity only in the Vair parasite population. These results suggest that parasites from the Vouge population already occupy the whole ecological niche offered by the host, even at lower resources, while those from the Vair population benefit from higher resources to establish. Differences in prevalence and intensity could then be due to a stronger resistance of the hosts against the Vair parasites.

Finally, consistent with several other studies (Franceschi *et al.*, 2008, 2010a), different *P. laevis* populations differed in behavioral manipulation. Franceschi *et al.* (2010a) underlined that differences observed among several natural populations of parasites could be due to variation in the levels of resources in their environment. However, according to our results, it is more likely that those differences could be explained by other factors, such as intrinsic parameters of parasites population.

Conclusions

While the experimental manipulation of the host food resources induced, as expected, significant differences in their body condition, our study suggests that resources are not likely to explain the observed inter-population variability in behavioral manipulation. However, overall, our results suggest that host in better condition may contribute to higher parasite success in populations, because they suffer less parasite virulence and can host more parasite larvae of slightly larger size.

Acknowledgements

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Chapter IV. Impact of temperature

Temperature is known to impact parasite-host systems in multiple ways (Marcogliese, 2001; Harvell *et al.*, 2002). Several traits in the association between gammarids and their acanthocephalan parasites are already known to be affected by temperature. For instance, acanthocephalan parasites develop faster at high temperature (Olson & Pratt, 1971; Tokeson & Holmes, 1982), and their success of infection in their definitive fish host is also known to depend on temperature (Barber *et al.*, 2016). In parallel, temperature is also known to alter gammarids in multiple ways, influencing for instance their metabolism (Roux & Roux, 1967; Issartel *et al.*, 2005; Foucreau *et al.*, 2014), growth (Moenickes *et al.*, 2011), or activity (Issartel *et al.*, 2005). However, the impact of temperature on many traits of the association between acanthocephalan parasites and their hosts, including manipulation, remain to be investigated.

In this chapter, three experiments exploring such impact are presented. First, the effect of temperature was investigated on the alteration of gammarids behavior induced by two different acanthocephalan species, *P. tereticollis* and *P. minutus*. Naturally infected gammarids were used, and three behaviors were tested after acclimatization of gammarids at different temperatures in the laboratory. Second, the impact of two temperatures during the development of *P. laevis* parasites in their gammarid hosts was studied using experimental infections. Several infection parameters were measured and the behavior of gammarids was tested. Finally, to improve our understanding on the effect of temperature on parasite development, in terms of rapidity and intensity of infection, the effect of temperature on the immune system of uninfected gammarids was investigated.

1. Natural infection with *P. tereticollis* and *P. minutus*

ARTICLE 3

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

Sophie Labaude, Frank Cézilly, Thierry Rigaud

Draft manuscript

Abstract

Many parasites with complex life cycles are known to alter the phenotype of their intermediate hosts, in a way that is believed to increase their transmission towards their definitive hosts. Through this manipulation, some parasites can alter the ecological role of their hosts leading to consequences at the scale of the ecosystem. In a context of global warming, understanding the impact of temperature on ecologically important species had become a major challenge. However, despite their ecological importance, the impact of temperature on behavioral alterations induced by manipulative parasites remains unknown. Acanthocephalan parasites are known to alter multiple behaviors of their gammarid hosts, ultimately leading to modifications in their ecological role in freshwater ecosystems. Despite indirect evidence suggesting an effect of temperature on the manipulation induced by acanthocephalan parasites, no study directly investigated such effect. Here, we explored the effect of temperature on the manipulation induced by two acanthocephalan parasites on their gammarid host, *Gammarus fossarum*. The fish parasite *Pomphorhynchus tereticollis* is known to induce an increase in the photophilic behavior of its host, and a lower use of refuges, while the bird parasite *Polymorphus minutus* alters its host geotaxis, with infected hosts swimming closer to the water surface. We relied on uninfected and naturally infected gammarids and exposed them for two weeks at different temperatures before testing their behavior. Our results show that higher temperature increased the phototaxis of gammarids, with a stronger effect on those infected by *P. tereticollis.* The effect of temperature was weak on the use of refuges. Overall, the manipulation on these two behaviors was more efficient at high temperature. In contrast, no effect of temperature was found on the geotaxis of gammarids, whether they were infected or not by *P. minutus*. Our results provide the first direct evidence that temperature could affect the extent of manipulation by certain species. The absence of effects in the manipulation induced by other species might result from different mechanisms of manipulation.

Keywords

Parasite manipulation, temperature, acanthocephalan parasite, gammarid, phototaxis, geotaxis, *Gammarus fossarum*, *Pomphorhynchus tereticollis*, *Polymorphus minutus*

Introduction

Numerous and diverse parasite species, ranging from bacteria and viruses to different groups of animals, such as nematodes, cestodes or acanthocephalans, are known to induce changes in the

phenotype of their hosts (Poulin & Thomas, 1999; Cézilly *et al.*, 2014; Heil, 2016). In particular, parasites with complex life-cycles that involve a trophic transmission often manipulate their intermediate hosts in a way that increases their probability of being transmitted to their definitive hosts, and thus their probability of completing their life cycle (Thomas *et al.*, 2005b). It is widely recognized that this phenomenon might be of major importance in ecosystems, for instance affecting the population dynamics of many species or modulating food webs and habitats (Lefèvre *et al.*, 2009; Labaude *et al.*, 2015a). In the current context of global change, understanding how environmental conditions might alter ecologically important species had become a major challenge, notably due to the necessity to make accurate predictions about the consequences of such changes. However, despite the important role played by manipulative parasites in their ecosystems, their interaction with environmental conditions received little attention (Labaude *et al.*, 2015a).

Environmental factors might be of great importance in the extent of manipulation. The impact of biotic factors, such as the presence of other parasites within the same host (Haine *et al.*, 2005; Dianne *et al.*, 2012; Hafer & Milinski, 2016), received substantial attention from scientists. However, only few studies investigated the effect of abiotic parameters. Among these studies, Perrot-Minnot *et al.* (2012) found that the difference of phototaxis between amphipods infected with an acanthocephalan parasite and control amphipods was reduced at low light intensity, due to a lower response of uninfected hosts to light. Benesh *et al.* (2005) found that the properties of light in terms of wavelength also affected the phototaxis of amphipods and the extent of the manipulation induced by another acanthocephalan species. On the other hand, the manipulation induced by acanthocephalan parasites was shown to be independent from the quality of food resources available for their gammarid hosts (Labaude *et al.*, 2015b). Apart from these studies, the evidence regarding the effects of abiotic environment on manipulation, including temperature, remain scarce despite its effect on numerous other biological traits of both hosts and parasites (Marcogliese, 2001).

Acanthocephalan parasites are an important biological model in the study of parasite manipulation, with all the species studied so far being able to induce phenotypic alterations in their hosts (Crompton & Nickol, 1985). In particular, several acanthocephalan species, using either birds or fish as definitive hosts, induce different types of modifications in the behavior of their gammarid intermediate hosts. Gammarids are crustacean amphipods that are themselves key species in freshwater ecosystems, where they constitute either an important prey (Degani *et al.*, 1987; Friberg *et al.*, 1994) or a predator (MacNeil *et al.*, 1997) for many species, and also have a major role in the maintenance of water quality through the shredding of dead leaves (Piscart *et al.*, 2009; Foucreau *et al.*, 2013a). Many studies showed that infection with acanthocephalan parasites induces modifications

in the behavior of gammarids that can ultimately lead to alterations in their ecological role (Fielding *et al.*, 2003; Médoc *et al.*, 2011b; Labaude *et al.*, 2016).

Temperature stands as a major parameter affecting many parasite-host systems (Marcogliese, 2001; Harvell *et al.*, 2002). In particular, several traits in the association between acanthocephalan parasites and their gammarid hosts have already been reported to depend on temperature, such as the time of development of the parasites (Olson & Pratt, 1971; Tokeson & Holmes, 1982), or the metabolism of their hosts (Pöckl & Humpesch, 1990). The success of infection in their definitive fish host is also known to depend on temperature (Sheath *et al.*, 2016). However, while temperature was suggested to modify manipulation in multiple ways, reviewed in Labaude *et al.* (2015a), only indirect evidence exists so far. For instance, Franceschi *et al.* (2010b) showed that gammarids experimentally infected during winter were slower to display altered behaviors than gammarids infected in spring with the same parasite populations. This trait was correlated with the development time of the parasites, with fastest parasites being unable to induce rapid changes in the phototaxis behavior of their hosts. Authors gave several hypotheses to explain these differences, such as a seasonality effect in the physiology of gammarids and their parasites, or differences in the environmental conditions experienced by acanthocephalan mothers in the field. They also acknowledged that laboratory temperature could be slightly different between the two experiments. Although this experiment gives indirect clues that abiotic environment, in particular temperature, could affect manipulation, this was not formally tested. Given the predicted increase of temperature in future years, improving our understanding of the consequences of temperature on host-parasite relationships proves particularly relevant (Labaude *et al.*, 2016).

Here, we tested the effect of temperature 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* is known to induce an increase in the photophily of its intermediate host (Tain *et al.*, 2006), as well as a decrease in its use of refuges (Perrot-Minnot *et al.*, 2007), infection by *P. minutus* leads to altered 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 these changes occur, 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) in September 2015, and in the Vèze River (eastern France, 47°14'01.9"N 5°34'37.4"E) in June 2016, using a kick sampling method with a hand net. While the Norges River is known to only contain *G. fossarum* species (Labaude *et al.*, 2016; Lagrue *et al.*, 2014), Lagrue *et al.* (2014) found that more than half of gammarids from the Vèze River belonged to the closely-related *G. pulex* species. However, more recent genetic analyses performed on 457 individuals sampled in the Vèze River in May 2015 showed that 90% of them (n = 410) belonged to the *G. fossarum* species (S. Labaude, unpublished data). The two populations were chosen because gammarids naturally harbor the acanthocephalan parasites *Pomphorhynchus tereticollis* and *Polymorphus minutus*, respectively. 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 divided into groups that were acclimatized for 12 days in the laboratory at different temperatures. Individuals from the Norges River were acclimatized to three temperatures (10, 14 or 18°C), while individuals from the Vèze River were separated into only two groups (14 or 18°C) because of the scarcity of individuals naturally infected by *P. minutus* and to ensure large enough sample sizes for data analyzes. Temperatures were chosen to be compatible 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 along the year 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 linked to the behavioral tests, water temperature was controlled in two different ways. Individuals from the Norges River, for which behavioral tests necessitated to be visible from above, were maintained into water baths, following Labaude *et al.* (2016). 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 fridges with transparent doors. 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

In total, 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 in 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 five minutes of habituation in the tube, the position of every individual was recorded every 30 seconds during five minutes 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 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 one centimeter hole in the convex part (see Dianne *et al.*, 2014). After five minutes of habituation in the device following the introduction of gammarids, the position of each individual was recorded every two minutes during 30 minutes, and scored as zero (inside the refuge) or one (outside the refuge). Summed refuge scores ranged from zero (always inside the refuge) to 16 (always outside) for each individual.

For the two tests, a random number was assigned to each individual, independently of its supposed parasite status, ensuring blind recordings.

Geotaxis

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

Geotaxis, which corresponds to 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 are known to be altered by the parasite (Bauer *et al.*, 2005). Each cylinder was placed in a fridge at the relevant temperature. It also ensured that the light came only horizontally, through the glass door, thus avoiding any confounding phototactic reaction. After two minutes of habituation in the cylinder, the position of each gammarid was recorded every 30 seconds for five minutes, 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 to 55 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, known to present a sexual dimorphism in amphipods (Hume *et al.*, 2005). All individuals were measured (height of the fourth coxal plate) using a 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 is known to depend both on 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 (*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 use 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 showed), 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 showed). Thus, sex was not considered.

First, the effect of temperature on each score was assessed with Kruskal-Wallis tests, except for geotaxis for which only two temperatures were tested. 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 deltas (Cliff, 1996). Cliff's deltas were 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 deltas were calculated using the R-package 'orddom' (version 3.1).

Statistical analyses were performed using R version 3.1.1 software (R Foundation for Statistical Computing).

Results

Temperature significantly affected the phototaxis score of *G. fossarum* individuals infected by *P. tereticollis* (Kruskal-Wallis, Chi2 = 24.02, d.f. = 2, $p < 0.0001$, Fig. 15A), as well as that of control individuals (Kruskal-Wallis, Chi2 = 22.44, d.f. = 2, $p < 0.0001$, Fig. 15A). The phototaxis score was significantly higher at 14°C and 18°C compared to 10°C, in both parasitized and control individuals (Fig. 16A). However, while there was a clear trend for phototaxis score to also 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. 16A), with even a tendency to decrease. As a result, the difference of behavior between control and parasitized individuals was only significant at 14°C and 18°C, with stronger effect at 18°C (Fig. 16B).

Temperature had no effect on the refuge use behavior of both control individuals (Kruskal-Wallis, Chi2 = 0.57, d.f. = 2, $p = 0.75$, Fig. 15B) and infected ones (Kruskal-Wallis, Chi2 = 1.83, d.f. = 2, $p = 0.40$, Fig. 15B). The score of refuge use of individuals infected by *P. tereticollis* was higher than that of controls (Fig. 15B), illustrating a lower tendency to use refuges, although this difference was significant only at 18°C (Fig. 16B). This might be explained by a weak tendency of parasitized individuals to spend less time in refuges at 14°C and 18°C compared to 10°C (Fig. 15B and Fig. 16A), some of these non-significant differences being probably due to high inter-individual variation relative to the small sample size.

Correlations between phototaxis scores and scores of refuge use were all non-significant. The only trend observed was a positive correlation in parasitized individuals at 18°C (Table 10), where the more photophilic animals also spent more time out of refuges.

Individuals infected by *P. minutus* were significantly affected by the parasite, with a strong higher geotaxis score for parasitized individuals compared to control ones (Fig. 17 and Fig 16B). Geotaxis scores were not influenced by temperature, with no difference between the two temperatures tested for both parasitized and control individuals (Fig. 17 and Fig 16A).

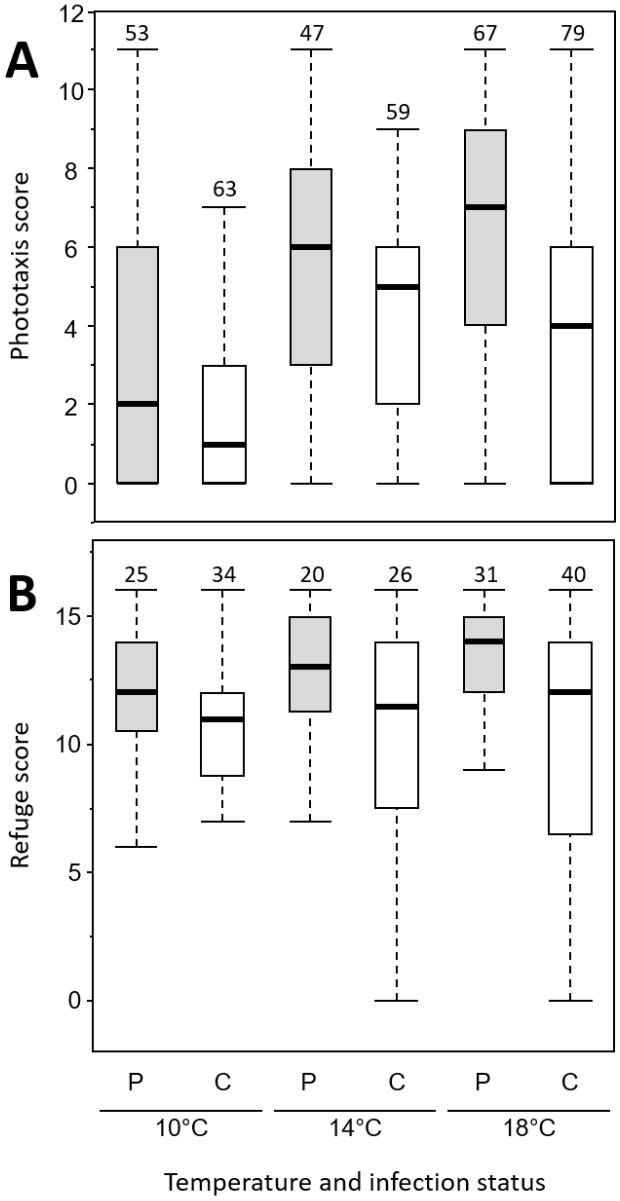

Figure 15. Individual *Gammarus fossarum* behavior scores for (A) phototaxis tests and (B) refuge use tests (higher scores representing a longer time exposed in the light or outside of refuges), according to their infection status (P or C, respectively parasitized by *P. tereticollis* or control) and the temperature (10, 14 or 18°C). Thick lines represent the medians, boxes represent the upper and lower quartiles, and dotted lines represent the upper and lower deciles. Sample sizes are indicated.

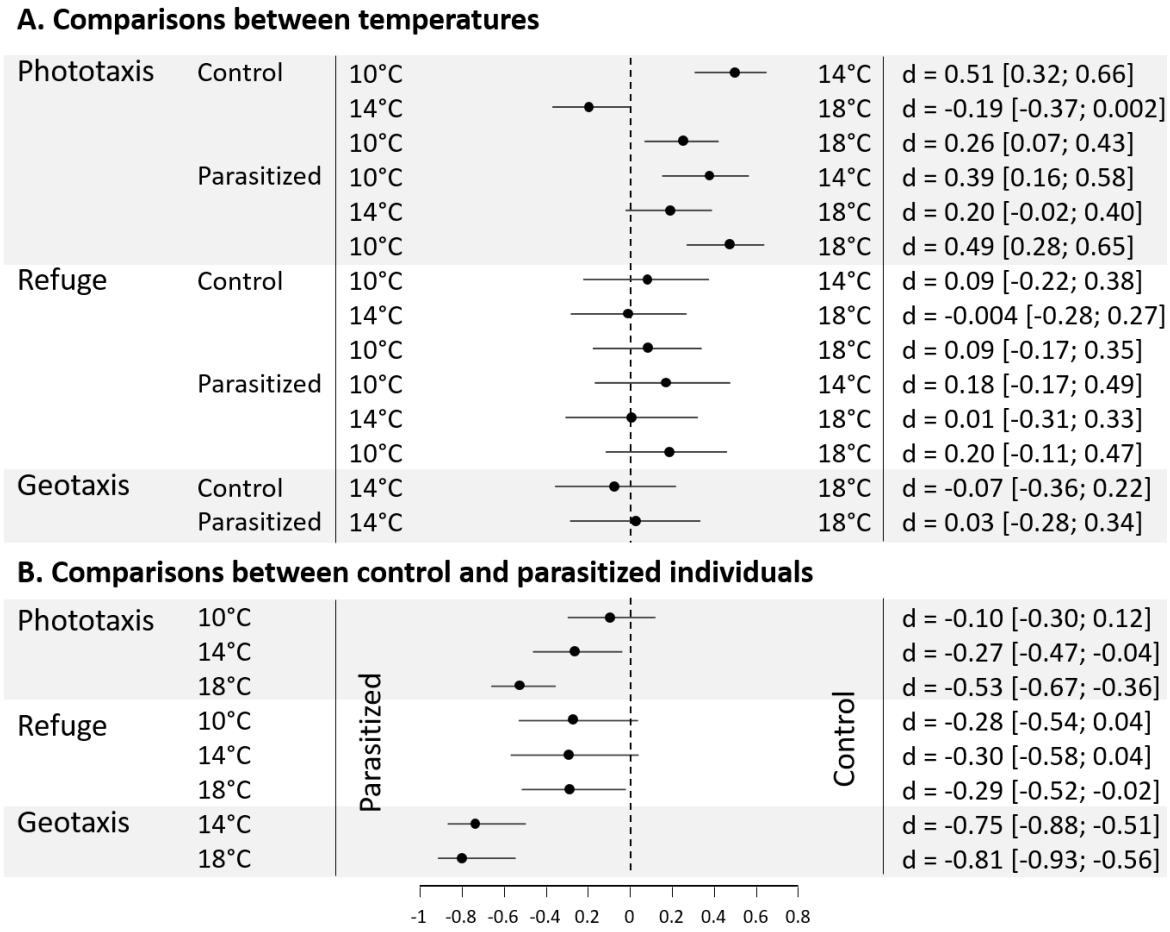

Figure 16. Effect sizes (Cliff's delta, d) of the behavioral differences (A) between temperatures, for each test and each infection status (parasitized and control), and (B) between parasitized and control *G. fossarum* individuals, for each test (phototaxis, refuge use and geotaxis) at each temperature (10, 14 and 18°C). Cliff's delta effect sizes are represented with their 95% confidence intervals, and their values are given. Values under 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 the phototaxis scores of control individuals were significantly higher at 14°C compared to 10°C.

Discussion

Our results show that temperature could influence the extent of manipulation on certain, but not all, behaviors of gammarids that are altered by acanthocephalan parasites. In particular, temperature affected the behavioral manipulation of *G. fossarum* gammarids by *P. tereticollis* parasites in terms of phototaxis, but had a more limited impact in terms of time spent inside refuges. Indeed, the differences in phototaxis observed between infected and control individuals suggest that parasite manipulation was less efficient at low temperatures. This result first arises from a photophobic behavior conserved in both infected and uninfected animals at low temperature. Then, at higher temperatures, a gradual

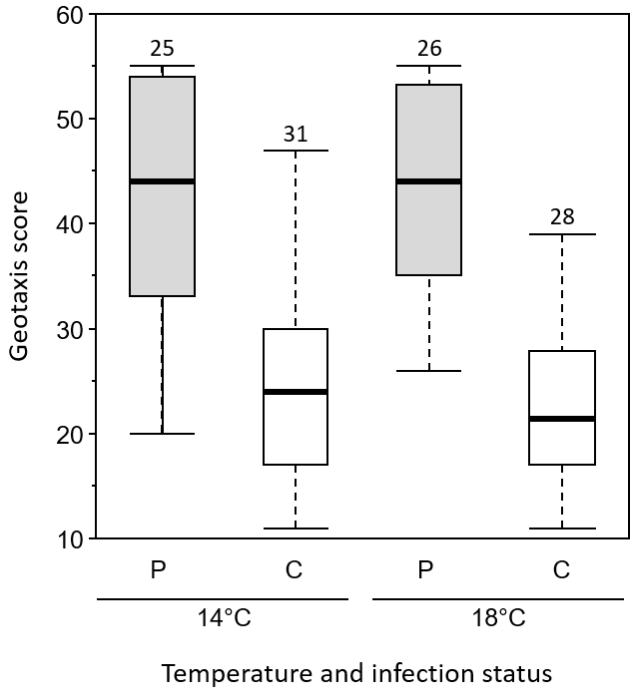

Figure 17. Individual *Gammarus fossarum* geotaxis scores according to their infection status (P or C, respectively parasitized by *P. minutus* at the cystacanth stage, or control) and the temperature (14 or 18°C). Thick lines represent the medians, boxes represent the upper and lower quartiles, and dotted lines represent the upper and lower deciles. Sample sizes are indicated.

increase in the photophilic behavior was observed in infected individuals, but this increase stabilized (with a tendency to decrease) in control individuals at the highest temperature. The same trends, but non-significant, was observed for refuge use in infected animals, while no effect of temperature was observed for control individuals, resulting in a difference between infected and uninfected individuals being significant only at 18°C for this behavior. In our experimental groups, correlations between phototaxis and refuge use scores were non-significant. Perrot-Minnot *et al.* (2012) showed that phototaxis intensity in itself might not be responsible for the increased predation rate of parasitized individuals. In contrast, the presence of refuges and the intensity of their use were shown to be linked with this differential of predation (Kaldonski *et al.*, 2007; Dianne *et al.*, 2011). Therefore, a direct link between these behaviors may not be as expected as an a priori reasoning. In another association between gammarids and manipulative parasites, Coats *et al.* (2010) observed positive correlations among behaviors in infected animals only, concluding that behavioral syndromes may only be manifested following parasite infection, whether due to parasite manipulation or mere physiological stress. Our finding that one correlation is stronger and marginally significant at high temperature in infected animals suggest that such syndromes may also be affected by abiotic environment, emphasizing the necessity to study behavioral manipulation in different ecological contexts.

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

Infection status	Temperature	Spearman's rho	P
Control	10°C	0.167	0.344
	14°C	0.301	0.124
	18°C	0.053	0.743
Parasitized	10°C	0.017	0.936
	14°C	0.189	0.429
	18°C	0.416	0.020[1]

[1] non-significant after Bonferroni correction

The differences in the phototaxis behavior observed at different temperatures might be due to several phenomena. First, the increased efficiency of manipulation at high temperature might be due to plasticity in parasite manipulation. Indeed, gammarids metabolism is known to increase with temperature to a certain extent (Roux & Roux, 1967; Pöckl & Humpesch, 1990; Issartel *et al.*, 2005), along with their mortality (Maazouzi *et al.*, 2011; Foucreau *et al.*, 2014). Parasites completely depend on the survival of their host before transmission. Thus, if parasites are able to manipulate the behavior of their hosts in a plastic way, they should increase their manipulative efforts when the life expectancy of their host decreases in order to secure their transmission (Thomas *et al.*, 2002a, 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, Labaude *et al.*, (2015b) observed no significant change in refuge use in *G. pulex* infected with *P. laevis* under different survival conditions.

Second, the increase in the manipulation of gammarids by parasites might be due to simple physiological effects of temperature. Indeed, the metabolism of ectotherm species is known to increase with temperature (Gillooly *et al.*, 2001). This effect is also found in acanthocephalan parasites, with a development time that is highly dependent on temperature (Olson & Pratt, 1971; Tokeson & Holmes, 1982). Thus, if manipulating the behavior of their host is linked with the physiology of parasites, it is possible that increased temperatures lead to more pronounced manipulation. The effect of temperature on the metabolism of gammarids might also explain their behavior. Indeed, the phototaxis of both control and infected individuals was shown to increase between 10°C and 14°C. This result might be explained by an increase in gammarids global activity with temperature (Issartel *et al.*, 2005). Resting gammarids might indeed benefit from staying in dark places, where they are *a priori*

less vulnerable to predators. In contrary, more active individuals might increase their exploration, thus spending more time in the light zone. However, this hypothesis does not explain the fact that the phototaxis of uninfected gammarids did not also increase at the highest temperature, neither the fact that infected gammarids, supposed to be manipulated, exhibited a photophobic behavior at the lowest temperature.

Third, the differences in manipulation observed between temperatures might be related to the level of stress in gammarids. Indeed, although temperature induces an increase in gammarids metabolism, thus potentially explaining the increase of phototaxis between 10°C and 14°C, it can also become a stressor when reaching high values (Maazouzi *et al.*, 2011). It was recently demonstrated that the food consumption of *G. fossarum* individuals increased between 10°C and 14°C, but decreased at 18°C only when individuals were kept in isolated conditions, which were interpreted as a stressful condition considering the aggregative habit of gammarids (Labaude *et al.,* 2016). Although harboring parasites might constitute a stress in itself, manipulative parasites could actually decrease the level of anxiety of their hosts. This hypothesis might explain the higher propensity of infected gammarids to spend a high proportion of their time out of refuges, or their absence of anti-predator behavior, such as aggregation or escape, in the presence of fish odor (Baldauf *et al.*, 2007; Kaldonski *et al.*, 2007; Perrot-Minnot *et al.*, 2007). Moreover, parasites have been shown to induce changes in the brain serotoninergic activity of their gammarid hosts (Tain *et al.*, 2006, 2007). These modifications are believed to be responsible for the alteration of several behaviors, forming altogether an infection syndrome (Cézilly & Perrot-Minnot, 2010). Experimental injections of serotonin confirmed its importance, leading to behavioral changes in uninfected gammarids that were comparable to changes observed in manipulated individuals (Perrot-Minnot *et al.*, 2014). Moreover, the presence of fluoxetine in the water, a widely prescribed anti-depressant drug, was also shown to induce changes in the behavior of gammarids that resemble those induced by parasites (Guler & Ford, 2010). Fluoxetine constitutes a serotonin reuptake inhibitor that can thus affect the serotonin level in amphipods brain. Although the mechanisms in which acanthocephalans induce modifications in gammarids serotoninergic activity are not clearly identified yet (Lafferty & Shaw, 2013), serotonin was pointed out to play a role in the regulation of fear and anxiety, including in invertebrates (Curran & Chalasani, 2012). It is thus possible that parasites manipulate their hosts by rendering them less anxious, decreasing their natural fear of predation and leading them toward places that are exposed to predators. Following this logic, we might also expect infected gammarids to be less susceptible to other stress, such as an elevated temperature. In parallel, an acclimatization for several days to high temperature was shown to induce an increase in serotonin levels in invertebrates (Stefano & Catapane, 1977; Stefano *et al.*, 1977). This could explain the fact that the phototaxis of infected individuals kept

increasing above 14°C, possibly due to either a higher metabolism or an effect on serotonin levels, while that of control gammarids stabilized or decreased, possibly due to stressful conditions. However, following this hypothesis, we might also have expected similar results in the refuge use of uninfected gammarids. In addition with the lack of power due to smaller sample size, already pointed out earlier, it is possible that our experimental conditions, where predator cues were absent, were not stressful enough to observe significant changes. However, it is interesting to note that, although serotonin was pointed out to modify the phototaxis of gammarids, its effect on their use of refuges was not significant (Perrot-Minnot *et al.*, 2014). Moreover, the intensity of changes induced by parasites was also shown to be more important for the phototaxis behavior compared to the use of refuges (Perrot-Minnot *et al.*, 2014), maybe explaining the absence of clear effect of temperature.

Interestingly, the manipulation of *G. fossarum* by *P. minutus* was not affected by temperature, contrary to that of *P. tereticollis*, albeit we acknowledge that the lower temperature was not tested. First, the behaviors that are modified by the parasite differ from *P. tereticollis* (Bauer *et al.*, 2005; Tain *et al.*, 2006), and manipulation might not rely on the same mechanisms. Indeed, Tain *et al.* (2006) showed that infection by *P. minutus* did not induce modifications in the serotoninergic activity of gammarids, while the geotaxis of gammarids was not affected by experimental injections of serotonin. In a recent paper, Perrot-Minnot *et al.* (2015) found that uninfected *G. 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, suggesting a role of anaerobic metabolism and hypoxia in the manipulation induced by *P. minutus*. It would thus be interesting to test the effect of temperatures that are different enough to induce higher changes in the quantity of oxygen dissolved in the water. If this mechanism is accurate, we would then expect the geotaxis of both infected and uninfected individuals to be increased at high temperatures.

Overall, our study suggests that temperature might be responsible for variations in the efficiency of the manipulation of gammarids by *P. tereticollis*. Although the metabolism of acanthocephalan parasites is known to be highly dependent on temperature, thus resulting in longer developmental time during cold periods (Olson & Pratt, 1971; Tokeson & 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 (VanCleave, 1916; Muzzall & Rabalais, 1975; Brown, 1989). To our knowledge, this study is the first to directly assess the effect of temperature on manipulation by acanthocephalan parasites on gammarids (but see Benesh *et al.*, 2009a, for contradictory results in isopods). However, it was not possible in this study to control the environmental conditions experienced by gammarids and their parasites during their development, and this can lead to other sources of variability. For instance,

acanthocephalans develop faster at high temperatures (Olson & Pratt, 1971; Tokeson & Holmes, 1982) and the efficiency of their manipulation increases with time after they reached the cystacanth stage (Franceschi *et al.*, 2008; Labaude *et al.*, 2015b). Thus, it cannot be discarded that gammarids maintained at 18°C harbored parasites that already reached their highest manipulative ability, while that of parasites from gammarids kept at lower temperatures was still increasing. Despite that the acclimatization period was chosen to be long enough to avoid such phenomenon, future studies might benefit in investigating the effect of temperature on a longer term, using experimental infestations to control environmental conditions during the development of the parasites.

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2. Experimental infestations with *P. laevis*

ARTICLE 4

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Changes induced by temperature on host-parasite interaction in the association between a gammarid and its acanthocephalan parasite: the exception of behavioral manipulation

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Draft manuscript

Abstract

Parasites are known to alter many traits of their hosts. In particular, several parasites with complex life-cycles induce phenotypic alterations in their intermediate hosts that are believed to increase their probability of transmission to their next hosts. Although such alterations can lead to profound modifications in the ecological role of key species, the proximate factors modulating this phenomenon remain poorly known. In particular, temperature is known to have many impacts on host-parasite associations. In a context of global warming, understanding the impact of temperature had become a major challenge. Gammarids are ecologically important freshwater crustaceans that play several roles in their ecosystems. They also constitute the intermediate host for several species of acanthocephalan parasites that are known to induce multiple effects on their hosts, including alterations of their behavior, ultimately leading to modifications in their functional role. Here, experimental infections were used to assess the effect of temperature on several traits of the association between *Gammarus pulex* amphipods and their acanthocephalan parasites *Pomphorhynchus laevis*. Gammarids were maintained in two different temperatures during the development of their parasites. Infection parameters were measured (infection success, parasite load, host survival), and the behavior of gammarids, in terms of general activity and use of refuges, was tested. Temperature affected most parameters measured in both parasites and their hosts. At high temperature, gammarids survival was decreased and their activity level was increased. In parallel, parasites developed faster, were more numerous and everted their proboscis sooner. Despite all these effects, neither the timing nor the intensity of manipulation in terms of use of refuges were affected by temperature, suggesting that manipulation could be independent from proximal environmental factors.

Keywords

Gammarus pulex, *Pomphorhynchus laevis*, parasite manipulation, climate change, refuge use

Introduction

The role of parasites within ecosystems was neglected for a long time, probably due to their small size and their life style that make them invisible to us. However, parasites are now highly recognized as important ecological actors that can modulate their ecosystems (Hatcher *et al.*, 2012). In particular, many parasites with complex life cycles rely on a trophic transmission between their intermediate and definitive hosts, and are thus embedded into food webs. Moreover, trophic-transmission is often accompanied with changes in the phenotype of parasites' intermediate hosts that makes them more

vulnerable to predation by definitive hosts, thus increasing the probability of transmission of parasites (Moore, 2002b; Thomas *et al.*, 2005b). Parasite manipulation has been pointed out in many examples to have profound consequences on ecosystems, such as alterations in food webs, modifications in the dynamic of host populations, or changes in habitats (see Labaude et al., 2015b and Lefèvre et al., 2009 for reviews). Understanding how abiotic conditions influence the stability of ecosystems had become a major challenge in the recent years, notably due to the need to anticipate the impact of global changes. Consequently, the effect of many factors, such as temperature or pollution, has been investigated in many ecologically important species (e.g. Salminen et al., 2001; Sanford, 1999). However, despite the growing recognition of their important role within ecosystems, little is known about how abiotic conditions might alter the influence of manipulative parasites on their hosts (Labaude et al., 2015b).

Gammarids are crustacean amphipods that are widespread throughout a large range of freshwater habitats (MacNeil *et al.*, 1997; Piscart *et al.*, 2009). They are considered as a key species because of their three-fold central place within food webs. First, because they are often among the dominant macroinvertebrate species in terms of biomass in their aquatic habitats (MacNeil *et al.*, 1997), they represent an important prey for many other species (Degani *et al.*, 1987; Friberg *et al.*, 1994). Second, gammarids are themselves a major predator for many species (MacNeil *et al.*, 1997; Kelly *et al.*, 2002), and their predation is known to be important enough to modulate the composition of freshwater macroinvertebrates communities (Kelly *et al.*, 2002; Piscart *et al.*, 2010). Third, they are also known to be involved in the maintaining of water quality as well as in the recycling of organic matter through their shredder role on dead leaves (Maltby *et al.*, 2002; Piscart *et al.*, 2009; Foucreau *et al.*, 2013a; Constable & Birkby, 2016).

Gammarids constitute a host for many parasitic species ranging from bacteria to macro-parasites such as helminths (Dunn & Dick, 1998; Grabner *et al.*, 2015). In particular, several acanthocephalan species use gammarids as intermediate hosts (Crompton & Nickol, 1985). These manipulative parasites are known to have multiple effects on their gammarid hosts, such as alterations in their behavior (Bethel & Holmes, 1973; Bauer *et al.*, 2000; Kaldonski *et al.*, 2007), their immune system (Cornet *et al.*, 2009a), their energetic reserves (Plaistow *et al.*, 2001) or their metabolic rate (Labaude et al., 2015a; Rumpus and Kennedy, 1974), ultimately leading to modifications in the role of gammarids within ecosystems. On the one hand, the feeding behavior of gammarids, be it on their consumption of dead leaves (McCahon *et al.*, 1988; Médoc *et al.*, 2011b; Labaude *et al.*, 2016) or on their predation (Fielding *et al.*, 2003; Médoc *et al.*, 2011b), has been shown multiple times to be decreased when they harbor acanthocephalan parasites. On the other hand, acanthocephalan parasites are known to induce multiple modifications in the anti-predator behavior of their

intermediate hosts, such as a decrease in their use of refuge (Kaldonski *et al.*, 2007; Perrot-Minnot *et al.*, 2007), modifications in their phototaxis (Tain *et al.*, 2006; Durieux *et al.*, 2012) or geotaxis (Cézilly *et al.*, 2000; Bauer *et al.*, 2005), or an attraction (or absence of repulsion) toward fish predator odor (Baldauf *et al.*, 2007; Perrot-Minnot *et al.*, 2007). Many studies have shown that gammarids infected by acanthocephalan parasites are more likely to be predated than uninfected ones (Hindsbo, 1972; Perrot-Minnot *et al.*, 2007; Dianne *et al.*, 2011), although not all the traits that are modified by the parasites are believed to be implicated in this increase (Perrot-Minnot *et al.*, 2012). For instance, the prevalence of *Gammarus pulex* infected with the acanthocephalan *Pomphorhynchus laevis* was found to be between ten times and 27 times more important in the stomach of a fish predator than that of free-ranging individuals from the same river (Lagrue *et al.*, 2007; Perrot-Minnot *et al.*, 2007), highlighting the impact of acanthocephalan parasites on the role of gammarids as a prey.

As for other key species, the ecological role of gammarids is likely to be tightly linked with abiotic conditions. In particular, temperature is known to be a key parameter in ectotherm species (Gillooly *et al.*, 2001), and was shown to alter gammarids in multiple ways, influencing for instance their metabolism (Roux & Roux, 1967; Issartel *et al.*, 2005; Foucreau *et al.*, 2014), growth (Moenickes *et al.*, 2011), or activity (Issartel *et al.*, 2005). On the other hand, most parasites are also affected by temperature in diverse ways (Barber *et al.*, 2016). For instance, acanthocephalan parasites develop faster in gammarids experiencing high temperatures (Olson & Pratt, 1971; Tokeson & Holmes, 1982). In parallel, their prevalence and abundance in their definitive fish host also depend on temperature (Sheath *et al.*, 2016). Furthermore, the effect of temperature on the interaction between parasites and their hosts might lead to drastic changes in their population dynamics (Mouritsen *et al.*, 2005; Poulin & Mouritsen, 2006), with subsequent consequences on their ecological role.

Labaude et al. (2016) recently showed that the impact of acanthocephalan parasites on the shredding role of gammarids was dependent on temperature. Although there is every reason to believe that their impact on the role of gammarids as a prey, through manipulated behaviors, might also be linked to abiotic conditions, only indirect evidence exists so far (see Labaude et al., 2015b, for a review). For instance, a seasonal effect has been reported in the abundance of acanthocephalans (VanCleave, 1916; Muzzall & Rabalais, 1975; Brown, 1989), with evidence that it might also exist on their manipulative ability. Indeed, Franceschi et al. (2010a) showed that gammarids displayed altered behaviors faster when they were experimentally infected in spring compared to gammarids infected in winter. They also found that parasites that developed faster were unable to induce rapid changes in the phototaxis behavior of their hosts. Although their experiment did not allow to identify the exact factors responsible for such variation, differences in abiotic conditions are a good candidate. Moreover, Benesh et al. (2009a) also documented seasonal differences in the manipulation induced

by acanthocephalan parasites on their isopod hosts, with hiding behavior more heavily modified in spring compared to late summer and fall. An experimental acclimatization of isopods to different abiotic conditions of light and temperature induced changes in their behavior, although no differences were found in the extent of their manipulation (Benesh *et al.*, 2009a). However, this study relied on naturally infected isopods, for which conditions during parasites development were thus not controlled. Moreover the effect of temperature and light were not investigated separately, while other studies showed that the manipulation of gammarids by acanthocephalans depended on light properties (Benesh *et al.*, 2005; Perrot-Minnot *et al.*, 2012). It thus remains unclear whether temperature might affect the manipulation of parasites, such as suggested by several authors (Labaude et al., 2015b; Thomas et al., 2011), ultimately leading to alterations in the ecological role of their gammarid hosts. A previous study allowed us to show that temperature significantly affected the changes in phototaxis induced by *P. tereticollis* infecting *G. pulex* (Labaude et al. 2016, in prep, Article 3 of this thesis), suggesting that at least a component of manipulation by *Pomphorhynchus* species may be sensitive to proximate abiotic conditions. However, this study also revealed no effect of temperature on the use of refuges in the same animals. In addition, as stated before for other studies, naturally-infected animals were used, leaving the possibility for uncontrolled factors in the experiments.

Here, we relied on experimental infections of *G. pulex* by the fish parasite *P. laevis* to investigate the effect of temperature on several parameters linked to their interaction, including the intensity and timing of manipulation. Gammarids were maintained in two temperatures during the development of their parasites. The survival of gammarids was recorded and infection parameters were measured (success of infection, parasite load, speed of parasites development). After parasites reached the cystacanth stage, at which they become infective for the definitive host, the behavior of gammarids, in terms of use of refuges, was tested three times. Although *P. laevis* is known to induce an infection syndrome (Perrot-Minnot *et al.*, 2014), altering several behavior of its host, the use of refuges is directly involved in parasite trophic transmission (Perrot-Minnot *et al.*, 2007; Dianne *et al.*, 2011). The activity level of gammarids was also measured, as well as the rapidity for parasites to evert their proboscis, an indispensable step for the establishment in their definitive host. Temperature is known to influence the time of parasites development (Olson & Pratt, 1971; Tokeson & Holmes, 1982), thus affecting both the time spent by gammarids in the laboratory and the time spent by parasites inside their intermediate host before becoming infective to their definitive host. Controls were therefore made to ensure that the potential differences observed between temperatures were not due to these two parameters.

Materials and methods

Sampling

Uninfected *G. pulex* gammarids were collected twice in a small tributary of the Suzon River (eastern France, 47°24'12.6"N, 4°52'58.2"E), in October and November 2015. Gammarids from this population have been widely used in previous studies for experimental infections, so the system is now well characterized (Franceschi *et al.*, 2008, 2010a, 2010b; Dianne *et al.*, 2012; Perrot-Minnot *et al.*, 2014; Labaude *et al.*, 2015b). Previous studies did not show any effect of the sex of gammarids on the extent of behavioral modifications (Bauer *et al.*, 2000, 2005; Cézilly *et al.*, 2000; Franceschi *et al.*, 2008). However, failure in parasite development is observed in female gammarids more often than in males (Franceschi *et al.*, 2008). Thus, only males were kept in this study. Before experimental infections, gammarids were maintained in the laboratory at 15 °C and under a 12:12 light:dark cycle.

Naturally infected chubs (*Leuciscus cephalus*) were sampled in October and December 2015 in the Vouge River (eastern France, 47° 9'34.36" N 5°9'2.50" E). The population of parasites from this river is known to have a high infection rate (Franceschi *et al.*, 2010a). Acanthocephalan eggs were extracted from adult parasites sampled in the intestines of the fish. Because both *P. laevis* and *P. tereticollis* parasites can be found in fish and cannot be distinguished visually, the species of adult parasites was determined using genetic analyses with the method described in Franceschi et al. (2008). Only eggs from the species *P. laevis* were used for experimental infections.

Experimental infections and treatments

Gammarids were experimentally exposed to *P. laevis* eggs following the procedure detailed in Franceschi et al. (2008). Pairs of gammarids that were previously starved for 24 hours in glass dishes were exposed for 48 hours to 200 parasite eggs (100 eggs per gammarid being a good compromise between a high infection success and low multiple infections; Franceschi et al., 2008). Gammarids were then placed in individual glass dishes, and randomly divided into the different treatments. Control individuals were maintained under the same conditions without eggs.

Two experimental infections were conducted (see Fig. 18). To investigate the effect of temperature during the development of parasites, 1200 gammarids from the first (October) sampling were infected using parasite eggs sampled the same month. In parallel, 420 control individuals were maintained in the same conditions, without eggs. Immediately following the exposure, gammarids were divided into two temperature treatments, either 14°C or 17°C.

Because the development time of parasites is known to be highly dependent on temperature (Olson & Pratt, 1971; Tokeson & Holmes, 1982), parasites were expected to reach the cystacanth stage latter at 14°C compared to 17°C in the first experimental infection, such that gammarids at 14°C would spend more time in the laboratory before being tested. Thus, a second experimental infection was conducted to test for the effect of the time spent by gammarids in the laboratory. Gammarids from the first sampling (October) were maintained in individual glass dishes for 40 days at 17°C before being exposed to parasite eggs sampled in December (n = 650), or kept as controls (n = 120). In parallel, 90 freshly sampled gammarids (second sampling, November) were also experimentally infected with the same parasite eggs, and 30 individuals were used as controls. Following the second infection, all gammarids were maintained in individual glass dishes at 17°C (see Fig. 18).

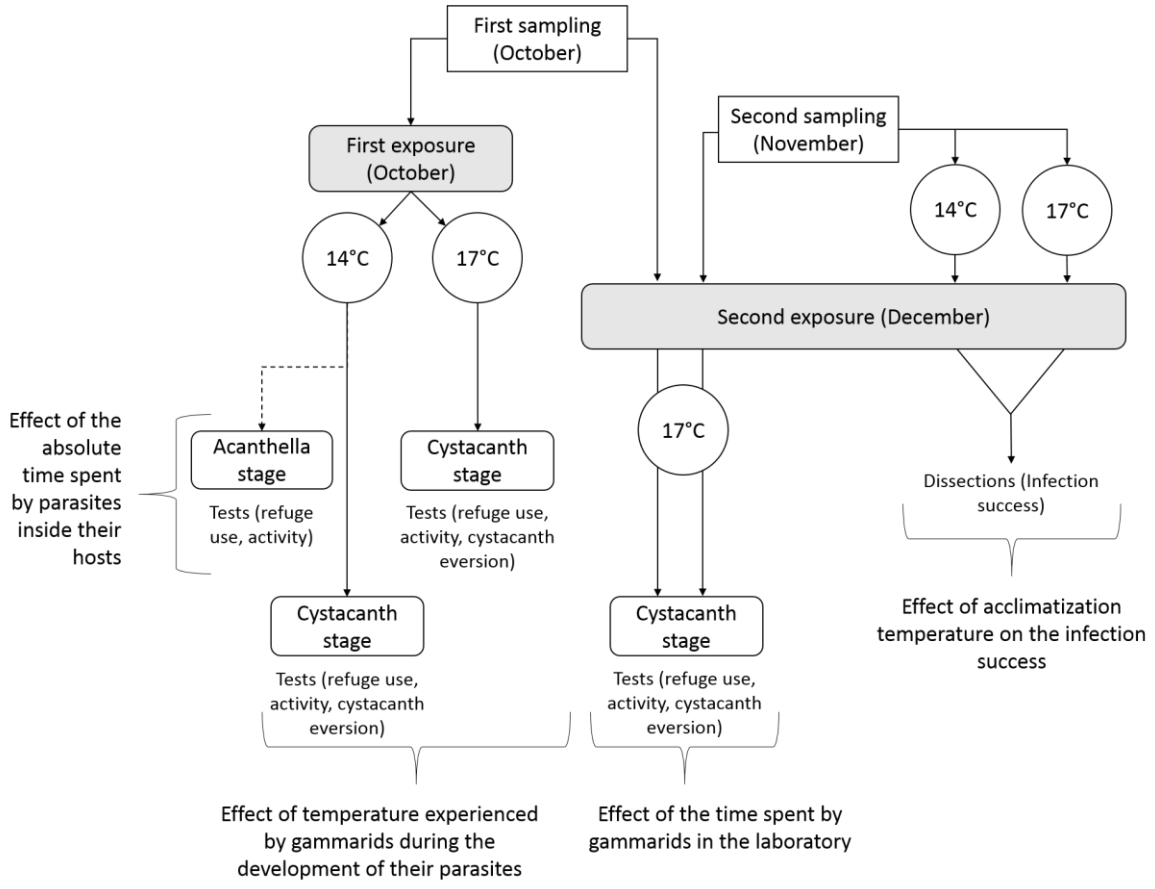

Figure 18. Overview of the protocol used in this experiment. Control individuals were maintained in the same conditions as exposed individuals and were simultaneously tested.

Individuals from the two experimental infections were maintained under a 12:12 light:dark cycle, and were fed ad libitum with conditioned elm leaves, with one additional frozen chironomid larva once every two weeks. Water was changed once every two weeks, using an oxygenated mix of water from the Suzon River and dechlorinated, UV-treated tap water.

Monitoring

All gammarids were checked on a daily basis to record the death of any individual. Individuals that were exposed to parasites were dissected immediately after their death to determine their infection status (number of parasites and their development stage). Moreover, 150 control and 150 gammarids exposed to parasite eggs from the first infestation were randomly selected at each temperature at the beginning of the experiment, and individuals found dead among them were also measured (height of the fourth coxal plate, see Bollache et al., 2000) using a microscope and Lucia G 4.81 software (Prague, Czech Republic).

When parasites were detected upon dissections at advanced acanthella stage (>1000 µm), all infected gammarids from the concerned group were checked daily under a dissecting microscope to monitor the exact date of the switch between the acanthella stage (ovoid shape, translucent orange color) and the cystacanth one (spherical and more pronounced opaque color, Dezfuli et al., 1991). Behavior tests were then conducted (see below).

At the end of the experiments, all gammarids that were used in behavior tests, including control individuals, were measured and dissected. Although *P. laevis* is not naturally found in the Suzon River, gammarids can be naturally infected with other acanthocephalan parasite species (*Echinorhynchus truttae* and *Polymorphus minutus*), as well as other macro-parasites such as *Cyathocephalus truncatus* (Cestoda). Such infected individuals were removed from the data.

Measurement of refuge use

Gammarids use of refuges was recorded three times (hereafter referred as “rounds”) on all infected individuals: one day, eight days, and 16 days after the cystacanth stage was detected. Control individuals were tested similarly three times in parallel. Gammarids were placed in individual boxes (10.5 × 16 cm) filled with 250 ml of water, containing a refuge at one extremity that consisted of a saucer terracotta pot (8.5 cm of diameter) cut in half, with a one centimeter hole in the convex part (see Dianne et al., 2014). Blind recordings were insured by labeling each test box independently from gammarids group treatment. After 10 minutes of acclimatization following the introduction of gammarids, the position of each individual was recorded every two minutes during 60 minutes. For

each observation, a score of zero was given to individuals that were outside the refuge, and one for individuals inside. Summed scores at the end of each round ranged from zero (always outside the refuge) to 30 (always inside) for each individual.

As expected, the development of parasites in gammarids maintained at 14°C was longer than those in gammarids maintained at 17°C. To test if the manipulation depended on the absolute time that parasites spent into their hosts, rather than their development stage, the behavior of 35 gammarids exposed to parasites and maintained at 14°C, thus expected to harbor parasites at the acanthella stage (further dissections confirmed infections), was tested at the same time as the behavior of gammarids maintained at 17°C. Control individuals maintained at 14°C were tested in parallel.

All gammarids were tested at the same temperature as their acclimatization temperature. To allow tests at different temperatures to occur in the same room, test boxes were placed in water baths, with surrounding water constantly recirculated through a temperature control device (TANK TK-1000 Chiller, Teco®, Ravenna, Italy; see Labaude et al., 2016).

Measurement of activity

The activity level of gammarids was tested for all individuals three days after the second round of refuge use tests (i.e. 11 days after the detection of the cystacanth stage for infected individuals). Each device consisted of a ten centimeters diameter glass dish containing a smaller dish (six centimeters diameters) preventing the gammarid to go in the center of the larger glass dish, thus forming a two centimeters wide annulus. To limit gammarids vertical movements, the device was filled with only one centimeter of water. Lines were traced under each device, intersecting in their center, thus dividing the annulus into eight equally large zones. After five minutes of acclimatization following gammarids introduction in the devices, the behavior of individuals was video-recorded from above for five minutes. The activity level of each individual was expressed as the number of lines crossed during five minutes.

Rapidity of proboscis eversion

The rapidity of cystacanth parasites to evert their proboscis was measured on old cystacanths (between 20 and 30 days after their detection) extracted from gammarids previously tested for their behavior. Immediately following the dissection of their hosts, each cystacanth parasite was carefully placed in a 96-well microplate. The eversion of cystacanth proboscis, which allows parasites to attach to the intestine wall of their fish host, is known to occur in reaction to a component of fish bile

(Kennedy *et al.*, 1978). Thus, bile extracted from chubs several months earlier, and frozen for conservation, was diluted 30 times with water and 30 µl were added to each microplate well. The microplate was immediately covered with aluminum foil to limit evaporation, and with an opaque box to ensure darkness (see Perrot-Minnot et al., 2011). Each microplate, containing no more than 20 cystacanths, was then checked every five minutes under a dissecting microscope, with reduced light, and quickly replaced in the darkness. The time needed for the proboscis of each parasite to start to evert was recorded. The temperature was kept at 15°C for all measurements of proboscis eversion, regardless of the treatment group.

Effect of acclimatization temperature on prevalence

In experiments described before, gammarids were all exposed to parasite eggs at the same temperature (15°C), and then maintained at 14°C or 17°C. Therefore, this experiment did not allow to fully conclude about the effect of temperature on the success of parasite infection. Indeed, although temperature might affect the success of establishment of parasites, it is also known to modify the consumption rate of gammarids (Foucreau *et al.*, 2016; Labaude *et al.*, 2016), thus maybe affecting their probability of consuming parasite eggs and getting infected at different temperatures. To measure the effect of temperature on the success of parasite infection, 100 gammarids from the second sampling (November) were acclimatized for three weeks in each of the two temperatures (14°C or 17°C), in individual glass dishes. Gammarids were then exposed to parasite eggs collected in December, following the protocol exposed above at their acclimatization temperature. Conditions were kept similar during parasite development. All gammarids were dissected immediately after their death or at the end of the experiment to determine their infection status.

Statistical analyses

All analyses were first conducted on gammarids from the first exposure to investigate the effect of temperature. Then, other analyses with individuals infected with acanthella parasites (first exposure, 14°C) were compared to gammarids infected with cystacanths from the same age (first exposure, 17°C), and their respective controls, to verify that the differences observed previously were due to the stage of the parasite and not the absolute time spent by parasites inside their hosts, due to longer development time at 14°C. Finally, the effect of the time spent by gammarids in the laboratory, which also differed between 14°C and 17°C, was controlled using individuals from the second exposure that spent long or short time in the laboratory before their infection (respectively first (October) vs second (November) samplings).

The size of gammarids was measured later at 14°C compared to 17°C, due to the differences in the development time of parasites. We therefore suspected that gammarids may have had more time to make an additional molt at 14°C than at 17°C, and thus could be larger. An ANOVA confirmed that the size of gammarids was globally larger at 14°C ($F_{399, 1}$ = 5.84, P = 0.016). To avoid any confounding effect with temperature, the size of individuals was not taken into account in between-temperatures analyses.

The survival of gammarids was analyzed using Cox regressions. First, the effects of infection status (control vs infected) and temperature were analyzed. A second Cox regression was performed using only infected individuals to investigate the effect of parasite load (one, two, or more than two parasites per gammarid) and temperature. To compare the virulence of parasites at the very same developmental stage, the survival of gammarids was also investigated 20 days after parasites reached the cystacanth stage, using a nominal logistic regression and odd-ratios. Once parasites were large enough to be detected upon dissections, individuals that were exposed to parasite eggs in which no parasite developed were removed from the analyses.

Nominal logistic regressions were used to investigate the success of infection (i.e. the proportion of gammarids harboring at least one parasite among those exposed to the infection). Mann-Whitney U tests were used to compare the parasite load between the groups and the time needed for parasites to reach the cystacanth stage.

The scores of refuge use were analyzed as repeated measures using the 'nparLD' R software package. This function is suitable for nonparametric analyses of right-censured longitudinal data, allowing the decrease in sample size along time, due to individuals' death (Noguchi *et al.*, 2012). First, the effect of temperature (14°C vs 17°C), infection status (control vs infected) and their interaction was investigated along time (rounds of measurements: one day, eight days and 16 days after parasites reached the cystacanth stage). A second analysis was conducted on infected individuals only, with temperature, parasite load, their interaction, and time as factors. For each analysis, 'ANOVA-type statistics' were performed, followed by post-hoc pair-comparisons when suitable (see Noguchi et al., 2012, for details). To verify that the changes in behavior were not solely linked to the time spent by parasites inside their hosts, Wilcoxon tests and post hoc comparisons were also used to compare scores between individuals at 14°C and 17°C tested simultaneously, when infected gammarids at the low temperature were still at the acanthella stage while those at the high temperature were at the cystacanth stage.

The activity level of gammarids was investigated using a linear model (ANOVA), followed by Tukey post hoc tests.

The speed of the eversion of parasites' proboscis was investigated using a Generalized Linear Model with a Poisson distribution corrected for over-dispersion. We tested for the effects of temperature, parasite load, the time needed for each parasite to reach the cystacanth stage, and their interactions. Spearman correlations were used to test if there was a link between the rapidity of parasites to evert their proboscis and their ability to manipulate the behavior of their hosts (refuge use scores and activity level) at each temperature.

Statistical analyses were performed using JMP version 10.0.0 software (SAS Institute, Cary, NC, U.S.A.) and R version 3.1.1 software (R Foundation for Statistical Computing, Vienna, Austria). For each analysis described above, all factors and their second order interactions were first entered in the models. Non-significant factors or interactions were then removed.

Results

Effects of the temperature

Survival

The Cox regression on gammarids from the first exposure (Chi2 = 208.97, d.f. = 2, $P < 0.0001$) showed that survival was significantly higher at 14°C (LR-Chi2 = 188.56, d.f. = 1, $P < 0.0001$, Fig. 19). Control individuals also survived significantly better than infected individuals (LR-Chi2 = 34.88, d.f. = 1, $P < 0.0001$, Fig. 19). The interaction between temperature and infection was not significant. When considering infected individuals only, no effect of the parasite load on gammarids survival was found, and only temperature remained in the model (LR-Chi2 = 144.09, d.f. = 1, $P < 0.0001$).

The survival of gammarids 20 days after parasites reached the cystacanth stage was lower at 17°C compared to 14°C (LR-Chi2 = 62.37, d.f. = 1, $P < 0.0001$) and in infected individuals compared to controls (LR-Chi2 = 49.82, d.f. = 1, $P < 0.0001$). However, the virulence of parasites was not different between temperatures, as indicated by the absence of significant interaction between these two parameters. Moreover, odd-ratios indicated similar effect size between control and infected individuals at 14°C (OR = 0.34, $CI_{95\%}$ = [0.20, 0.56]) and 17°C (OR = 0.36, $CI_{95\%}$ = [0.25, 0.52]), confirming results of the Cox analysis.

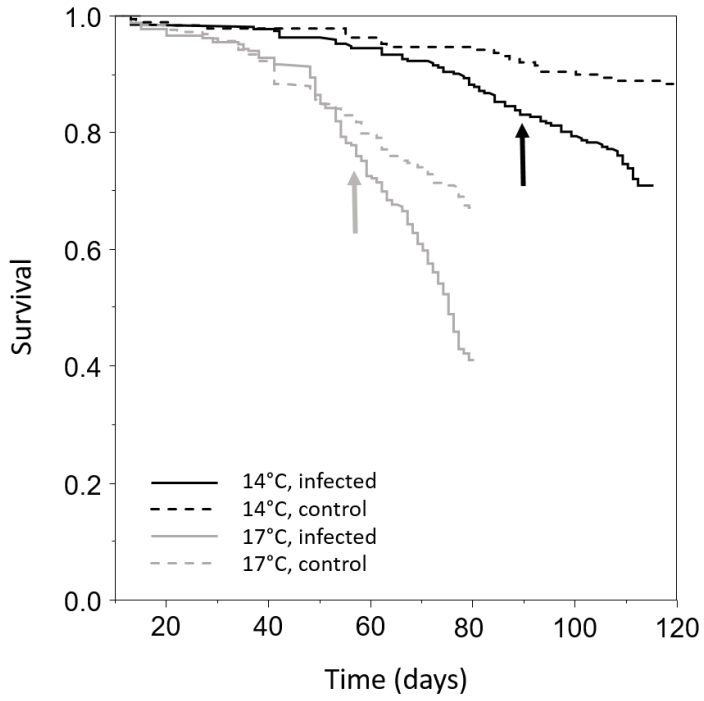

Figure 19. Gammarids survival according to infection status (control or infected by *P. laevis* parasites) and temperature (14°C or 17°C). Time 0 was considered as the day from which gammarids were exposed to parasite eggs. Arrows indicate the average day of switching of parasites between acanthella and cystacanth stages at each temperature.

Infection parameters

Overall, 53.8 % of individuals that were exposed to parasite eggs were successfully infected, with no difference in the success of infection between the two temperatures (LR-Chi2 = 0.003, d.f. = 1, P = 0.96). No difference was observed either in the parasite load between the two temperatures (mean ± standard deviation = 1.67 ± 1.04 parasites per gammarid, Mann-Whitney U test: Z = 1.06, P = 0.29). The development time of parasites (from exposure to cystacanth stage) was significantly longer at 14°C (mean ± standard deviation = 89.95 ± 2.49 days) than at 17°C (57.27 ± 1.39 days; Mann-Whitney U test, Z = -14.69, P < 0.0001; Fig. 19).

When gammarids were acclimatized for three weeks at the two temperatures before exposure to parasite eggs, the infection success reached 84.9 % at 17°C (n = 86) and 74 % at 14°C (n = 77), although this difference was not significant (LR-Chi2 = 2.97, d.f. = 1, P = 0.08). However, significantly more parasites developed in gammarids at 17°C (mean ± standard deviation = 4.58 ± 2.76 parasites per gammarid) compared to 14°C (mean ± standard deviation = 3.86 ± 2.55 parasites per gammarid; Mann-Whitney U test, Z = -1.96, P = 0.05).

Gammarids behavior

ANOVA-type results from the nparLD model showed that temperature did not influence the use of refuges by gammarids (Fig. 20), and thus this parameter was removed from the model. The remaining model showed that infection status, time (behavioral rounds), and the interaction between these two factors significantly influenced refuge use (Table 11). The use of refuges decreased with time for infected individuals, while it increased for control individuals (Fig. 20).

When considering only infected individuals to investigate the effect of parasite load (one, two or more than two parasites), temperature had no effect either on the use of refuges, and was again removed from the analysis. Although the remaining model showed again that infected gammarids decreased their use of refuges over time, we found that parasite load and its interaction with time also significantly affected gammarids use of refuges (Table 12). Pair-comparisons showed that gammarids with one or two parasites overall used refuges less than gammarids with more than two parasites (Fig. 21, pair-comparisons 2 and 3 in Table 12). In addition, while all groups reached similar scores at the third round, their dynamics was different across time (this difference being significant only between gammarids harboring one and those harboring more than two parasites): gammarids harboring more than two parasites decreased more their use of refuges between the second and third rounds compared to gammarids harboring one parasite (Fig. 21, pair-comparison 2 in Table 12).

The ANOVA of the level of activity of gammarids ($F_{3, 500} = 29.05$, $P < 0.0001$) showed that individuals were significantly more active at 17°C compared to 14°C ($F_{1, 500} = 77.14$, $P < 0.0001$, Fig. 22). Moreover, although infection status alone was not significant ($F_{1, 500} = 0.89$, $P = 0.35$), its interaction with temperature also influenced gammarids activity level ($F_{1, 500} = 7.99$, $P = 0.005$). Tukey's HSD post hoc tests showed that the activity was significantly higher for infected individuals at 14°C, but not at 17°C (Fig. 22).

Table 11. Results of the model from the nparLD R package, testing for the effects of infection status (infected with *P. laevis* cystacanths or control) and rounds of measurement on the scores of refuge use of *G. pulex* individuals.

Factor	Statistic	d.f.	P
Status	323.51	1	< 0.0001
Round	124.56	1.96	< 0.0001
Status x round	393.86	1.96	< 0.0001

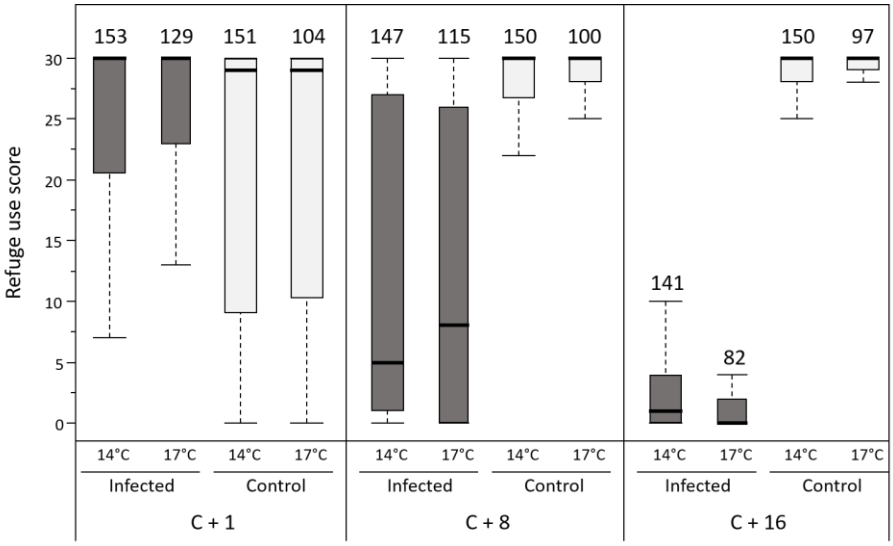

Figure 20. Gammarids scores of refuge use according to infection status (infected or control) and temperature (14°C or 17°C), measured during three rounds: one day (C+ 1), eight days (C+ 8) and 16 days (C+ 16) after detection of cystacanth stages. Scores range from 0 (individuals always outside the refuge) to 30 (individuals always inside the refuge). Thick lines represent the medians, the boxes represent the upper and lower quartiles and dotted lines represent the upper and lower deciles. Sample sizes are given above each plot.

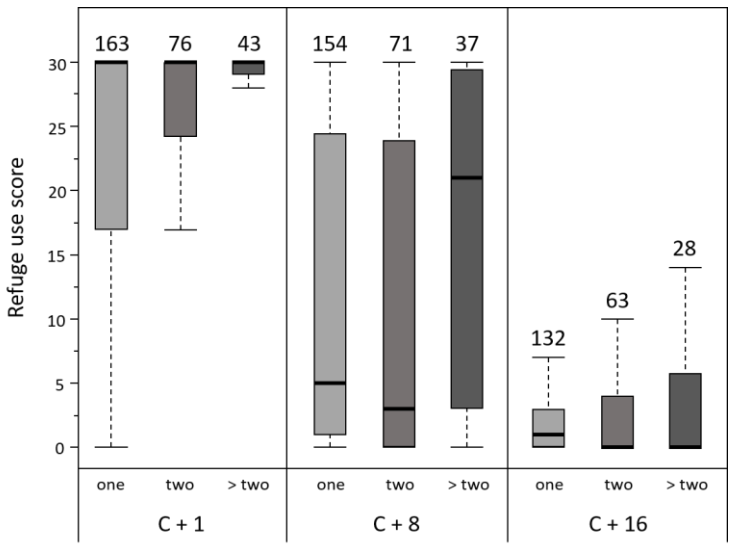

Figure 21. Scores of refuge use of infected gammarids according to parasite load (one, two or more than two parasites per gammarid), measured during three rounds: one day (C+ 1), eight days (C+ 8) and 16 days (C+ 16) after detection of cystacanth stages. Scores range from 0 (individuals always outside the refuge) to 30 (individuals always inside the refuge). Thick lines represent the medians, the boxes represent the upper and lower quartiles and dotted lines represent the upper and lower deciles. Sample sizes are given above each plot.

Table 12. Results of the model from the nparLD R package, testing for the effects of parasite load (one, two or more than two *P. laevis* cystacanths per gammarid) and rounds of measurement on the scores of refuge use of *G. pulex* individuals. Here, only infected individuals were considered in the analysis.

Factor	Statistic	d.f.	P
ANOVA TEST			
Parasite load	3.51	1.82	0.034
Round	310.64	1.91	< 0.0001
Parasite load x round	2.93	3.17	0.030
PAIR-COMPARISONS			
1) Gammarids infected with one and two parasites			
Parasite load	0.026	1	0.87
Round	313.45	1.93	< 0.0001
Parasite load x round	2.76	1.93	0.065
2) Gammarids infected with one and more than two parasites			
Parasite load	4.93	1	0.026
Round	183.76	1.86	< 0.0001
Parasite load x round	3.49	1.86	0.033
3) Gammarids infected with two and more than two parasites			
Parasite load	4.39	1	0.036
Round	178.42	1.87	< 0.0001
Parasite load x round	2.55	1.87	0.082

Rapidity of proboscis eversion

The Generalized Linear Model (Chi2 = 6.13, d.f. = 2, P = 0.047) showed that parasites everted their proboscis slightly but significantly faster when they developed in hosts maintained at 17°C (median and interquartile range = 40 [35; 45] minutes) compared to hosts maintained at 14°C (median and interquartile range = 45 [40; 50] minutes, LR-Chi2 = 5.57, d.f. = 1, P = 0.018). Moreover, there was a compromise between the time to reach the cystacanth stage and the time to evert their proboscis, with eversion starting sooner for parasites that took more time to develop (LR-Chi2 = 4.97, d.f. = 1, P = 0.026).

Spearman correlations showed that the parasites inducing low manipulation in the third round of refuge use test were overall faster to evert their proboscis, although the correlation was only found at 17°C (Table 13). No other behavior of gammarids was correlated with the rapidity of proboscis eversion of their parasites (Table 13).

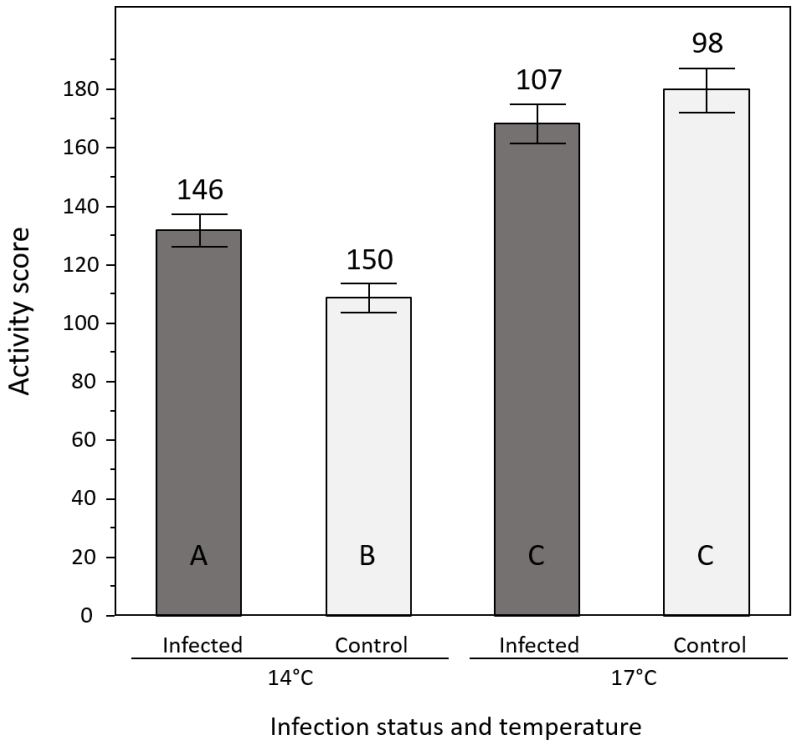

Figure 22. Gammarids activity level according to infection status (infected by *P. laevis* cystacanths or control) and temperature (14°C and 17°C). The activity is given by a score corresponding to the number of zones entered during five minutes in an annulus arena. Thick lines represent the medians, boxes represent the upper and lower quartiles, and dotted lines represent the upper and lower deciles. Sample sizes are given above each bar. Significant differences are indicated by different letters (Tukey's HSD post hoc tests; $P < 0.05$).

Table 13. Spearman correlations between the time for *P. laevis* cystacanth parasites to start the eversion of their proboscis and the behavior scores of their hosts.

Group	Factor	rho	n (parasites)	P
14°C	Activity	0.005	199	0.95
	Refuge, first round	0.02	199	0.75
	Refuge, second round	-0.04	199	0.59
	Refuge, third round	-0.08	199	0.29
17°C	Activity	0.17	94	0.10
	Refuge, first round	-0.07	94	0.51
	Refuge, second round	-0.08	94	0.43
	Refuge, third round	-0.26	94	**0.01**

Significant value (after Bonferroni correction) is highlighted in bold

Effect of the absolute time spent by parasites into their hosts

The scores of refuge use differed between individuals tested simultaneously, according to temperature and infection status (Wilcoxon, Chi2 = 80.96, d.f. = 3, P < 0.0001). Post hoc comparisons showed that the scores of individuals infected with cystacanth stages (17°C) were significantly higher compared to individuals infected with acanthella stages (14°C) and control individuals at both temperatures (Fig.

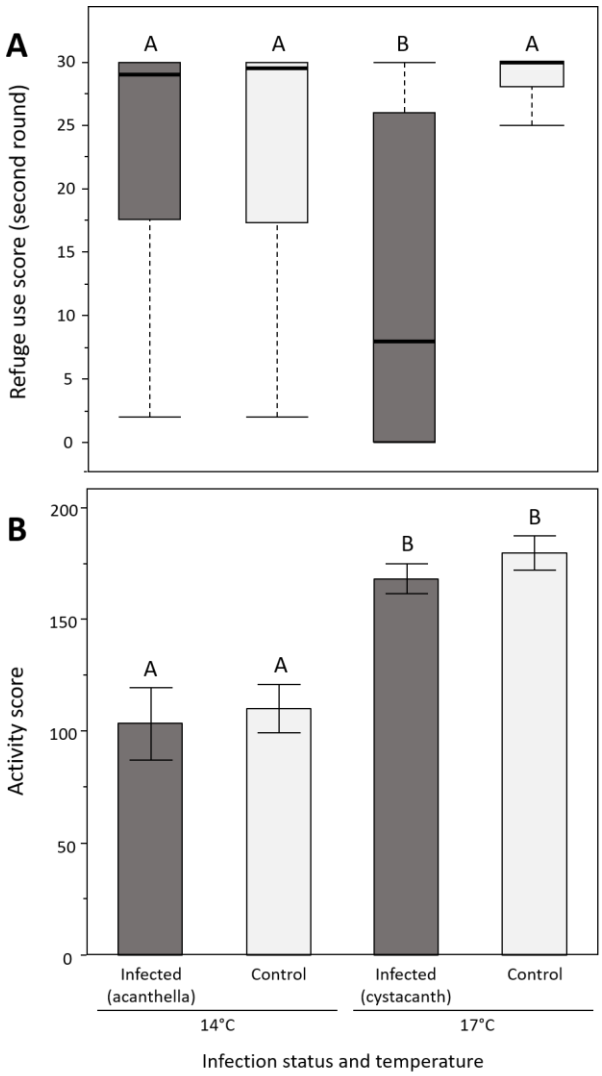

Figure 23. Behavioral scores according to temperature (14°C and 17°C) and infection status (infected and control). (A) Score of refuge use (median, upper and lower quartiles and deciles) measured during the second round (eight days after the detection of cystacanth stages at 17°C) and (B) activity level of gammarids (mean and standard error). All individuals were tested simultaneously, such that infected gammarids at 17°C harbored parasites at the cystacanth stage while parasites were still at an acanthella stage in gammarids maintained at 14°C. Significant differences are indicated by different letters (Tukey's HSD post hoc tests; P < 0.05).

23A). The scores of individuals infected with acanthella parasites did not differ from that of control individuals (Fig. 23A). Gammarids activity was significantly affected by temperature ($F_{245, 1}$ = 30.45, P < 0.0001) but not by infection status. Tukey post hoc tests showed that activity was higher at 17°C compared to 14°C, in all infection status (Fig. 23B).

Effect of the time spent by gammarids in the laboratory (17°C)

No differences were observed in infection parameters between gammarids maintained for short time or long time in the laboratory (development time: Mann-Whitney U test, Z = 0.61, P = 0.54; infection success: LR-Chi2 = 0.36, d.f. = 1, P = 0.55; parasite load: Mann-Whitney U test, Z = -0.22, P = 0.82). The refuge use was not affected either by the time spent by gammarids in the laboratory. After the removal of this factor from the analysis, the same factors as before were shown to influence the use of refuges (status: Statistic = 29.11, d.f. = 1, P < 0.0001; round: Statistic = 45.46, d.f. = 1.92, P < 0.0001; and their interaction: Statistic = 44.96, d.f. = 1.92, P < 0.0001). There was no effect of the time of maintenance on the rapidity of proboscis eversion, and this parameter was thus removed from the Generalized Linear Model. The remaining model (Chi2 = 13.18, d.f. = 2, P = 0.0014) showed that the time needed

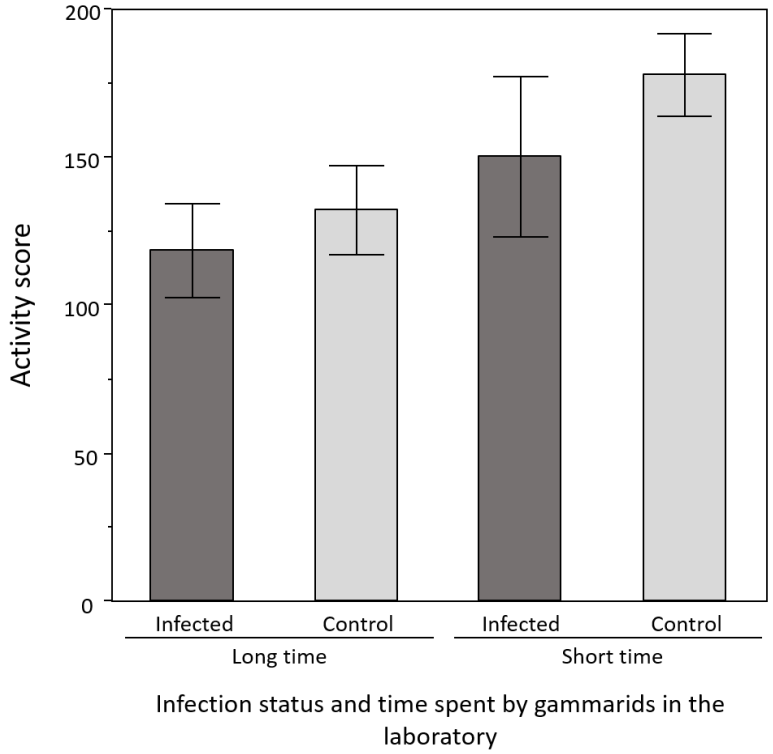

Figure 24. Scores of activity level of gammarids according to infection status (infected and control) and the time they spent in the laboratory before being infected (long time vs short time, respectively first sampling and second sampling of gammarids). Mean values and standard errors are indicated.

by parasites to evert their proboscis was negatively and significantly influenced by the size of their hosts (Chi2 = 6.86, d.f. = 1, P = 0.0088) and by the parasite load (Chi2 = 6.53, d.f. = 1, P = 0.011). In our study, these two parameters were independent (Spearman's rho = -0.026, P = 0.85). Only the activity level differed, with lower activity scores for gammarids maintained for a longer time in the laboratory ($F_{1, 59}$ = 4.63, P = 0.036), while the effect of infection status was not significant (Fig. 24).

Discussion

Our results show that temperature affected several parameters linked to the physiology of gammarids, with a lower survival and a higher activity level at high temperature. Parasites were also affected by temperature, with a faster development and eversion of their proboscis at high temperature, as well as an increased parasite load when exposition to parasite eggs was made at different temperatures. Despite all these effects, neither the timing nor the intensity of manipulation in terms of use of refuges were affected by temperature.

As expected based on other studies (Olson & Pratt, 1971; Tokeson & Holmes, 1982), the time of development of parasites was much longer at 14°C compared to 17°C, with a remarkable synchrony in the timing of their switch between acanthella and cystacanth stages within each temperature. Because of this difference, gammarids at 14°C spent more time in laboratory conditions than those at 17°C before being tested for behavior. In addition, behavioral tests were conducted at the same stage of the parasites, but not at the same age in terms of days. However, control tests allowed us to discard these two effects. First, when gammarids spent different amounts of time in the laboratory, with all other parameters being equal, no infection parameter differed, in terms of development time of parasites, infection success, parasite load and behavioral manipulation. Only activity was reduced in gammarids that spent more time in laboratory conditions. Second, gammarids tested at the same absolute parasite age, in terms of days, showed differences in their behavior consistent with the idea that manipulation is linked to parasite stage, and not to the time parasites spent in gammarids. Indeed, gammarids infected with acanthella stages displayed similar behavior than that of uninfected gammarids, in terms of use of refuges, and behaved significantly differently than gammarids infected with cystacanth stages from the same age.

In addition with the parasite's development time, temperature also modified other infection parameters in our study. First, when the exposure to parasite eggs occurred at the same temperature, the subsequent development of parasites at two different temperatures did not lead to any difference in infection success or parasite load. However, due to an overall low number of parasites per gammarid in the first infection, such a difference would have been difficult to point out. On the other hand, when

exposure to parasite eggs occurred at different temperatures with hosts previously acclimatized at these temperatures, the infection success was slightly higher at 17°C compared to 14°C, although this difference was only marginally significant. In addition, more parasites per host developed at 17°C compared to 14°C in these conditions. These results line up with those found in *P. laevis* definitive fish host, *Squalius cephalus*, for which a higher probability of infection and a higher parasite load was found at 22°C compared to 18°C (Sheath *et al.*, 2016). The fact that a difference was observed only when exposure to parasite eggs occurred at different temperatures suggests that the effect of temperature on these two parameters was due to a higher consumption of eggs rather than a higher success of establishment of the parasites after consumption. This hypothesis is supported by several studies that highlighted an effect of temperature on gammarids food consumption (Pellan *et al.*, 2015; Foucreau *et al.*, 2016; Labaude *et al.*, 2016), thus probably affecting their probability of consuming parasite eggs. It is also interesting to note that both the infection success and the parasite load differed in our experiment between the two experimental infections, highlighting once more the strong effect of parasites origin, even within the same population (Franceschi *et al.*, 2010b; Labaude *et al.*, 2015b).

Finally, parasites were also affected by temperature in the speed of the eversion of their proboscis, with parasites that developed at 17°C starting the eversion sooner after the adding of fish bile compared to those which developed at 14°C. During this test, the temperature was similar between the two groups, such that only the temperature experienced during parasite development could affect their proboscis eversion. This result might be linked, along with their faster development inside their hosts, to an increased metabolism of parasites at 17°C compared to 14°C. There also seemed to be a compromise between the rapidity of proboscis eversion and the importance of manipulation in infected gammarids from the first exposure, although the correlation was very weak and significant only at 17°C for the third behavior round. Parasites from the second exposure everted their proboscis faster when they developed in larger hosts and within hosts with higher parasite load.

In parallel to its effect on parasite traits, temperature also affected the interaction between hosts and parasites. First, gammarids survival was decreased at high temperature, as already shown in other studies (Moenickes *et al.*, 2011; Foucreau *et al.*, 2014). Parasites also led to a higher mortality of gammarids, but their virulence was not affected by temperature, with a similar impact on gammarids survival when it was investigated at the same stage of parasite development.

The activity level, a parameter tightly linked to metabolism in gammarids (Issartel *et al.*, 2005), was also affected by temperature. As expected, gammarids were globally more active at high temperature (Issartel *et al.*, 2005; Maazouzi *et al.*, 2011). This difference could however partly be linked to the fact that gammarids at the low temperature were tested after a longer time in laboratory

conditions, as the second part of the experiment showed that a longer maintenance led to a decrease in the activity level of gammarids. In parallel, infection with acanthocephalan parasites was shown to influence gammarids activity in many studies. Although most of them concluded with an increase of activity in infected individuals compared to uninfected ones (Maynard *et al.*, 1998; Dezfuli *et al.*, 2003; Stone & Moore, 2014), contradictory results were also observed (Thünken *et al.*, 2010; Jacquin *et al.*, 2014; Perrot-Minnot *et al.*, 2014). Interestingly, our results suggest that abiotic conditions could be responsible for such contradictions. Indeed, infected individuals were significantly more active than control individuals only at 14°C. On the contrary, although this difference was never significant, average activity level was slightly higher in control individuals compared to infected ones at 17°C, in all our experimental infections.

Altogether, these results suggest that both host and parasite metabolisms were accelerated at 17°C compared to 14°C. In opposition, no difference was observed according to temperature in the behavior of gammarids in terms of use of refuges, neither in the timing of manipulation nor in its intensity. As already shown before, the use of refuges tended to increase with time for control individuals, while infected individuals decreased their use of refuges along time (Dianne et al., 2010; Labaude et al., 2015a). These two trends were observed in both temperatures, with an identically progressive manipulation of infected individuals. Only the number of parasites per hosts was shown to influence the use of refuges. Manipulation was delayed in gammarids harboring more than two parasites. However, this phenomenon might be linked to our protocol. Indeed, the behavior of individuals was tested as soon as a cystacanth was detected through gammarids cuticle. Variation was shown in the growth of parasites sharing the same host (Dianne *et al.*, 2012), and a small asynchrony is thus expected to occur in the exact day of switch to the cystacanth stage in gammarids infected with several parasites. It is thus likely that multi-infected gammarids still harbored acanthella parasites when they were first tested, or parasites at an earlier cystacanth stage in the second test. In addition, the manipulation of gammarids by their cystacanth parasites is known to be reduced by the presence acanthella parasites (Dianne *et al.*, 2010), a stage known to enhance the anti-predatory behaviors of their hosts (Dianne *et al.*, 2011), thus maybe explaining the delayed manipulation in our study.

The absence of any effect of temperature on manipulation suggests that manipulation might not be plastic, a hypothesis already proposed before (Labaude et al., 2015a). Indeed, as the survival of gammarids decreased with high temperature, one would expect parasites to adopt a strategy to increase their chances of being transmitted before the death of their hosts, such as a faster manipulation (Thomas *et al.*, 2002a). In a similar study investigating the effect of host nutritional condition, Labaude et al. (2015a) also found that, although the survival of gammarids was altered by a

poor diet, along with effects on other metabolic traits, the amount of host resources had no effect on *P. laevis* manipulation of *G. pulex*.

Nevertheless, it cannot be excluded that temperature affects parasite manipulation in a way that could not be detected in our study. First, tests occurred in the absence of any predator cue. Although such conditions are sufficient to induce alterations in behaviors, the differences between control and infected individuals might be exacerbated by the presence of predator odor. Indeed, Durieux et al. (2012) found that the effect of *P. laevis* on the phototaxis of *G. pulex* was more pronounced in scented water compared to control water. The presence of fish odor is also necessary to observe certain alterations induced by parasites in gammarids, such as differences in aggregation (Durieux *et al.*, 2012; Lewis *et al.*, 2012) or attraction of infected individuals toward fish odor compared to repulsion for uninfected gammarids (Baldauf *et al.*, 2007; Kaldonski *et al.*, 2007; Perrot-Minnot *et al.*, 2007). Second, variations induced by temperature might be linked to other behaviors than the use of refuges. Indeed, the seasonal variation of manipulation observed by Franceschi et al. (2010a) was highlighted on gammarids phototaxis, although the use of refuges was not tested. A recent study investigating the effects of a shorter acclimatization time at different temperatures on control and naturally infected gammarids by *P. tereticollis* showed a significant effect of temperature on the phototaxis of both infected and control gammarids, but not on their use of refuges (Labaude et al. in prep, Article 3 of this thesis). On the contrary, sheltering behavior was shown to be linked to abiotic conditions (temperature and light) and seasonality in isopods infected with acanthocephalan parasites, suggesting that variation in the use of refuges might also exist under certain circumstances (Benesh *et al.*, 2009a).

In their study, Benesh et al. (2009a) showed that different experimental conditions of light and temperature, chosen to mimic seasonal differences, altered the use of refuges of both infected and uninfected isopods. However, the difference of behavior between infected and uninfected individuals remained similar under such experimental conditions, although the two parameters were not investigated separately. On the contrary, they found that this difference varied among isopods collected at different seasons. A seasonal effect of manipulation was also documented in gammarids infected by acanthocephalan parasites (Franceschi *et al.*, 2010b), although the mechanisms explaining such seasonality were not explained. Our study supports the hypothesis made by Benesh et al. (2009a) who suggested that seasonal changes in manipulation might not be caused by proximal abiotic conditions. In other studies, only light properties were shown to affect manipulation (Benesh *et al.*, 2005; Perrot-Minnot *et al.*, 2012), while other factors such as the quantity of resources available did not affect manipulation either (Labaude et al., 2015a). Thus, seasonality in manipulation could be linked to other parameters. First, the conditions experienced by female parasites in their definitive fish

hosts might differ among seasons, for instance in the availability of resources, and effect their offspring. The effect of abiotic parameters on other stages of the parasites thus deserves more attention. Second, Benesh et al. (2009a) suggested that such seasonality might be adaptive, with higher manipulation occurring in accordance with the seasonal preference of fish for amphipods. We might thus expect such variation to rely on a genetic basis, although the mechanisms responsible for such timing need to be investigated. In this case, global changes might alter the seasonal distribution and/or the diet of definitive fish hosts and ultimately lead to a maladaptation of the degree of manipulative efforts of parasites.

Although temperature did not plastically affect the manipulation of acanthocephalan parasites in our study, indirect effects are likely to happen (Labaude et al., 2015b). Indeed, temperature was shown to be linked with gammarids consumption of leaves (Pellan *et al.*, 2015; Foucreau *et al.*, 2016; Labaude *et al.*, 2016), ultimately leading to higher infection success and parasite load. Such effect was also observed in parasites definitive hosts (Sheath *et al.*, 2016). Moreover, parasites developed faster at high temperature. Other conditions, such as the availability of resources known to modulate parasite load in their gammarid hosts (Labaude *et al.*, 2015b), are also likely to be affected by temperature. Altogether, these effects might lead to modifications on the intensity of infection, known to influence manipulation (Franceschi *et al.*, 2008), as well as in the prevalence of acanthocephalan parasites in gammarids populations, thus, provided that prevalence is high enough, modifying behaviors on a population scale.

To our knowledge, this study is the first to investigate the effect of temperature on the timing and intensity of behavioral changes using experimental infections. Our results provide solid evidence that temperature might affect many parameters of host-parasite associations, with no direct effect on the extent of manipulation. However, although temperature might not be directly responsible for changes in the behavior of gammarids, further studies are needed to investigate its effect in the whole picture, and conclude about how its interaction with manipulative parasites might alter gammarids role as a prey. In order to do so, other development stages of parasites, as well as the behavior of the definitive host, should be included in more integrative experiments.

Acknowledgments

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3. Impact on gammarids immune system

Temperature is known to be one of the most important factors affecting host-parasite relationships (Gillooly *et al.*, 2001, Harvell *et al.*, 2002; Barber *et al.*, 2016). My previous experiments confirmed the importance of temperature on the interaction between gammarids and their acanthocephalan parasites. In particular, the development time of acanthocephalan parasites was shown to be highly dependent upon the temperature experienced by their hosts, a result also found in other studies (Olson & Pratt, 1971; Tokeson & Holmes, 1982). The infection success of several macroparasite species is known to depend on temperature, such as cestode and trematode species (Okaka, 1989; Mouritsen & Jensen, 1997; Studer & Poulin, 2013), while replication of microsporidia within amphipods is inhibited at low temperatures (Dunn *et al.*, 2006). My previous experiments suggest that infection intensity, and possibly infection success, could also depend on temperature in acanthocephalan parasites infecting gammarids. Moreover, Sheath *et al.* (2016) recently demonstrated that the prevalence and abundance of the acanthocephalan *Pomphorhynchus laevis* also increased with temperature in its definitive fish host. The reasons behind such effect of temperature are not clear. Indeed, a higher infection rate of gammarids could be due either to a higher consumption of parasite eggs or a better survival of parasites inside gammarids, possibly due to a lower ability to eliminate the parasite.

The immune system of amphipods relies on several components (Söderhäll & Cerenius, 1992; Vazquez *et al.*, 2009), including the prophenoloxidase system known to induce the deposition of melanin on the surface of foreign organisms (Cerenius & Söderhäll, 2004), and circulating hemocyte cells that are also involved in several defence mechanisms, such as phagocytosis, encapsulation, or coagulation (Johansson *et al.*, 2000; Vazquez *et al.*, 2009). Only a few observations of acanthocephalans or trematodes found dead and melanised or coated with hemocytes inside live gammarids have been reported (Hynes & Nicholas, 1958; Thomas *et al.*, 2000b; Dezfuli *et al.*, 2008a). Indeed, parasites such as acanthocephalans have been shown to be able to induce immunosuppression in their local gammarid hosts (Rigaud & Moret, 2003; Cornet, 2011), albeit not in invasive species of gammarids (Rigaud & Moret, 2003; Cornet *et al.*, 2010). Together with the higher prevalence (Dunn & Dick, 1998) and stronger virulence (Bauer *et al.*, 2000) of parasites on their local hosts compared to invasive ones, such results point to the importance of the immune system of gammarids in resistance against helminth parasites. Moreover, the immune system is of great importance in defence against bacteria, with immunosuppression induced by helminths leading to a decrease in the efficiency of

gammarids to eliminate bacterial infection (Cornet *et al.*, 2009a) and a lowered survival when exposed to micro-organisms (Cornet & Sorci, 2010).

The effect of temperature on the immune system of gammarids is investigated in the following article, in order to better understand the reasons of the differences in infection parameters due to temperature.

ARTICLE 5

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Effect of water temperature on the immune system of Gammarus pulex *(Crustacea: Amphipoda)*

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Draft manuscript

Abstract

Ambient temperature is known to impact host-parasite interactions in various ways. In particular, high temperatures are often associated with increased parasite prevalence and abundance, and a shorter development time of parasites. Such effects are often regarded as the consequence of the increased metabolism of parasites with increasing temperature. However, the effect of increased temperature on hosts, especially on their immune system, could also be determinant. Gammarids are ecologically important crustacean amphipods, which roles within the ecosystem can be altered by several different parasite species, such as helminths. While temperature is known to modify infection parameters for many of these parasites, the effects of temperature on the immune system of gammarids, which could partly explain this result, are still unclear. Here, we investigated the consequences of three weeks of acclimatization at four different temperatures, ranging from 9°C to 17°C, on different immunological parameters of uninfected *Gammarus pulex* individuals. Our results show that hemocyte concentration and phenoloxidase (PO) enzymatic activity were at their lowest value at intermediate temperatures, and increased at lower and higher temperatures. The total enzymatic activity (PO and its inactive form, prophenoloxidase), however, was found to be independent of temperature. In addition, the ability of gammarids to clear bacterial infection was shown to increase with temperature at low temperature, while decreasing at high temperature. Our results suggest that, at high temperature, the immune system of gammarids might function to contain infection with macro-parasites, while making them more susceptible to bacterial infection.

Keywords

Gammarids, Immunocompetence, Environmental conditions, Phenoloxidase, Hemocytes, Bacterial resistance

Introduction

Freshwater gammarids are crustacean amphipods that are widespread and abundant in many rivers worldwide. They occupy a central place within food webs, where they constitute both an important prey for many species (Degani *et al.*, 1987; Friberg *et al.*, 1994) and a predator that can modulate other macroinvertebrate populations (MacNeil *et al.*, 1997; Kelly *et al.*, 2006). Freshwater gammarids also feed on leaf litter, participating in their breakdown (Piscart *et al.*, 2009; Foucreau *et al.*, 2013a), and thus in the maintenance of water quality (Maltby *et al.*, 2002). Gammarids can be infected with various parasitic and pathogen species, ranging from bacteria to macro-parasites such as cestodes, trematodes

or acanthocephalans (Van Maren, 1979; Thomas *et al.*, 2002b). Infection of gammarids can ultimately affect their functional role as predator or shredder species (Fielding *et al.*, 2003; Médoc *et al.*, 2011b; Labaude *et al.*, 2016).

Temperature is known to be one of the most important factors affecting host-parasite relationships. Temperature may first affect parasite biology or physiology (Gillooly *et al.*, 2001; Harvell *et al.*, 2002; Barber *et al.*, 2016). In amphipods, the development time of acanthocephalan parasites has been shown to be highly dependent upon the temperature experienced by their hosts (Olson & Pratt, 1971; Tokeson & Holmes, 1982). Infection success was also found to depend on temperature for both cestode and trematode species (Okaka, 1989; Mouritsen & Jensen, 1997; Studer & Poulin, 2013), while replication of microsporidia within amphipods is inhibited at low temperatures (Dunn *et al.*, 2006).

However, temperature can also affect host species, and part of the observed variation in the consequences of infection could stem from the interaction between parasites and their hosts. In particular, the immunocompetence of hosts might be of crucial important for the successful establishment, growth and survival of parasites within their hosts (Marcogliese, 2001).

Crustaceans have an innate immune system that relies on several components (Söderhäll & Cerenius, 1992; Vazquez *et al.*, 2009). The prophenoloxidase (ProPO) cascade, in particular, is involved in the recognition of non-self (Söderhäll & Cerenius, 1998). The active catalyzing phenoloxidase (PO) enzyme has the capacity to adhere to foreign organisms, ranging from micro-organisms to macro-parasites, and induces the deposition of melanin on their surface (Cerenius & Söderhäll, 2004). The inactive form of the enzyme is stored in the hemolymph and in hemocytes, and is rapidly activated upon infection. Hemocytes are circulating cells that are also involved in several defense mechanisms, such as phagocytosis, encapsulation, or coagulation (Johansson *et al.*, 2000; Vazquez *et al.*, 2009). Variation in the immunocompetence of gammarids has been found to be negatively related to parasite prevalence among natural populations (Cornet *et al.*, 2009b), and environmental conditions, in particular food resources, have been shown to modulate the immune system of gammarids (Babin *et al.*, 2010, 2015).

Temperature is known to affect several components of gammarids physiology. Common species in European rivers, such as *Gammarus fossarum*, *G. pulex* or *G. roeseli*, have a high thermal plasticity. For example, the thermal optimum range for adult survival is comprised between 3°C and 17°C (e.g. Issartel *et al.*, 2005, for *G. fossarum*, Maazouzi *et al.*, 2011, for *G. pulex*), with a variation between populations (southern French populations surviving better at temperatures higher than 20°C than northern ones, Foucreau *et al.*, 2014). Pöckl & Humpesch, (1990) also showed that the optimal temperature for reproductive success and egg survival was around 12°C for *G. fossarum* and 15°C in *G. roeseli*. Several physiological parameters (e.g. oxygen consumption or glycogen contents) are impacted

by temperature in gammarids, although almost only relatively high temperatures were investigated, showing that temperatures above 15°C negatively impacted the physiology of Northern French populations of *G. pulex* (Foucreau *et al.*, 2014). However, precise effects of temperature on gammarids immune system remain unknown. Temperature is known to influence the immune system of crustaceans (reviewed by Le Moullac & Haffner, 2000), although, so far, most studies have focused on crustacean species of economic interest, such as those raised for farming, and mostly tropical ones (e.g. Cheng & Chen, 2000; Cheng *et al.*, 2003, 2005). Overall, these studies suggest that increasing temperature until some value tends to have a positive effect on different immunological parameters, after which a decreased is observed, though this pattern varies between studies and/or crustacean species. To what extent this may apply to crustacean amphipods is unknown at the moment.

The aim of this study was therefore to measure the immunocompetence of *Gammarus pulex* amphipods exposed to different temperatures. Both the inactive ProPO enzyme, representing the maintenance of the ProPO system, and its active form, indicative of its activity, were measured. The number of hemocytes was counted, as well as the ability of gammarids to clear bacterial infection.

Material and methods

Sampling and acclimatization

Gammarus pulex individuals were collected in April 2014 from a small tributary of the Suzon river (eastern France, 47°24'12.6"N 4°52'58.2"E), using the kick sampling method with a hand net. Gammarids were first maintained during one week under standard laboratory conditions at 10°C, what corresponds to the temperature of the river at the time of collection. Only males showing no sign of infection with acanthocephalan parasites were kept for the experiments to avoid any confounding effect of sex or parasitic infection.

Individuals were randomly assigned to a temperature (9, 11, 14 or 17°C) and then placed in acclimatization at this temperature for three weeks. This range of temperatures corresponds to naturally fluctuating temperatures experienced by gammarids in their habitat (Pöckl *et al.*, 2003) and current temperatures in Burgundy (Gunn & Crumley, 1991; Rowell, 2005). Gammarids were individually maintained in plastic tubes (1.5 x 4 cm) closed at both ends with fine mesh, allowing water exchanges. All tubes were then placed in a tank, with one different tank being used for each temperature. Tanks were filled with a mix of water from the river and dechlorinated, UV-treated tap water. Water from each tank was constantly pumped toward a device (TANK TK-1000 Chiller, Teco US) controlling its temperature. The temperature was measured every five minutes during the experiment, using automatic thermometer recorders. As the actual temperatures slightly diverged from the

selected temperatures, we took into account the mean of real temperatures measured over the total duration of the experiment (respectively 8.8°C, 11.1°C, 14.2°C and 17.0°C). The actual water temperature was not constant, but was oscillating around an average value with a variation of ± 2°C. Individuals were fed once a week with conditioned elm leaves, and maintained under a 12:12 light:dark cycle regime.

Resistance to bacterial challenge

To assess the ability of the immune system of gammarids to clear bacterial infection, resistance to bacterial challenge was tested using the protocol described in Cornet *et al.* (2009b). The day before the experiment, tetracycline-resistant *Escherichia coli* bacteria (strain CIP 103410, Pasteur Institute, Paris, France) were allowed to grow in 10 ml of broth (10 g of bactotryptone, 5 g of yeast extract and 10 g of NaCl per liter of distilled water, pH 7.0) containing 10 µl of tetracycline (2.5 mg/ml), at 30°C in a shaking incubator. Before the experiment, the solution of bacteria was centrifuged (4°C, 15000 rpm, 30 min), and bacteria were rinsed twice using PBS buffer. Bacteria were diluted in PBS buffer and their concentration was set at 1×10^5 bacteria per microliter using a counting chamber (Neubauer Improved) under a compound microscope.

Gammarids were briefly anesthetized on ice and gently immobilized on sticky gum under a dissecting microscope. Their second or third coxal plate was then laterally perforated with a fine sterile needle. Using a Hamilton syringe with a fine needle, 0.5 µl of the bacterial solution was injected through the hole. Each injected individual was immediately put in an individual hermetic glass bottle, to avoid water contamination, and placed back at its acclimatization temperature for seven hours and fifteen minutes. After this time, about half of bacteria are expected to have been cleared, and variation between individuals is likely to be visible (Cornet *et al.*, 2009b). A small hole was then pierced again in the cuticle of the individual, and 2 µl of hemolymph were extracted using a sterile glass capillary, and immediately diluted in 198 µl of PBS buffer. After homogenization, this mixture was divided into two replicates of 100 µl that were spread on Petri dishes containing 5 µg/ml of tetracycline. Petri dishes were incubated at 30°C until bacterial colonies were clearly visible, and CFUs (colony-forming units) were counted using an automatic colony counter (Scan® 500 version 6.1.2.0, Interscience, Saint Nom, France). For each gammarid, the number of colonies was adjusted to 1 µl of pure hemolymph and the mean between the two replicates was considered. Petri dishes where no colony or only a single one developed were removed from the data (n = 4).

E. coli optimum growth occurs at around 37°C. Therefore, the temperatures used in our experiments might not only have an effect on the immune system of gammarids, but also on the

growth and survival of bacteria. To quantify this effect, a control was made using a similar protocol as described above, where gammarids were replaced by plastic tubes containing 50 µl of PBS buffer. After the addition of 1 µl of the bacterial solution (1 x 10^5 bacteria/µl), five tubes were put at each temperature during 6.5 hours. Two microliters of the solution were then diluted in 198 µl of PBS buffer and bacteria were spread on Petri dishes and counted as described above.

Hemolymph extraction, hemocyte concentration and enzyme activity of the ProPO system

Hemolymph extractions were made on gammarids that were not used for the bacterial challenge. Following a brief anesthetization on ice, individuals were immobilized on sticky gum under a dissecting microscope and a hole was dorsally made on their cuticle using a fine sterile needle. Hemolymph was extracted using sterile pre-chilled graduated glass capillaries, and the total volume that was extracted was recorded. Hemolymph was immediately mixed with 20 µl of PBS buffer. Ten microliters of the mix were immediately used to assess hemocyte concentration under a microscope, using a Neubauer counting chamber. The rest of the mix was frozen in liquid nitrogen and stored at -80°C for enzymatic activity assays.

For all individuals, the activity of naturally activated phenoloxidase enzymes (hereafter referred as “PO activity”) and the combined activity of PO and its ProPO (prophenoloxidase) proenzyme (hereafter referred as “total activity”) were measured using a spectrometric assay (Cornet *et al.*, 2009a). Hemolymph samples were thawed on ice and 5 µl were added to microplate wells containing 20 µl of PBS buffer and either 140 µl of distilled water to measure PO activity or 140 µl of chymotrypsin solution (0.07 mg per ml of distilled water), used to activate the ProPO into PO, to measure total activity. The enzymatic reaction started with the addition of 20 µl of cold L-Dopa solution (4 mg per ml of distilled water) in each well. The reaction was allowed to proceed for 40 min at 30°C, and change in optical density was followed in a microplate reader (Versamax, Molecular Devices) at 490 nm. Enzymatic activity was analyzed using the SOFT-MaxPro 4.0 software (Molecure Devices), and measured as the maximum slope (Vmax value) of the linear phase of the reaction curve.

Measures of hemocyte concentration and enzymatic activities were both adjusted for 1 µl of pure hemolymph for all individuals.

Measurements and genetic analyses

All the gammarids used in the experiment were measured (from the height of the fourth coxal plate, see Bollache *et al.*, 2000), using a microscope and Lucia G 4.81 software. Although the cryptic species *Gammarus fossarum* can also occur in the river, *G. pulex* is known to be largely dominant (Lagrue *et*

al., 2014). Genetic analyses were however conducted on 20% of tested individuals (n = 88) that confirmed the high majority of *G. pulex* (95%). Thus, apart from these *G. fossarum* individuals that were removed, all other individuals were considered as belonging to this species.

Statistical analyses

Data from resistance to bacterial challenge were natural-log transformed to meet homoscedasticity conditions and analyzed using a linear model with gammarid size and temperature as fixed factors. A quadratic effect of temperature was also considered, after visual inspection of the results. Similarly, bacterial dynamics in tubes under different temperatures was natural-log transformed and analyzed separately using a linear model, with temperature and its quadratic effect as factors.

Data on hemocyte concentration (number of hemocytes per microliter of pure hemolymph) were square-root transformed. PO and total enzymatic activities were transformed using Box-Cox procedures. As the three parameters were measured on the same individuals, a multivariate analysis of variance (MANOVA, Pillai's trace) was conducted, testing for the influence of gammarid size, temperature, temperature's quadratic effect, and their interactions. Linear models were also used for each parameter, with the same factors.

Non-significant interactions were removed for all models. Data were analyzed using JMP version 10.0.0 software (SAS Institute, Cary, NC, U.S.A.).

Results

Bacterial survival

The number of bacterial colonies was negatively affected by gammarid size, and showed a convex relationship with temperature (Table 14, Fig 25A). Overall, the clearance ability of gammarid hemolymph increased between 8.8 and 14.2°C, while a higher number of bacteria was found at 17°C (Fig. 25A). The number of *In vitro* bacterial colonies was significantly affected by temperature, with a linear increase of surviving bacteria with increased temperature (Table 14, Fig 25B).

Immunological parameters

All three immunological parameters were significantly affected by gammarid size, temperature and its quadratic effect (MANOVA, Table 15). The quadratic effect of temperature on the combination of the three immunological parameters (as estimated by the coordinates on the first canonical axis, which

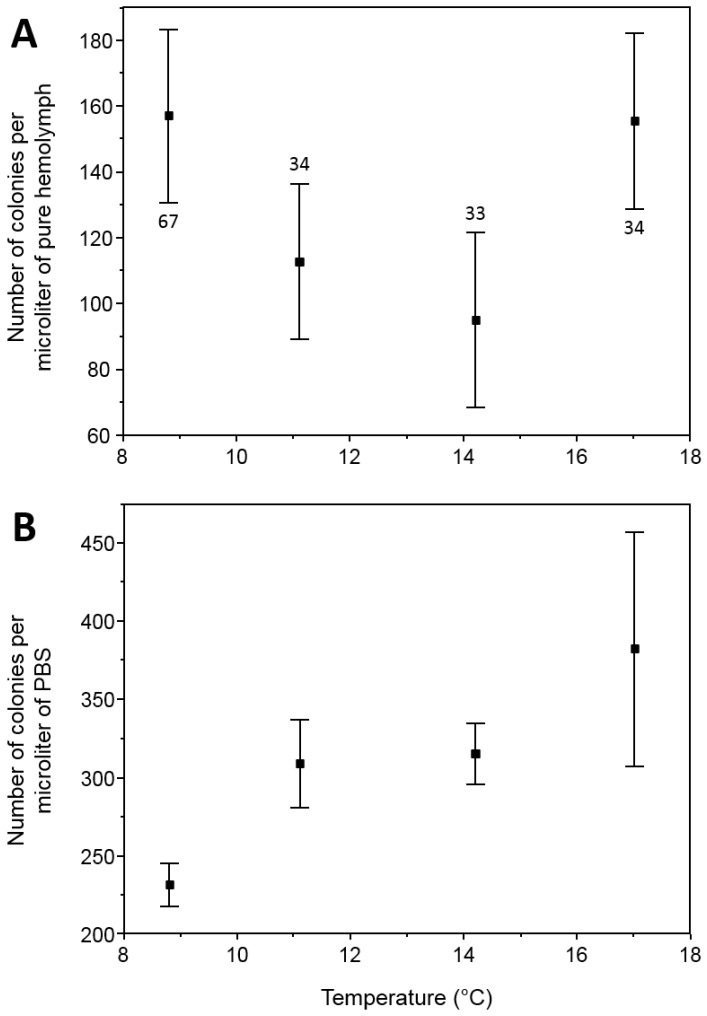

Figure 25. Effect of temperature on the number of bacteria (colony-forming units) remaining in 1 µl of (A) pure gammarid hemolymph or (B) PBS buffer. Dots represent mean values, error bars stand for standard error and sample size of gammarids are indicated.

were generated using the eigenvectors used to construct multivariate test statistics), is illustrated in Figure 26A. Global immunocompetence of gammarids was higher at low and high temperatures, and lower at intermediate ones. When analyzing these immunological parameters separately with linear models, gammarid size was found to have a significant effect only on PO activity (Table 15). This parameter was also close to be significantly affected by the quadratic effect of temperature, with a decrease of PO activity between 8.8 and 11.1°C, followed by an increase (Table 15, Fig 26C). The effect of temperature on hemocyte concentration was also marginally significant (Table 15, Fig 26B). No factor was found to significantly influence the total enzymatic activity (Table 15, Fig 26D).

Table 14. Linear model (ANOVA) analyzing the number of bacteria remaining in 1 µl of pure gammarid hemolymph ("bacteria in gammarids"), or remaining in PBS ("bacteria *in vitro*"), as a function of gammarids size, temperature and its quadratic effect (Temperature²). Non-significant interactions were removed from the model. Significant values are presented in bold.

Models	Source of variation	*num d.f.*[a], *den d.f.*[b]	F	P
Bacteria in gammarids	Global Model	3, 167	4.90	**0.027**
	Size	1, 167	7.56	**0.0067**
	Temperature	1, 167	2.24	0.14
	Temperature^2	1, 167	6.94	**0.0093**
Bacteria *in vitro*	Global Model (temperature)	1, 23	5.01	**0.036**

[a] Degrees of freedom of the numerator

[b] Degrees of freedom of the denominator

Table 15. Multivariate (MANOVA, Pillai's trace) and univariate (ANOVA) analyses of variance for the three immunological parameters (hemocyte concentration, PO and total enzymatic activity) as a function of gammarids size, temperature and its quadratic effect (Temperature²). Non-significant interactions were removed from the model. Significant values are presented in bold.

Models	Source of variation	*num d.f.*[a], *den d.f.*[b]	F	P
MANOVA	Global Model	9, 633	4.83	**< 0.0001**
	Size	3, 209	9.28	**< 0.0001**
	Temperature	3, 209	3.21	**0.024**
	Temperature^2	3, 209	4.69	**0.0034**
ANOVA hemocyte concentration	Global Model	3, 211	2.22	0.086
	Size	1, 211	2.32	0.13
	Temperature	1, 211	3.24	0.073
	Temperature^2	1, 211	2.02	0.16
ANOVA PO activity	Global Model	3, 211	7.36	**0.0001**
	Size	1, 211	15.49	**0.0001**
	Temperature	1, 211	3.14	0.078
	Temperature^2	1, 211	3.61	0.059
ANOVA Total activity	Global Model	3, 211	0.25	0.86
	Size	1, 211	0.29	0.59
	Temperature	1, 211	0.05	0.82
	Temperature^2	1, 211	0.19	0.66

[a] Degrees of freedom of the numerator

[b] Degrees of freedom of the denominator

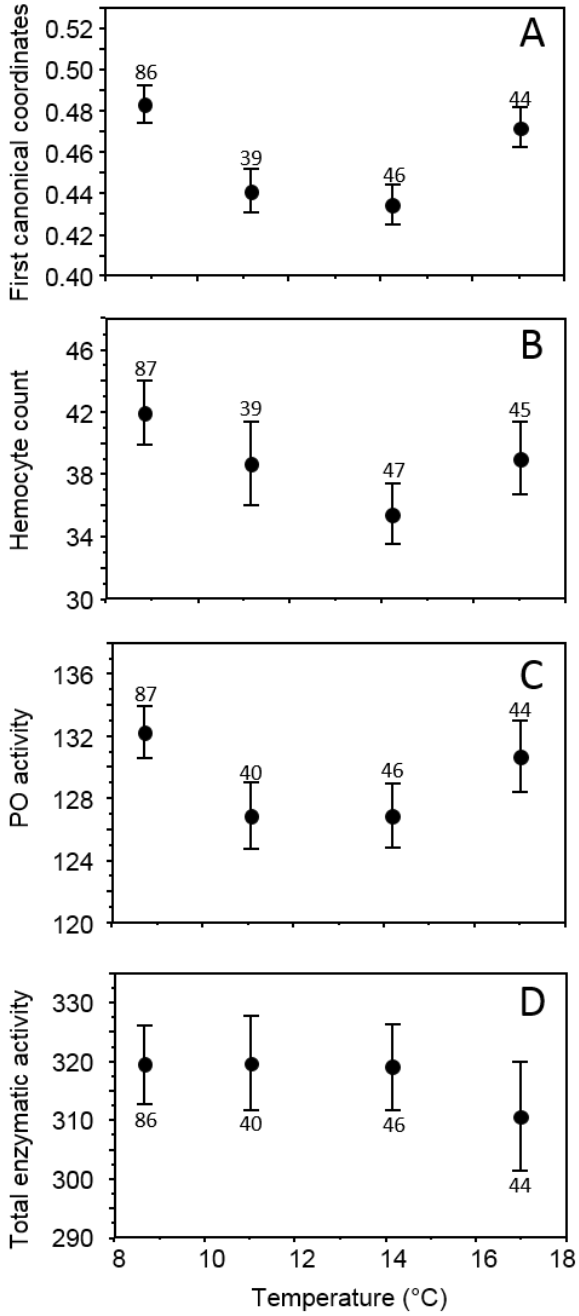

Figure 26. Effect of acclimatization temperature on the different immunological parameters: (A) Coordinates of the first canonical axis generated by the MANOVA (combination of the three immunological parameters), (B) hemocyte concentration, (C) PO activity and (D) total enzymatic activity. The transformed data are presented here. Dots represent mean values, error bars stand for standard error and sample size are indicated.

Discussion

Our results show that the maintenance of *Gammarus pulex* individuals at different temperatures (around 9, 11, 14 and 17°C) during three weeks led to differences in several components of their basal immune system. Overall, immunological parameters were shown to be linked with a quadratic effect of temperature. Combination of hemocyte concentration, PO activity and total (ProPO + PO) enzymatic activity was lower at intermediate temperatures, but the ability to clear bacterial infection was higher at these intermediate temperatures.

Temperature affects the ability of gammarids to clear bacterial infection. Indeed, although bacterial survival in vitro presented a positive linear relationship with increasing temperature, the CFU concentration in the hemolymph of gammarids decreased between 9°C and 14°C, before increasing again. Thus, gammarids ability to deal with bacteria increased with temperature, but only to a certain extent, consistently with other results found in crustaceans (Cheng *et al.*, 2003) or in insects (Catalán *et al.*, 2012). For example, Chrisholm & Smith (1994) evidenced, in the crab *Carcinus maenas*, a seasonal change in the antibacterial activity by hemocytes according to temperature. The antibacterial activity was the lowest at the lowest and highest water temperatures of the year. Although this result seems contradictory with the other immune parameters measured in our study, defense against *E. coli* was already found to show no correlation with basal PO activity in *G. pulex* (Cornet *et al.*, 2009b), suggesting that resistance against bacteria might go through other pathways. Some authors even suggested the existence of a trade-off between antibacterial activity and PO activity (Siva-Jothy *et al.*, 2005; Moret & Schmid-Hempel, 2009).

The overall lower level of other components of the immune activity at intermediate temperatures seemed to be mostly due to the hemocyte concentration and the PO activity. Indeed, the two parameters were at their lowest value at intermediate temperatures (respectively 14°C and 9°C), and increased at lower and higher temperatures. This similitude is consistent with the positive correlation already reported between the two parameters in crustaceans (Cheng et al., 2005). However, the range of temperatures chosen in our study did not induce any effect on the total enzymatic activity of gammarids. PO activity represents the amount of naturally active enzymes, while total enzymatic activity also takes into account the inactive ProPO enzymes present in the hemolymph, thus rather measuring the maintenance of the ProPO system (Söderhäll & Cerenius, 1992). It seems therefore that the acclimatization temperature had an effect on the use of the ProPO system, without influencing its maintenance. Consistent with this result, the total enzymatic activity was found to show low variations between years in several populations of *G. pulex*, while their PO activity was highly variable (Cornet *et al.*, 2009b), suggesting that investment in the maintenance of the ProPO system resulted from local adaptation, whereas its activation depended on proximate environmental

conditions (Cornet *et al.*, 2009b). Our results suggest that one of the environmental conditions could be temperature.

While it is widely recognized that temperature can alter the immune system of crustaceans, there is no consensus as to the direction of these alterations, and many studies led to contradictory results (Le Moullac & Haffner, 2000). However, it is generally accepted that increased temperatures might have a positive effect on crustacean immunocompetence, at least to a certain extent (see Le Moullac & Haffner, 2000; Matozzo *et al.*, 2011 and references therein). Here, highest levels of immune defenses were found for both the highest and lowest temperatures tested. Contrary to many studies testing the effects of extreme temperatures (e.g. Gomez-Jimenez *et al.*, 2000; Pascual *et al.*, 2003; Brockton & Smith, 2008), the temperature conditions chosen here framed the optimal thermal range of gammarids (Pöckl & Humpesch, 1990; Issartel *et al.*, 2005), and are consistent with the current temperatures in Burgundy (Gunn & Crumley, 1991; Rowell, 2005), thus providing information about modifications probably experienced by gammarids following fluctuations of temperatures in their natural habitat (Pöckl *et al.*, 2003; Cornet *et al.*, 2009b). The effects of temperature on different components of the immune system of ectotherms were found to depend on the time of exposure, with rapid modifications early after temperature modification, followed by a stabilization of the different parameters over time (Raffel *et al.*, 2006; Pan *et al.*, 2008). In bivalves transferred from 17°C to 11°C, 23°C or 28°C, the quantity of hemocytes was shown to be the highest at 23°C and the lowest at 28°C after one hour. However, after three days of acclimatization, the shape of the effect of temperature was similar to ours, with the highest value being at the lowest temperature (Chen *et al.*, 2007). Thus, the relatively long acclimatization time chosen in our study allowed us to observe effects of temperatures due to individual plasticity, rather than short-time stress effects.

The higher immunocompetence found in our study at low and high temperatures could a priori suggest that extreme temperatures are better for immune protection in gammarids. This is, however, not consistent with the optimum of other physiological functions already suggested for *Gammarus* (e.g. Pöckl & Humpesch, 1990), where intermediate values represent the most comfortable temperature for individuals. It is often suggested that environmental variations lead to stress-induced immunosuppression in crustaceans (Le Moullac & Haffner, 2000). However, crabs transferred from 17°C to 4°C or 30°C for seven days also displayed higher PO activity, as well as increased hemocyte proliferation, compared with those who stayed at the intermediate temperature (Matozzo *et al.*, 2011). Similar results were found in a mollusk exposed for one day to temperatures ranging from 20 to 32°C, with hemocyte counts being at the minimal value for intermediate temperatures, while PO activity decreased between 20°C and 24°C, increased at 28°C before decreasing again at 32°C (Cheng *et al.*, 2004). Therefore, we might consider that the optimal level of hemocyte counts or PO activity (in the absence of parasite infection, i.e. without any immune response) would not be the highest values,

but rather the lowest ones. One interpretation could be that extreme temperatures would induce tissue damages, as evidenced for some crustacean shrimps exposed to high temperatures (Madeira *et al.*, 2015), damages that could be cope by the immune system. Whatever the underlying cause, this would mean that extreme temperatures would induce an over-functioning of some immune pathways, with the risk of increasing immune pathologies. Indeed, the activation of cytotoxic compounds can lead to damages on self-tissues (Sadd & Siva-Jothy, 2006). In gammarids, the inter-population variation in the level of PO or ProPO enzymes positively correlates with the level of circulating carotenoids, suggesting that these anti-oxidant molecules are important to limit the negative effects of high levels of toxic compounds delivered by this immune pathway (Cornet *et al.*, 2007). Experimental results verified this hypothesis (Babin *et al.*, 2010, 2015), suggesting that high levels of immunocompetence can be detrimental if not compensated by high levels of carotenoid storage.

Our results suggest that a decrease in gammarids ability to resist bacterial infection might lead to higher micro-organism infections at high temperatures. However, increased macro-parasite infections or growth observed at high temperatures can hardly be explained by a negative effect of high temperature on gammarids basal immunocompetence. On the contrary, their immune system might actually prevent more severe infection at high temperatures, especially since infection itself could induce an increase in gammarids immune system through its activation (Cerenius & Söderhäll, 2004), at least before an immunosuppression induced by certain parasite species (Rigaud & Moret, 2003; Cornet, 2011). Higher infection parameters at high temperatures could partly derived from other effects of temperature on gammarids. First, high temperature, even when not inducing any effect by itself on the immune system of an organism, might constitute a general physiological stress. For instance, the effects of ocean acidification on a lobster were more pronounced at high temperatures, while temperature alone did not influence its immune system (Hernroth *et al.*, 2012). Moreover, high temperatures can be linked to other stressors, such as lower water oxygenation. Second, the interactions between parasites and their hosts are not restricted to the hosts' immune system, and modifications on other traits might as well have an impact on infection parameters. For instance, temperature has been shown to induce an increase in gammarids food consumption (Pellan *et al.*, 2015; Foucreau *et al.*, 2016; Labaude *et al.*, 2016), thus also increasing their probability of consuming parasite eggs and becoming infected. Finally, this study investigated the effects of temperature on individuals. If considering longer time-scales, we might expect gammarids to adapt to changes in temperature, and eventually present differences in their immune system. Indeed, Cornet *et al.* (2009b) found a negative relationship between parasite prevalence and PO activity in different gammarids populations.

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Chapter V. Ecological consequences on gammarids

The experiments presented in previous chapters suggest that some environmental parameters could affect the relationship between acanthocephalan parasites and their gammarid hosts. Such effects are likely to have ecological consequences that are detailed in the following chapter. First, infected and uninfected gammarids might present different thermic preferences, such that the actual effect of temperature might also depend on their microhabitat choice. An experiment exploring the thermic preferences of infected and uninfected gammarids was conducted to investigate such assumption. Second, the shredder role of gammarids is known to be of great importance in their rivers, and was shown to be affected by parasites and temperature. The cumulative effect of these two parameters was thus investigated, with consumption tests conducted on naturally infected gammarids acclimatized at three different temperatures in the laboratory. Third, because manipulative parasites are mostly known to alter the probability of predation of their intermediate hosts by their definitive hosts, it is likely that the role of gammarids as preys might also depend on a cumulative effect of temperature and parasites. Although only preliminary experiments could be conducted in the frame of this thesis, this aspect is discussed at the end of this chapter.

1. Modification of the thermal preferences of gammarids

Introduction

Temperature plays a major role in many parameters of host-parasite associations (Barber *et al.*, 2016). Along with other studies, my work confirmed that acanthocephalan parasites are also affected by temperature in many ways. At high temperature, acanthocephalan parasites develop faster in their gammarid intermediate hosts (Olson & Pratt, 1971; Tokeson & Holmes, 1982, see also Article 4). Their prevalence and abundance are also increased, both in their intermediate hosts (Article 4) and in their definitive fish hosts (Sheath *et al.*, 2016). On the other hand, temperature also affects gammarids, modifying their metabolism and accelerating their death (Issartel *et al.*, 2005; Foucreau *et al.*, 2014).

Because of these combined effects, the optimal temperature might differ between acanthocephalan parasites and their hosts, and the resulting thermal preference of infected gammarids might diverge from that of uninfected individuals. First, infected gammarids could benefit from preferring colder temperatures, increasing their survival but also decreasing the speed of development of their parasites, and thus delaying their potentially fatal manipulation. On the contrary, parasites might manipulate the thermal preferences of their hosts to lead them toward a higher thermal range, such as a better compromise between their host survival and their own development. Moreover, the thermal optimum of parasites might also change according to their development stage, as the effect of temperature might differ among stages (Altman *et al.*, 2016). Indeed, acanthella parasites are still growing inside gammarids, while cystacanth parasites are ready to be transmitted to their definitive hosts.

Experimental studies investigating the effect of temperature on host-parasite associations often rely on obligate thermal conditions. However, differential thermal preferences might divide gammarids population according to their infection status, such that infected and control individuals would always experience different thermic conditions based on micro-habitat choices.

In the present preliminary experiment, we tested the thermal preferences of uninfected and infected gammarids. First, naturally infected gammarids were used to assess the impact of acanthocephalans at a cystacanth stage. Second, experimentally infected gammarids were used to test the impact of acanthella parasites.

Material and methods

Experimental device

The experimental device, inspired by Chen & Chen (1999), consisted of a 3.5 meters long horizontal plastic gutter (five centimeters deep and six centimeters large at its top). In the bottom of the device, two copper tubes were used to create a counter-current heat-exchanger system (Fig. 27). Each tube was connected to a temperature control device (TANK TK-1000 Chiller, Teco®, Ravenna, Italy) and a pump, forming a closed system with constant circulation of water inside the tube. Water circulated in opposite directions in the two tubes, with cold water in one tube and hot water in the other tube. The thermal gradient in the device was created thanks to the contact between the water contained in the gutter and the two copper tubes. A fine plastic mesh was used to cover the copper tubes, to avoid any direct contact between the tubes and gammarids. The device was virtually divided into 35 zones (10 centimeters long), numbered from the coldest to the hottest zone, allowing the recording of the position of individuals during the test.

The thermal gradient in the device was very sensitive to variations from both the room temperature and the temperatures set up on the temperature control devices. Moreover, the thermal gradient also depended on the speed of circulation of water inside each tube that could be modified at the pumps and temperature control devices. Settings were adjusted for each of the two experiments to obtain a thermal gradient as wide as possible. However, this gradient differed between the two experiments. Thus, regular measurements of the temperature were made along the device during each experiment.

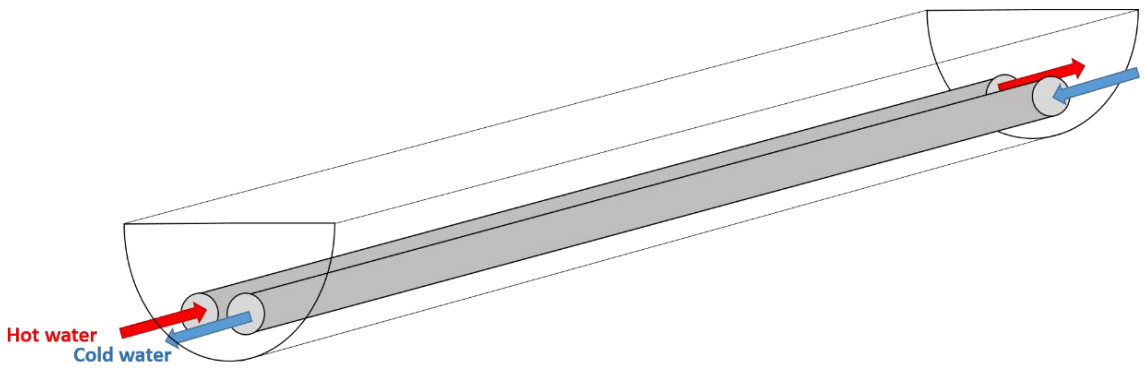

Figure 27. Experimental device allowing the creation of a thermal gradient (hottest part represented on the left) thanks to a counter-current heat-exchanger system.

Test 1: Naturally-infected gammarids with cystacanth stages

Gammarids were collected in May 2015 in the Norges River (eastern France, 47°21'41.38"N, 5°9'30.16"E). Only males were kept. Genetic analyses conducted on 36 individuals showed that this population only consisted of *Gammarus fossarum* individuals, confirming the results found by Lagrue *et al.* (2014). Gammarids from this population can harbor several acanthocephalan species. Individuals infected with the species most commonly found at the cystacanth stage, *Pomphorhynchus tereticollis*, were used. Gammarids were maintained at 14°C under a 12:12 light:dark cycle regime and fed ad libitum with conditioned elm leaves before the test.

For each test, a single gammarid was introduced at the center of the device. After two minutes of acclimatization, its position was recorded every two minutes for 30 minutes. All gammarids were dissected at the end of the experiment, to assess their infection status. Gammarids with no parasites were kept as controls, and gammarids harboring *P. tereticollis* parasites at the cystacanth stage were considered as parasitized. Individuals harboring only acanthella stages or cystacanth parasites from other species were removed from the data.

Test 2: Experimentally infected gammarids with acanthella stages

Uninfected *Gammarus pulex* individuals were collected in a small tributary of the Suzon River (eastern France, 47°24'12.6"N, 4°52'58.2"E) in November 2015. Only males were kept. Individuals were acclimatized for three weeks at 14°C before being exposed to *Pomphorhynchus laevis* eggs collected in adult female parasites from chubs (*Leuciscus cephalus*) sampled in December 2015 in the Vouge River (eastern France, 47°9'34.36" N, 5°9'2.50" E). Gammarids were maintained at 14°C during the development of their parasites, under a 12:12 light:dark cycle regime, and with food ad libitum.

Individuals were tested when they were harboring acanthella stages, between one day and three weeks before parasites turned from the acanthella stage to the cystacanth stage. For each test, a single gammarid was introduced at the center of the device. Because gammarids were highly active in preliminary tests, the acclimatization period inside the device was extended to half an hour. Then, the position of the gammarid was recorded every 15 seconds for 20 minutes. All gammarids were dissected at the end of the experiment.

Results

Temperatures measured along the gradient during the test 1 ranged from 14.6°C to 20.3°C with a progressive rise from the first to the last zone (Fig. 28A). The gradient obtained for the test 2 was much narrower, with temperatures ranging from 13.7°C to 16.9°C, and the shape of the thermal gradient was very irregular, especially at the hot extremity of the device (Fig. 28B).

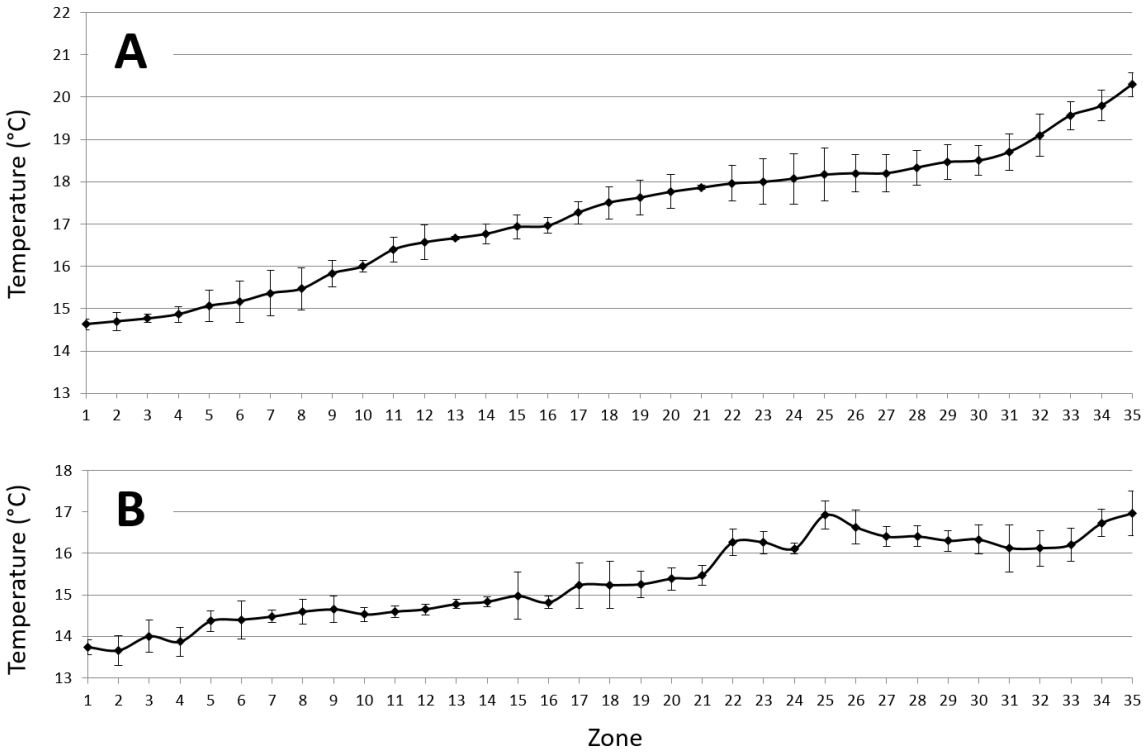

Figure 28. Temperatures (mean ± standard error) measured in each zone of the thermal gradient during the test 1 (A) and the test 2 (B). At least three measures were taken in each zone, using a digital thermometer plunged in the water, in the center of the zone.

Because of this shape, two statistical tests were used to analyze the data. First, Student tests were used to compare the average position observed for each gammarid depending on their group (thus considering that temperature increased gradually along the device). In the test 1, gammarids infected with *P. tereticollis* cystacanths were found, on average, significantly more in colder zones compared to control individuals (Fig. 29A, $t = 2.43$, $P = 0.021$). The same trend was found in the test 2 for gammarids infected with *P. laevis* acanthella parasites (Fig 29B), but no significant difference was evidenced ($t = 1.22$, $P = 0.23$).

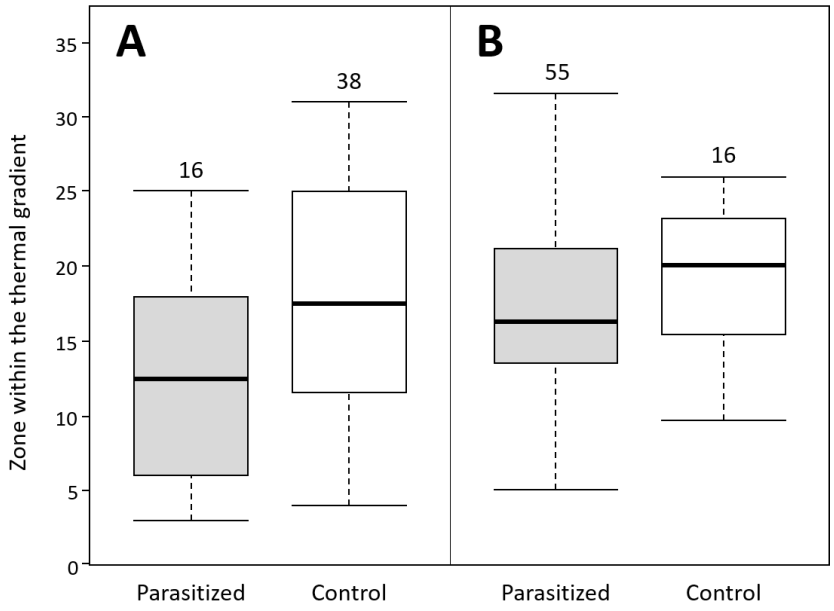

Figure 29. Zones from the thermal gradient visited by gammarids according to their infection status. (A) *G. fossarum* from the test 1, either uninfected (control) or naturally infected with *P. tereticollis* cystacanth parasites. (B) *G. pulex* from the test 2, either uninfected (control) or experimentally infected with *P. laevis*, at the acanthella stage at the moment of the test. Thick lines represent the medians, boxes represent the upper and lower quartiles, and dotted lines represent the upper and lower deciles. Sample sizes are indicated.

In a second statistical test, we divided the device into two areas ('cold' area, from zone 1 to zone 17, and 'warm' area, from zone 18 to zone 35, Fig. 28). We then assigned each gammarid to one of these areas, based on its average score. In the first test, only 12.5 % of parasitized individuals had their average position in the warm area, while 47.4 % of uninfected individuals were found in the warm area (Fig. 30). This difference between infected and control individuals was, once again, significant for the test 1 (Chi2 = 6.56, P = 0.01). A similar trend was found in test 2, where 36.4 % of infected individuals and 56.2 % of uninfected ones were mostly found in the warm area (Fig. 30). The difference between infected and control individuals was, however, not significant in the test 2 (Chi2 = 2, P = 0.16).

Discussion

Our results provide evidence that the thermal preferences of gammarids might be affected by the presence of acanthocephalan parasites. Infections from both acanthella and cystacanth stages led to

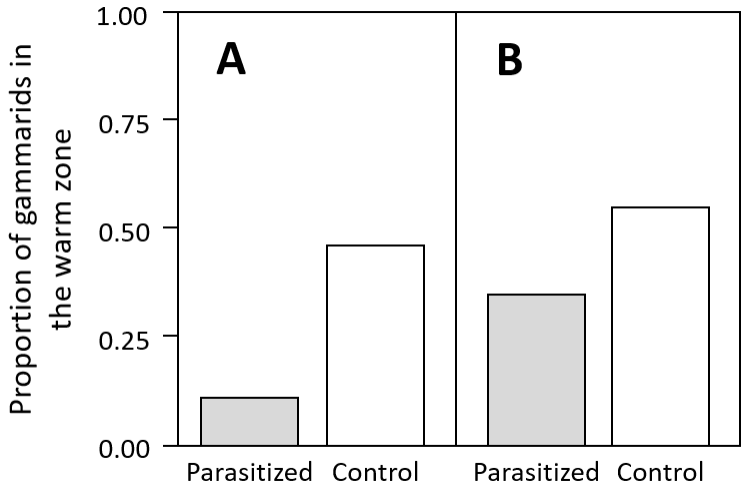

Figure 30. Proportion of gammarids found in the warm area, according to their infection status. (A) *G. fossarum* from the test 1, either uninfected (control) or naturally infected with *P. tereticollis* cystacanth parasites. (B) *G. pulex* from the test 2, either uninfected (control) or experimentally infected with *P. laevis*, at the acanthella stage at the moment of the test. Total sample sizes are indicated in Figure 3.

preferences for colder environment compared to uninfected individuals, although the difference was significant only for gammarids infected with cystacanth parasites.

This experiment is only preliminary. The device needs to be improved to obtain a more reliable thermal gradient, with wider differences between each extremity. For instance, Chen & Chen (1999) managed to create a 22°C gradient in a 7.14 meters long pipe. Due to the small size of gammarids, such a long device might not be appropriate as individuals' choice, measured by their position, depends on the distance they can swim during the test. A more effective counter-current heat-exchanger system, along with a better thermic isolation of the gutter, might however allow to obtain a better thermal gradient in a smaller device. Moreover, the shape of the device might influence the position of gammarids. Indeed, active individuals have no choice but to turn back when they arrive at an extremity of the apparatus. On the contrary, the two extreme zones, where the fine mesh emerged from the water, provided a place in which many individuals chose to rest. To avoid such effects, other devices could be used. For instance, Kivivuori (1994) used a toroidal temperature gradient apparatus, providing a more homogeneous environment, with no extremities (see also the device created by Kivivuori & Lagerspetz, 1990, for an alternative). Classical Y maze devices could also be used to confirm the difference of preferences between two temperatures (similarly to Perrot-Minnot *et al.*, 2007).

Although many points need to be improved, this preliminary experiment brings evidence that thermal preferences might differ between infected and control individuals. Contrary to what was found in other host-parasite interactions (Bates *et al.*, 2011; Catalán *et al.*, 2012; Macnab & Barber, 2012), we found that infected gammarids preferred colder temperatures compared to uninfected individuals. Thus, rather than manipulating the thermal preferences of their host to increase their own fitness, as suggested by Bates *et al.* (2011), parasites might actually face a response of their hosts limiting their development. However, such response of infected individuals was found for both development stages of the parasites, including the cystacanth stage at which parasites are believed to have reached their development inside gammarids. Catalán *et al.* (2012) suggested that the preference for warmer temperatures of infected insects could be linked with a higher efficiency of their immune system. The effect of temperature on the immune system of gammarids was not linear (Article 5 from this thesis), and it is also possible that infected gammarids preferred temperatures increasing its efficiency. Further investigation of thermal preferences of infected and uninfected gammarids thus requires more attention. Moreover, to understand the consequences of such differences in thermal preferences, it would be interesting to investigate infection parameters from parasites developing in gammarids that can choose their preferred temperature, for instance by providing experimentally infected gammarids with different micro-habitats.

2. Alteration of the shredder role of gammarids

ARTICLE 6

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Additive effects of temperature and infection with an acanthocephalan parasite on the shredding activity of Gammarus fossarum *(Crustacea: Amphipoda): the importance of social context*

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Abstract

Climate change can have critical impacts on the ecological role of keystone species, leading to subsequent alterations within ecosystems. The consequences of climate change may be best predicted by understanding its interaction with the cumulative effects of other stressors, although this approach is rarely adopted. However, whether this interaction is additive or interactive can hardly be predicted from studies examining a single factor at a time. In particular, biotic interactions are known to induce modifications in the functional role of many species. Here, we explored the effect of temperature on leaf consumption by a keystone freshwater shredder, the amphipod *Gammarus fossarum*. This species is found at high densities in the wild, and relies on aggregation as an anti-predator behavior. In addition, gammarids regularly harbor acanthocephalan parasites that are known to induce multiple effects on their hosts, including modifications on their functional role. We thus assessed the cumulative effect of both intra-specific interactions and parasitism. Consumption tests were conducted on gammarids, either naturally infected with *Pomphorhynchus tereticollis* or uninfected, feeding alone or in groups. Our results show that increased temperatures induced a significant increase in consumption, but only to a certain extent. Interestingly, consumption at the highest temperature depended on amphipod density: whereas a decrease was observed for single individuals, no such effect on feeding was observed for individuals in groups. In addition, infection by acanthocephalan parasites per se significantly negatively impacted the shredding role of gammarids. Overall, the combined effects of parasitism and temperature appeared to be additive. Thus, future studies focusing on the impact of climate change on the functional role of keystone species may benefit from a multimodal approach under realistic conditions to derive accurate predictions.

Keywords

Global change, rising temperatures, thermal stress, keystone species, leaf litter decomposition, freshwater ecosystem, gammarid, trophic ecology, stressor, cumulative effects

Introduction

The ability to make accurate predictions about the consequences of climate change on ecosystem functioning and services depends for a large part on our understanding of how changes in temperature can interact with other sources of ecological disturbance, including biotic and abiotic factors. In this context, the impact of climate change on the functional role of keystone species deserves particular

attention. By definition, keystone species play a major role in the structure of communities and ecosystem functioning (Mills *et al.*, 1993; Jordán, 2009). Several studies have shown that climate change can affect the behavior of keystone species (Sanford, 1999; Zamani *et al.*, 2006; Pincebourde *et al.*, 2008; Kidawa *et al.*, 2010; Englund *et al.*, 2011; Luque *et al.*, 2014; Hamilton *et al.*, 2015), with potential consequences at the level of ecosystems. However in terms of predictions, an important issue is whether the effect of temperature on keystone species acts in synergy or antagonistically with other biotic and abiotic stressors (Crain *et al.*, 2008; Darling & Côté, 2008). In particular, meta-analyses suggest that the effect of climate change on organisms could lead to unpredictable changes in inter and intra-specific interactions (Tylianakis *et al.*, 2008; Rosenblatt & Schmitz, 2014). Indeed, biotic interactions can strongly affect the functional role of species within ecosystems. Hawlena *et al.* (2012) showed, for instance, that the effectiveness of plant-litter decomposition by grasshoppers was reduced under predation risk. Biotic interactions could then strongly influence the effect of climate change on organisms and their functional roles (Gilman *et al.*, 2010), although the shape of such complex interactions cannot be predicted from studies conducted on single species.

Parasitic infections constitute one of the most common biotic interactions, considering that most living organisms represent a potential host for many parasites (Poulin & Morand, 2000). Furthermore, parasites often constitute a stressor for these organisms. Parasites are well known to impact their hosts in multiple ways, potentially affecting their functional role (Thomas *et al.*, 1999; Hernandez & Sukhdeo, 2008). For example, parasitic infections can alter the diet of hosts foraging opportunistically (Médoc *et al.*, 2011b), or alter food web structure by diverting predators from host preys as a consequence of parasite-induced behavioral changes in hosts (Sato *et al.*, 2012). In addition, parasites are themselves highly affected by changes in temperature (Marcogliese, 2001; Morley & Lewis, 2014). Although parasites and global warming are likely to have cumulative effects on the role of key species within ecosystems (Lafferty & Kuris, 1999; Labaude *et al.*, 2015a), it is not clear whether such effects would be additive or interactive.

Gammarid amphipods are widespread and often abundant throughout diverse aquatic habitats (MacNeil *et al.*, 1997; Piscart *et al.*, 2009). They are regarded as key species within freshwater food webs as they constitute an important trophic resource for many species (Degani *et al.*, 1987; Friberg *et al.*, 1994), and are themselves a major predator for many invertebrate species (MacNeil *et al.*, 1997; Kelly *et al.*, 2002), thus modulating the composition of freshwater macroinvertebrates community (Kelly *et al.*, 2006). More to the point, gammarids have important scavenger and shredder roles (MacNeil *et al.*, 1997; Felten *et al.*, 2008), controlling the leaf litter breakdown in certain ecosystems (Piscart *et al.*, 2009; Constable & Birkby, 2016) and maintaining water quality (Maltby *et*

al., 2002). They are therefore of significant economic and ecological importance, as water quality plays a crucial role in the emergence of water-borne diseases, ecosystem dysfunction and loss of biodiversity (Klaphake *et al.*, 2001). However, the functional role of gammarids as shredders has been shown to depend upon several parameters including both biotic interactions, such as the presence of other gammarid species (Piscart *et al.*, 2011; Constable & Birkby, 2016) or predators (Åbjörnsson *et al.*, 2000), and abiotic parameters, such as microhabitat characteristics (Felten *et al.*, 2008) or the presence of pollutants (Dedourge-Geffard *et al.*, 2009; Andreï *et al.*, 2015). Recently, it has been demonstrated that the consumption of leaves by *Gammarus pulex* amphipods tended to increase, though not linearly, with rising temperatures (Foucreau *et al.*, 2016; see also Pellan *et al.*, 2015).

Gammarid amphipods constitute an intermediate host for many helminth parasites, and particularly acanthocephalans (e.g. Voigt, 1991). This widespread biotic interaction (Poulin & Morand, 2000) also constitutes a stressor for amphipods, since such parasites have multiple effects on their hosts, e.g. altered behavior (Bauer *et al.*, 2000; Haine *et al.*, 2005; Perrot-Minnot *et al.*, 2007), immune system (Cornet *et al.*, 2009a), metabolic rate (Rumpus & Kennedy, 1974; Labaude *et al.*, 2015b) or energetic reserves (Plaistow *et al.*, 2001). Acanthocephalan parasites can also alter the ecological role of their hosts by affecting their food consumption, although previous studies provided contradictory results. Predation by gammarids infected by acanthocephalan parasites has been reported to either decrease (Fielding *et al.*, 2003; Médoc *et al.*, 2011b) or increase (Dick *et al.*, 2010), whereas no effect of the parasite on their scavenging activity was evidenced (Fielding *et al.*, 2003; Médoc *et al.*, 2011b). Similarly, the consumption of leaves was found to be either unchanged (Fielding *et al.*, 2003) or reduced (McCahon *et al.*, 1988; Médoc *et al.*, 2011b) in infected individuals compared to uninfected conspecifics.

Despite that most gammarid populations are exposed to one or several acanthocephalan species (Cézilly *et al.*, 2000), thus leading to subsequent consequences on gammarids functional role, studies investigating the effects of temperature have so far failed to take into account these biotic interactions. Parasites are themselves affected by the temperature, with a longer time of development at low temperature (Tokeson & Holmes, 1982), or a reduced success of establishment in the definitive hosts at high temperature (Lackie, 1972). Variation in the seasonal distribution of acanthocephalan parasites also suggests that their prevalence could be linked to climatic conditions (VanCleave, 1916; Muzzall & Rabalais, 1975; Brown, 1989). In parallel, some effects of temperature on gammarid traits, such as their immune system (Le Moullac & Haffner, 2000), their growth (Moenickes *et al.*, 2011), their locomotor activity (Issartel *et al.*, 2005) or their metabolism (Roux & Roux, 1967; Issartel *et al.*, 2005; Foucreau *et al.*, 2014), are also likely to modify the interaction between gammarids and their parasites,

such as the prevalence or the probability of encounters. For instance, Guinnee and Moore (2004) found that the effect of an acanthocephalan parasite on its insect host depended on temperature: infection negatively impacted host fecundity only at the highest temperature tested. Although temperature and parasitism are highly likely to also affect the shredding efficiency of gammarids, the resulting combination of the two factors will depend on whether they have additive or interactive effects.

In this study, we investigated the cumulative effect of different temperatures and infection by the acanthocephalan parasite *Pomphorhynchus tereticollis* on the leaf consumption of *Gammarus fossarum*, two species that are commonly found in European rivers (Westram *et al.*, 2011; Emde *et al.*, 2012). First, we investigated leaf consumption by uninfected and infected single gammarids in isolated conditions. Second, we assessed leaf consumption by gammarids in a more ecologically relevant context, using individuals maintained in groups in microcosms. Indeed, gammarid individuals often occur at high density in the wild, and aggregation has been shown to constitute a relevant antipredator behavior that is disrupted by parasites (Durieux *et al.*, 2012; Lewis *et al.*, 2012).

Materials and methods

Sampling

Gammarus fossarum individuals were collected in September and October 2015 in the Norges river (Burgundy, eastern France, 47°21'42.7"N 5°09'29.6"E), using a kick sampling method with a hand net. This population was chosen because gammarids from this site naturally harbor the acanthocephalan parasite *Pomphorhynchus tereticollis*. Although this population is known to contain only *G. fossarum* gammarids (Lagrue *et al.*, 2014), we conducted genetic analyses on 36 individuals to control for the presence of the cryptic, closely-related species *G. pulex*. Individuals from both sexes and both infection status (uninfected or naturally infected with *P. tereticollis*) were collected.

Gammarids are the intermediate host for *P. tereticollis* parasites, in which they develop successively in two distinct larval stages: acanthella and cystacanth stages. At this second stage, which is infective to fish final hosts, parasites induce a whole range of modifications in their gammarid hosts, including changes in behavior (Tain *et al.*, 2006; Perrot-Minnot *et al.*, 2012) or immune system (Cornet *et al.*, 2009a), although, to our knowledge, the effect of this parasite species on food consumption by gammarids has not been investigated. Therefore, gammarids infected with the cystacanth stage of the parasite were used in this experiment. Thanks to their yellow-orange coloration, cystacanths are

clearly visible through the translucent cuticle of gammarids, allowing a preliminary selection of infected individuals directly upon collection in the field.

Acclimatization of animals

Gammarids collected in the field were immediately placed in acclimatization in the laboratory for ten days. They were maintained in groups of several dozens of individuals, in boxes (10.5 × 16 cm) filled with an oxygenated mix of water from the Norges river and dechlorinated, UV-treated tap water. All amphipods were fed *ad libitum* with conditioned elm leaves, and maintained under a 12:12 light:dark cycle regime. Groups were randomly assigned to one temperature (10, 14 or 18°C), for both the acclimatization period and the subsequent consumption test. This range of temperatures was chosen based on naturally fluctuating conditions in the habitat of *G. fossarum* (Pöckl *et al.*, 2003), current temperatures in Burgundy, and predictions for 2100 (Gunn & Crumley, 1991; Rowell, 2005). Boxes containing amphipods were placed in water baths, allowing all three temperatures to be tested simultaneously. The surrounding water was constantly recirculated through a temperature control device (TANK TK-1000 Chiller, Teco US). Water temperature was monitored daily.

Individual food consumption

Before each test, individuals were checked under a binocular microscope to identify gammarids containing no visible parasite (controls) and gammarids containing *P. tereticollis* cystacanths. Single individuals were placed into individual glass dishes (six cm diameter), at acclimatization temperature (10, 14 or 18°C), and starved for 24 hours. Individuals were then provided a leaf disc of known dry weight, and were allowed to feed for 24 hours. Three replicates were conducted at each of the three temperatures (one replicate per day). For each replicate, paired glass dishes at each temperature were left free of gammarids, serving as control for the natural deterioration of leaves. A total of 145 males and 156 females were used for this experiment.

Leaf discs were prepared before each test. Humidified elm leaves were sterilized at 120°C for 20 minutes, and cut into 1.5 cm diameter discs using a cork borer. Discs were then dried at 50°C until they stopped loosing water, as estimated by weighing leaves regularly during a pre-test. This took three hours for the 1.5 cm diameter disks. Disks were then weighed to the nearest tenth of milligram, and re-hydrated for 24 hours before the test. After the test, each leaf disc was dried again for three hours and weighed.

Food consumption in microcosms

After acclimatization (at 10, 14 and 18°C), gammarids were placed in groups of ten individuals, either all infected or all uninfected (based on visual inspection under a binocular microscope), in larger glass dishes (10 cm diameter) containing a few stones under which they could hide. Apart from their infection status, individuals were randomly chosen, such that the sex-ratio was variable between groups. Gammarids were starved for only five hours, to avoid cannibalism. They were then provided with pieces of elm leaves of known dry weight, and were allowed to feed for 48 hours. Several glass dishes at each temperature were free of gammarids, serving as controls for the natural deterioration of leaves. A total of 270 infected gammarids were used (27 groups), while 390 uninfected gammarids were tested (39 groups). Five replicates were conducted at each of the three temperatures (one replicate per day).

Elm leaves were prepared and weighed as described before, except that they were dried at 50°C for a longer time. Indeed, due to larger amount of leaves, as well as their bigger size, pre-tests showed that at least six hours were necessary to dry them completely. Batches of approximately 200 mg (dry weight) were provided to gammarids.

Measurements and dissections

After each test, all individuals were sexed based on the size and shape of their first and second pairs of gnathopods, which present a sexual dimorphism in amphipods (Hume *et al.*, 2005). Following Bollache *et al.* (2000), body size was measured as the height of the fourth coxal plate using a microscope and Lucia G 4.81 software. Gammarids were then dissected to determine their infection status (presence or absence of parasites), parasite developmental stage and parasite species based on morphological identification (as the acanthocephalans *P. laevis*, *Polymorphus minutus* and *Echinorhynchus truttae* can also be found in the Norges river, although at much lower frequencies than that of *P. tereticollis*). Individuals were categorized as “parasitized” when they were harboring at least one *P. tereticollis* cystacanth stage, and “control” if no parasite was found. Individuals harboring only acanthella (immature stage) or harboring cystacanths from other acanthocephalan species were excluded from the data set. Individuals which died during the tests were also removed from the data set.

Although consumption is usually expressed on a dry weight basis (Foucreau *et al.*, 2013a; Dianne *et al.*, 2014; Schmidlin *et al.*, 2015a), the need to dissect individuals made this impossible. Instead, linear measures of gammarids were recorded (i.e. the height of the fourth coxal plate). In

addition to gammarids used in consumption tests, we also measured 137 uninfected *G. fossarum* from the Norges river (68 males and 69 females). Those individuals were then dried in a 50°C chamber during 64 hours, and immediately weighed. The relationship between the height of the fourth coxal plate and the dry weight of individuals was then calculated for this population, such that the size of individuals could be considered in the calculation of individual consumption.

Data analyses

Estimation of food consumption

The quantity of food (Qi, in mg) consumed for each individual or group of individuals was calculated as follows:

$Qi = MBi^* - MAi$

where (MAi) represents the mass of the disc or batch of leaves after consumption and (MBi*) the mass before, corrected to take into account the quantity of food that was lost due to natural deterioration. To estimate this value, we calculated for each replicate the mean ratio between MCA, the mass of control leaves (leaves left without gammarids) measured at the end of the experiment, and MCB, the mass of control leaves measured at the beginning of the experiments. We then multiplied these estimates of natural deterioration by the mass of disc or batch of leaves after consumption (MBi), such that:

$$Qi = \left[\left(mean\left(\frac{MCA}{MCB}\right)\right) \times MBi\right] - MAi \ .$$

The relationship between the wet mass and the height of the fourth coxal plate was found to follow a linear relationship ($R^2 = 0.71$, $P < 0.0001$) that did not differ between males and females. Thus, the size of gammarids was considered in the estimation by dividing Qi with the height of the coxal plate for individual consumption, or by the mean value for all remaining individuals in each group for microcosm consumption.

Individual food consumption

Food consumption data were (log +1) transformed in order to normalize residuals. Transformed data were analyzed with a linear model, considering temperature (as an ordinal variable), sex, status (parasitized vs control), and their interactions, and including replicate as a random factor. Non-

significant interactions were removed from the model. Tukey HSD post-hoc tests were then used to compare food consumption differences between pairs of temperatures.

To analyze the strength of the effects of both temperature and parasitism on food consumption, Cohen's d effect sizes (Cohen, 1988) and their 95% confidence intervals were calculated for differences between parasitized and control individuals at each temperature and for each sex, and between all the temperatures for each status and for each sex. Cohen's d is a scale-less parameter used to represent the size of the effect, such as a difference between two groups, and the direction of this difference (Nakagawa & Cuthill, 2007). Cohen's d is calculated using the means and standard deviations of those groups, and confidence intervals are calculated with a bootstrap technique.

Food consumption in microcosms

After controlling for normality of model residuals using Q-Q plots, microcosm consumption was also analyzed using a linear model, with temperature, group status (parasitized vs control) and group sex-ratio as fixed factors, and replicate as a random factor. Non-significant interactions were removed from the model. Tukey HSD post-hoc tests were used to compare food consumption differences between pairs of temperatures. Cohen's d were then used to analyze the effect sizes, as described before.

Models were performed using JMP version 10.0.0 software (SAS Institute, Cary, NC, U.S.A.) and Cohen's d effect sizes were calculated using R version 3.1.1 software (R Foundation for Statistical Computing).

Results

Individual food consumption

The three single factors were significantly affecting gammarids individuals food consumption (temperature: $F_{2, 294} = 3.96$, $P = 0.02$; sex: $F_{1, 294.5} = 5.36$, $P = 0.02$ and infection status: $F_{1, 294.3} = 33.22$, $P < 0.0001$), whereas no interaction was significant. Overall, food consumption was higher for males compared to females, and in control individuals compared to parasitized ones (Fig. 31). The overall consumption tended to increase between 10°C and 14°C, but then decreased at 18°C (Fig. 31). Tukey's range tests showed that the difference was only significant between 14°C and 18°C.

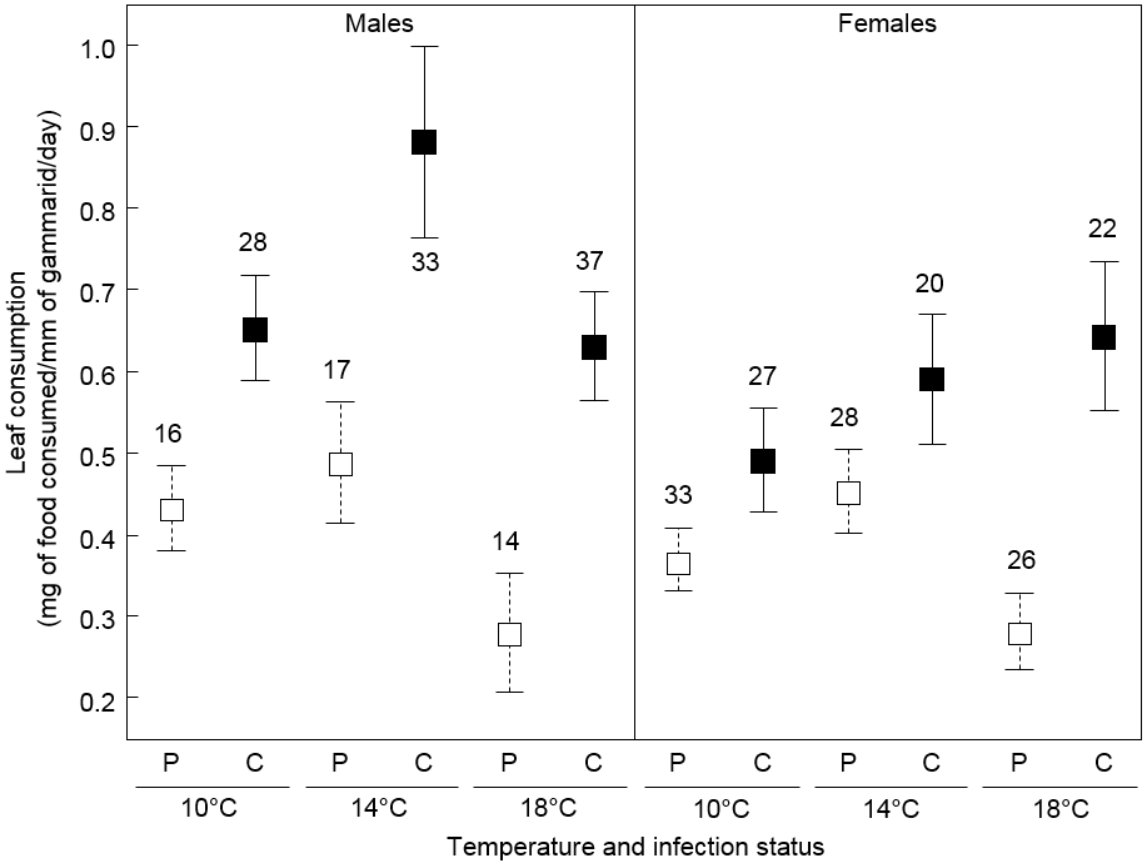

Figure 31. Individual *Gammarus fossarum* leaf consumption, according to infection status (P or C, respectively parasitized or control), their sex, and the temperature (10, 14 or 18°C). Squares represent means for the quantity of leaf (in mg) consumed by individual gammarids during 24 hours, corrected by their size (height of the fourth coxal plate, in mm). Standard errors and sample sizes are indicated.

Cohen's d confirmed that consumption was more important for control individuals compared to parasitized ones, with all differences being significant except between females at 10°C and 14°C, for which there was however a clear trend (Fig. 32). Comparisons between temperatures showed that parasitized individuals, males and females, consumed significantly more food at 14°C compared to 18°C, with a marginally-significant decrease between 10°C and 18°C. Uninfected males also ate more at 14°C compared to 18°C, while all other differences were non-significant, especially in females (Fig. 33).

Food consumption in microcosms

Mortality during the test occurred for 15 groups out of 66, with no difference between parasitized and control groups (Chi-squared test: 3.34, d.f. = 2, p = 0.16). Thus, all the groups were kept in the model.

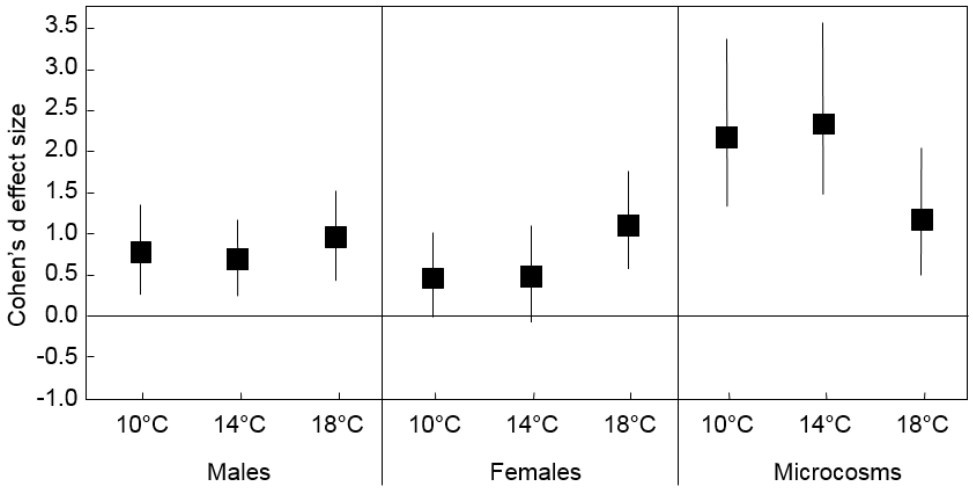

Figure 32. Effect sizes of consumption differences between parasitized and control *Gammarus fossarum* maintained as individuals (males or females) or in microcosms, at each temperature. Values above zero indicate that the consumption is higher for control individuals/groups compared to parasitized ones. The difference is significant when the bar does not overlap zero. Cohen's d effect sizes are represented with 95% confidence intervals.

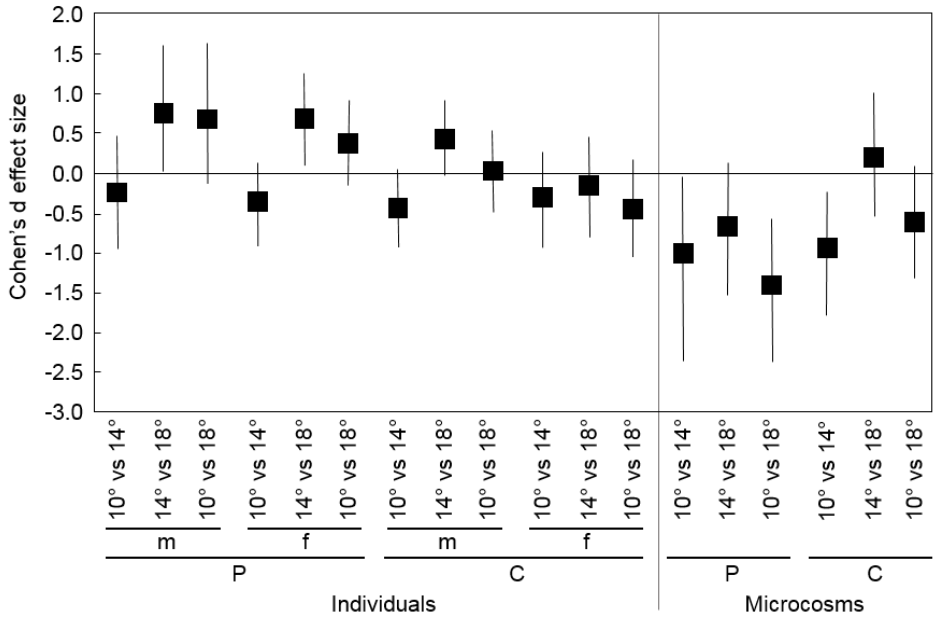

Figure 33. Effect sizes of the differences in consumption at the three experimental temperatures, for individuals (males and females, respectively m and f) and grouped (microcosms) *Gammarus fossarum*, and for infection status (parasitized or control, respectively P or C). Values above zero indicate that the consumption is higher for the first temperature cited, and dots under zero indicate higher consumption for the second temperature cited. The difference is significant when the bar does not overlap zero. Cohen's d effect sizes are represented with 95% confidence intervals.

The consumption of individuals in microcosms was significantly greater for control groups than the parasitized groups ($F_{1, 58.21}$ = 42.38, P < 0.0001, Fig. 34). There was a positive, but not significant relationship between food consumption and the proportion of males ($F_{1, 59.78}$ = 3.19, P = 0.079). Temperature also significantly influenced food consumption ($F_{2, 58.5}$ = 5.75, P = 0.005, Fig. 34), and Tukey's HSD post hoc tests showed that differences in consumption were significant only between 10°C and the two other temperatures.

Cohen's d confirmed that parasitized groups consumed significantly less food at all temperatures, compared to control groups (Fig. 32), and that these differences were stronger than those observed during individual tests (Fig. 32). Comparisons between temperatures showed that the pattern was different between parasitized and control groups: while consumption increased with temperature for parasitized groups, there was no difference in consumption for control groups between 14°C and 18°C (Fig. 33).

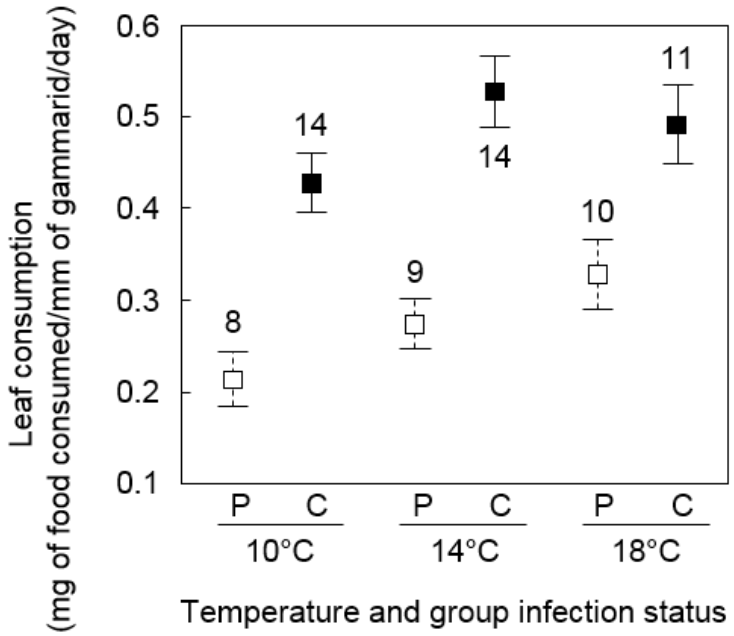

Figure 34. Leaf consumption of grouped *Gammarus fossarum* individuals in a microcosm, according to infection status (P or C, respectively parasitized or control), and the temperature (10, 14 or 18°C). Squares represent means for the quantity of leaf (in mg) consumed by average individuals in each group during 24 hours, corrected by their size (mean height of the fourth coxal plate of all individuals within the group, in mm). Standard errors and sample sizes are indicated.

Discussion

Our results clearly show that temperature had an important impact on the shredding role of gammarids, with a positive effect between low and medium temperatures, followed by either a stagnation or a diminution of consumption by gammarids at the highest temperature. A negative effect of the parasite was found on gammarid consumption at all temperatures, suggesting an overall additive effect of temperature and parasitism. Feeding alone or in a group was also found to be of significant importance for food consumption by gammarids, influencing response to temperature.

Assuming that infection is costly and increases gammarids metabolism (Labaude *et al.*, 2015b), we would have expected infected gammarids to increase leaf consumption in compensation. However, in accordance with (Médoc *et al.*, 2011b), parasitism was found to negatively affect the consumption of leaves by gammarids in all the situations that we tested. *P. tereticollis* cystacanths are known to alter the behavior of their hosts, possibly increasing the probability of trophic transmission (Perrot-Minnot *et al.*, 2007). A decrease in food consumption could thus be linked to such manipulation, although Dianne *et al.* (2014) found that gammarids infected by the acanthella stage (i.e. not infective for the definitive host) of the closely-related *P. laevis* also consumed less food than uninfected individuals. More likely, the negative effect of parasitism could result from some stress induced by the parasite. Indeed, although the fitness of *P. laevis* is highly dependent on the survival of its intermediate host before trophic transmission occurs, acanthocephalan-infected gammarids have a higher mortality than uninfected ones under laboratory conditions (Cornet & Sorci, 2010; Labaude *et al.*, 2015b). Most studies have reported a lower resistance of female gammarids to different stress (pollutants, hypoxia, salinity, temperature: McCahon & Pascoe, 1988; Hoback & Barnhart, 1996; Sornom *et al.*, 2010), potentially explaining the lower food consumption of females compared to males, relative to their size, as observed in our study. Several other investigators have reported sex-related differences in food consumption by amphipods, but, contrary to our study, in most cases, food consumption was lower in males (Foucreau *et al.*, 2013b, 2014; Pellan *et al.*, 2015).

As expected, temperature affected gammarids food intake, with significant effects on leaf consumption in all conditions of infection and aggregation. An increase in temperature is known to increase metabolic rate in ectothermic species (Brown *et al.*, 2004), thus leading to higher energy requirements. Accordingly, and as observed in previous studies (Coulaud *et al.*, 2011; Pellan *et al.*, 2015; Schmidlin *et al.*, 2015a), leaf consumption increased between low and medium temperatures in the present study. However, at the highest temperature, food consumption dramatically decreased in most single individuals. Many parameters linked to the metabolic rate of gammarids, such as

locomotor activity (Issartel *et al.*, 2005; Maazouzi *et al.*, 2011), oxygen consumption (Issartel *et al.*, 2005; Foucreau *et al.*, 2014), ventilatory activity (Issartel *et al.*, 2005) or glycogen content (Maazouzi *et al.*, 2011; Foucreau *et al.*, 2014), are known to first increase with temperature to a certain extent, before decreasing. Maazouzi *et al.* (2011) found, in the case of glycogen content, that such a decrease is tightly linked with thermal tolerance in *G. pulex*. The decrease in food consumption observed in the present study could thus be associated with thermal stress occurring at temperature well above the thermal optimum for gammarids. Considering that the thermal optimum for *G. fossarum* is approximately 11.5°C (Pöckl & Humpesch, 1990), with potential variations depending on the geographical origin of the population (Foucreau *et al.*, 2013b, 2014), it is not surprising that a temperature of 18°C would induce a non-lethal thermal stress, with consequences on food consumption. Similarly, (Schmidlin *et al.*, 2015b) observed a slightly reduced feeding activity of *G. fossarum* above 16°C, while that of males *Dikerogammarus villosus* decreased between 20°C and 25°C (Pellan *et al.*, 2015).

The effect of temperature on *G. fossarum* consumption was dependent on whether gammarids were feeding alone or in groups. Indeed, although a marked decrease was observed in lone individuals (apart from uninfected females) at the highest temperature, no such effect was observed in individuals feeding in groups. Gammarids are however a gregarious species, often present at high density in the wild, and relying on aggregation as an antipredator behavior (Durieux *et al.*, 2012). Our results thus suggest that experimental studies addressing the influence of climate change on keystone species may benefit from measuring their behavior under realistic conditions, i.e. as close as possible to natural conditions. Maintaining individuals in isolated conditions could actually result in a supplementary stress, especially at the highest temperature, at which a thermal stress is suspected. Moreover, the addition of a third stressor, parasitism, could contribute to explain why consumption decreased at the highest temperature in infected isolated females, whereas it only levelled off in uninfected ones. Thus, although several studies investigated food consumption by gammarids in isolated individuals (Bundschuh *et al.*, 2011; Foucreau *et al.*, 2013a, 2013b, 2016; Dianne *et al.*, 2014), their shredding efficiency could actually be highly dependent on the presence or absence of conspecifics. Results from studies measuring food consumption within groups (e.g. Coulaud *et al.*, 2011; Schmidlin *et al.*, 2015a, 2015b) might then be more reliable to extrapolate the influence of environmental factors on the functional role of keystone species such as amphipods.

Finally, the effect of high temperature on the shredder role of amphipods could be amplified by its direct effects on parasitic infection. Indeed, an increase in temperature can lead to an increase in both the prevalence and intensity of infection with parasites (Mouritsen *et al.*, 2005; Karvonen *et*

al., 2010; Schoebel *et al.*, 2011). Thus, through increasing the number of parasites within infected individuals, as well as the proportion of infected individuals, the overall effect of increased temperatures on the shredding efficiency of gammarids might be even more pronounced than what was estimated in the present study, and therefore impact negatively the ecological sanitation role of gammarids in freshwater ecosystems. Other effects of increased temperature on the quality and distribution of plant species (Kelly & Goulden, 2008; Lenoir *et al.*, 2008) or on the impact of rival species, especially invasive ones (Dukes & Mooney, 1999; Walther *et al.*, 2009), may also affect the shredding efficiency of gammarids. Additionally, the interaction between parasitism and such invasive species (Prenter *et al.*, 2004) may add further complexity. Thus, although our approach analyzing the combined effects of abiotic and biotic factors (inter and intraspecific) provides a useful basis to understand the consequences of increasing temperatures on the shredding activity of detritivores, improving predictions might require further investigations to address this complexity. Future studies would benefit in accuracy by using approaches relying on long-term experiments and/or monitoring (Lepetz *et al.*, 2009).

Our results indicate that rising temperatures might induce an increase in the shredding efficiency of gammarids, at least to a certain extent. However, this effect might be countered by parasitism, which may depend on the prevalence and intensity of infection in natural populations. In addition, the intricate link between temperature, parasitism and other biotic interactions, such as conspecifics or individuals from other gammarid species, as well as combined effects on the shredder role of gammarids, highlight the difficulty in predicting the consequences of global change on the functional role of keystone species from studies based on a limited number of factors.

Acknowledgments

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3. Impact on gammarids role as a prey

Because manipulative parasites often induce changes in their intermediate hosts that make them more susceptible to predation, one of their major impacts resides in their role as drivers within food webs (Labaude *et al.*, 2015a). For instance, the susceptibility of killifish (*Fundulus parvipinnis*) to predation was found to be increased up to 31 times when they were parasitized by the trematode *Euhaplorchis californiensis*, compared to uninfected individuals (Lafferty & Morris, 1996). Gammarids parasitized by *Cyathocephalus truncatus*, a tapeworm that is also known to induce behavioral modifications in its intermediate host (Franceschi *et al.*, 2007), were also shown to be predated approximately eight times as often as uninfected gammarids (Knudsen *et al.*, 2001). In parallel, there is some evidence that the increased probability of predation induced by some manipulative parasites on their hosts might be of substantial importance in certain ecosystems. The most impressive example comes from nematomorph parasites (*Gordionus sp*.) that drive their terrestrial insect hosts into jumping in the water (Sato *et al.*, 2011). In this case, the increased probability of predation is a consequence of the necessity of parasites to find water to reproduce. Here, manipulated hosts that fall into rivers represent a new prey for fish, although fish do not constitute a host for nematomorph parasites (Sato *et al.*, 2011). This new food resource can be so important for fish that their predation on benthic invertebrate community dramatically decreases, leading to a subsequent increase in algae consumption by invertebrates, thus affecting algae biomass, and, ultimately, leading to modifications in the whole ecosystem (Sato *et al.*, 2012).

Many studies confirmed that the manipulation induced by acanthocephalan parasites on their gammarid hosts might lead to a higher probability of predation by parasites' definitive hosts (Hindsbo, 1972; Bethel & Holmes, 1977; Kaldonski *et al.*, 2007; Perrot-Minnot *et al.*, 2007; Dianne *et al.*, 2011; Jacquin *et al.*, 2014), although not all the traits that are modified by the parasites are believed to be implicated in this increase (Kaldonski *et al.*, 2009; Perrot-Minnot *et al.*, 2012). In particular, the proportion of gammarids infected by *P. laevis* was found to be between 10 and 27 times higher in the stomach contents of bullheads (*Cottus gobio*) compared to the proportion of infected individuals found in free-ranging gammarids from the same river (Lagrue *et al.*, 2007; Perrot-Minnot *et al.*, 2007). These results indicate that acanthocephalan parasites might play an important role in the facilitation of fish predation, possibly affecting the populations of fish and their gammarid preys.

In this work, the temperature experienced by gammarids during the development of their *P. laevis* parasites was found to have no effect on the intensity of the manipulation of their use of refuges (article 4). In contrary, a shorter acclimatization time experienced by the cystacanth parasitic stage, possibly better reflecting natural variations of temperature in the wild, was found to affect the manipulation of *P. tereticollis* parasites, with more important differences between infected and uninfected *G. fossarum* individuals at high temperature (article 3). However, the effect of temperature on the increased probability of predation induced by parasites can hardly be predicted solely based on its effect on manipulation. Indeed, the resulting increase in predation depends on a complex interaction between the parasite, its intermediate host and the predator.

First, apart from its influence on the intensity of manipulation in the intermediate host, temperature might also affect several traits of the predator that might modify its predatory efficiency. For instance, temperature is known to alter many physiological functions in fish such as swimming behavior and energetic requirements (Brett, 1971; Elliott, 1976). Thus, temperature might affect the predatory ability of fish by itself. To verify this hypothesis, we ran preliminary tests about the effects of temperature on the predation of *G. pulex* gammarids by goldfish (*Carassius auratus*). Goldfish is an unfamiliar fish predator for gammarids, supposed to induce low repulsive reaction behavior from gammarids exposed to its odor (Perrot-Minnot *et al.*, 2012).

The results of this preliminary experiment showed that fish predation on uninfected gammarids was highly dependent on the temperature. Both fish and gammarid individuals were acclimatized in groups for at least one week at 14°C or 17°C (12:12 light:dark cycle, daily feeding) before predation tests. Following 36 hours of fish starvation, 40 gammarids (either only males or females, or at an equilibrated sex-ratio) were introduced in an aquarium (30 x 80 x 40 cm) containing two air bricks as refuges (following Kaldonski *et al.*, 2007), and maintained at the same temperature as acclimatization. After 30 minutes of acclimatization, single fish were introduced in aquaria and were

Table 16. Mann-Whitney tests comparing the predation of gammarids (*Gammarus pulex*) by goldfish (*Carassius auratus*) between 14°C and 17°C. Three conditions were tested: gammarids available for fish were either at an equilibrated sex-ratio (mixed sexes), or only females or males were introduced.

	Chi 2	d.f.	P
Mixed sexes	12.43	1	0.0004
Females	6.53	1	0.011
Males	4.87	1	0.027

allowed to feed on gammarids for 30 minutes. Remaining gammarids were counted at the end of the experiment. As expected, the quantity of gammarids consumed was always more important at 17°C than 14°C (Table 16, Fig. 35A). A higher proportion of males was eaten compared to females at 17°C when both sexes were introduced (Table 16, mixed sexes in Fig. 35B), which might be due to a choice from fish toward larger individuals (i.e. the males) or a consequence of the higher intensity of refuge use observed in females compared to males (Dianne *et al.*, 2011). A higher consumption of gammarids by fish at 17°C was also found when only male or only female amphipods were introduced (Table 16, Fig. 35A).

Along with a possible increase of manipulation with temperature (article 3), these results suggest that the success of parasite's transmission could be increased at high temperature. However, other parameters need to be considered in order to draw pertinent conclusions about the effect of temperature on the increased probability of predation. Indeed, the effects of temperature on parasite manipulation and on fish predation were investigated separately here. However, while the presence

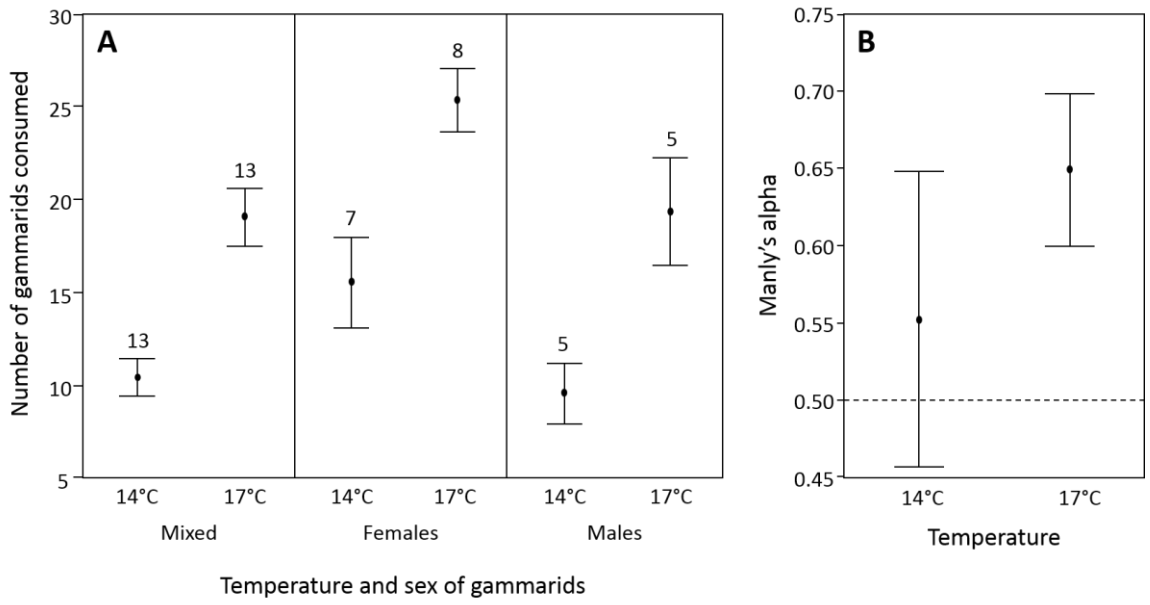

Figure 35. Impact of temperature on the consumption of gammarids (*Gammarus pulex*) by goldfish (*Carassius auratus*). (A) Number of gammarids consumed after 30 minutes of consumption, according to the temperature (14°C or 17°C) and the sex of gammarids (40 gammarids introduced, either in an equilibrated sex-ratio, or only females/males). Dots represent means and bars stand for standard errors. Sample sizes (i.e. the number of fish tested) are indicated. (B) Manly's alpha (means and 95% confidence intervals, see Seppälä *et al.*, 2004) representing the selective predation of goldfish on male versus female gammarids in the "mixed" condition, according to temperature (14°C or 17°C). The dotted line indicates no predation bias between the two sexes. A value above the dotted line indicates a selective predation on males. The bias is significant when the confidence interval does not overlap 0.5.

of parasites inside gammarids is expected to increase fish predation through manipulation, we might also expect a reaction of gammarids to fish. Indeed, gammarids behavior was shown to depend on the presence of predators. First, odor of usual predatory fish is known to induce modifications in many behaviors of uninfected gammarids, such as a decrease in their general activity (Andersson *et al.*, 1986; Dezfuli *et al.*, 2003; Wellnitz *et al.*, 2003; Durieux *et al.*, 2012), an increased aggregation with conspecifics (Kullmann *et al.*, 2008; Durieux *et al.*, 2012; Lewis *et al.*, 2012) or an increase in the time spent in refuges (Kaldonski *et al.*, 2007; Médoc *et al.*, 2009; Dianne *et al.*, 2011). Most importantly, this reaction to fish cue is altered when gammarids are parasitized. Indeed, no aggregation was observed for gammarids infected by acanthocephalan parasites, even in the presence of fish odor (Durieux *et al.*, 2012; Lewis *et al.*, 2012). Moreover, parasite manipulation can be exacerbated by the presence of predatory cues. Indeed, Durieux *et al.* (2012) found that *G. pulex* individuals infected by *P. laevis* were more photophilic than uninfected amphipods, both in the presence and in the absence of predator cues, and that the effect of the parasite on phototaxis was more pronounced in scented water compared to control water. Finally, the very presence of predator cues might be necessary for the expression of manipulation, such as the attraction of infected gammarids toward fish odor compared to repulsion for uninfected gammarids (Baldauf *et al.*, 2007; Kaldonski *et al.*, 2007; Perrot-Minnot *et al.*, 2007). Thus, temperature might not only affect parasite manipulation on the intermediate host on one hand, and predation ability of the definitive host on the other hand, but also the interaction between gammarids and their predators in the presence of parasites. Future studies need to consider at the same time all the components of the system to draw conclusions about the effect of temperature, for instance by predation tests on both infected and uninfected gammarids at different temperatures after proper acclimatization. The probability of predation for infected and uninfected gammarids, as well as the ecological significance of parasitic manipulation, might also depend on the prevalence of parasites (Franceschi *et al.*, 2008). However, acanthocephalan prevalence could also be increased by temperature (Sheath *et al.*, 2016), increasing in turn the ecological impact of parasitic manipulation. Therefore, only long term studies might properly explore the intricate links of all the components in this system and conclude accurately about how temperature can affect the impact of acanthocephalan parasites on gammarids role as preys.

Chapter VI. Conclusion and perspectives

1. Summary of the results

Along this thesis, the impact of some environmental parameters was investigated on the interaction between gammarids and their acanthocephalan parasites, using several protocols. In particular, using experimental infections, both the level of food resources and the temperature experienced by *G. pulex* gammarids during the development of their *P. laevis* parasites were shown to have many consequences on this interaction (Article 2 and Article 4). In gammarids fed with a deprived diet, compared to a normal diet, parasites were less numerous (although this result depended on the population of parasites) and tended to be smaller. The metabolism of their hosts was also altered by a poor diet, with a lower survival and a lower oxygen consumption. Some of these parameters were also affected by temperature. Indeed, the survival of gammarids was reduced and their activity level was increased at 17°C compared to 14°C. Their parasites developed faster at high temperature. When exposure occurred at the two temperatures, parasites were more numerous per hosts at the higher temperature, and there was a slightly higher infection success. Parasites were also faster to evert their proboscis when they developed at high temperature. Thus, food resources and temperature affected many parameters, suggesting an effect of the two parameters on the metabolism of both hosts and parasites. However, and remarkably, the timing and intensity of manipulation, in terms of alteration of the sheltering behavior of gammarids, seemed to be completely independent from these two environmental parameters. In particular, contrary to what was suggested in Franceschi et al. (2010a), important differences in development time of parasites did not lead to any difference in the rapidity of parasites to manipulate their hosts.

These two studies suggest that manipulation (i) might not depend on the metabolic state of the hosts, (ii) might not depend on the metabolic state of the parasites, and (iii) might not be a plastic trait (see discussions in Article 2 and Article 4). In particular, it was suggested that parasites should adjust their exploitation strategy to the physiological state of their hosts, possibly manipulating their behavior sooner or stronger in hosts in poor condition, to increase their probability of being transmitting before the death of their hosts (Thomas *et al.*, 2002a). On the other hand, it was also suggested that the cost associated with the display of manipulated behavior for the host could prevent those in poor condition to fully express the manipulation (Thomas *et al.*, 2011). The results of my studies do not support any of these hypotheses.

It cannot be excluded, however, that other protocols might lead to different conclusions. In particular, the population of gammarids used in these two experiments is naïve to the acanthocephalan species used. Franceschi et al. (2010a) showed that this population was more

affected by the manipulation of *P. laevis* parasites compared to populations of gammarids where the parasite can be found. It thus cannot be excluded that the manipulation was too strong for any subtle variation linked to environmental conditions. It is also possible that such variations in the manipulation result from an answer from the host, in terms of resistance to manipulation, requiring some degree of co-adaptation with the parasite. Moreover, it cannot be excluded either that stronger differences in the environmental factors would lead to variations in manipulation. However, the conditions were different enough in the present experiments to induce significant and substantial variations in many other parameters.

Temperature was shown to have other impacts on hosts during experiments conducted on naturally-infected *G. fossarum* gammarids with *P. tereticollis* parasites (Article 3 and Article 6). Contrary to experiments involving experimental infections, the temperature treatments were applied on gammarids already harboring cystacanth parasites, and thus this parameter did not influence their development. Moreover, the experiments relied on shorter acclimatization periods, possibly better reflecting natural variations of temperature in the wild. These studies highlighted an effect of temperature and parasitism on both phototaxis and leaf consumption of gammarids. More interestingly, there was also an interaction between parasitism and temperature, suggesting that the impact of parasites on their gammarid hosts, in terms of manipulation of phototaxis behavior and alteration of their shredder role, depends on abiotic factors. However, the temperature did not significantly influence the use of refuges of gammarids, a manipulated trait also found to be independent from temperature in the study based on experimental infections.

The experiments presented in this thesis also support several results already found in acanthocephalan parasites. In particular, consistent with results found by Franceschi et al. (2010a, 2010b), infection parameters seemed to be linked to the origin of parasites. Indeed, we found strong variations in the success of infection of parasites from different populations, as well as in their infection intensity (parasite load). There was also differences between populations in their impact on their hosts, with variations in virulence (host survival), effects on the metabolism of their hosts, and manipulation. The reasons behind such differences are not clear, since both genetic and environmental differences, such as the conditions experienced by adult parasites in the definitive host, could play a role. However, it is worth noting that differences were also found between parasites from different mothers, but originating from the same river (Article 4).

Overall, the manipulation induced by parasites on their gammarid hosts could be modified by temperature, under certain circumstances that require some clarification. On the contrary, the environmental parameters experienced during the development of parasites seem to have only

indirect effects on manipulation (see Article 1 for a review). Whether in a direct or indirect way, environmental parameters were thus shown to influence many traits in the association between gammarids and their hosts, including some leading to modifications in their ecological role. However, further studies are needed to better understand the impact of environment on the many interactions between acanthocephalan parasites and their gammarid hosts. Moreover, although this thesis might help to better understand the mechanisms by which acanthocephalan parasites manipulate their hosts, their complete comprehension would be a condition *sine qua none* for understanding the reasons behind such environmental impacts. Such perspectives for future studies are discussed below.

2. Effect of the environment: beyond parasite-induced facilitation of predation

A major part of my work was focused on the impact of environment on the use of refuges by gammarids. The choice to study this trait was based on two assumptions. First, sheltering behavior has been shown to be decreased by the presence of several species of acanthocephalan parasites (Kaldonski *et al.*, 2007; Perrot-Minnot *et al.*, 2007, 2014; Stone & Moore, 2014). Second, sheltering is an important anti-predator behavior for gammarids, and its alteration might be responsible for the increased probability of predation of gammarids harboring cystacanth parasites. Indeed, Kaldonski et al. (2007) found that the predation of *G. pulex* parasitized by *P. laevis* was increased compared to control individuals only when refuges were available for gammarids. On the contrary, no causal link could be found between trophic transmission and phototaxis (Perrot-Minnot *et al.*, 2012), another trait that is modified by many acanthocephalan species (see table 2 for a review).

Although sheltering behavior seem to be a major trait in parasite manipulation, the changes induced by parasites on their hosts is multidimensional, with many traits altered as part of an "infection syndrome" (Cézilly & Perrot-Minnot, 2010; Perrot-Minnot *et al.*, 2014). In particular, Perrot-Minnot et al. (2014) showed that the impact of acanthocephalan parasites on other behaviors, such as phototaxis and geotaxis, was more important than their impact on gammarids use of refuges, as suggested with different effect sizes. Moreover, injections of serotonin, a neuromodulator highly suspected to play a major role in the mechanisms in which fish acanthocephalan parasites manipulate their gammarid hosts (e.g. Perrot-Minnot et al., 2014; Ponton et al., 2006, see discussion below), was shown to induce alterations in many behaviors, such as phototaxis, geotaxis and swimming activity (Perrot-Minnot *et al.*, 2014). However, the use of refuges was not significantly altered by serotonin injections (Perrot-Minnot *et al.*, 2014). It is thus likely that alterations in other behaviors than sheltering might also be important in acanthocephalan manipulation, especially since the use of refuges by gammarids might actually be a result from a combination of other behaviors, such as phototaxis and thigmotaxis. Considering that one of my studies highlighted an effect of temperature on phototaxis, the effect of environment on all behavioral alterations induced by parasites cannot be deducted from its impact on refuge use. Thus, further studies are required to investigate the effect of environment on other traits that are considered as "manipulated", such as phototaxis, geotaxis or aggregative behavior (see table 2 for other examples).

Although widely ignored for a long time in studies about parasite manipulation, there is now growing evidence that parasites might also alter the behavior of their host before they reach their transmissible stage (e.g. Dianne et al., 2011; Hafer and Milinski, 2016; Hammerschmidt et al., 2009). In particular, acanthocephalan parasites at the acanthella stage have been shown to induce an increase in the use of refuges, interpreted as a strategy to protect their hosts from being predated at a time when no successful transmission can occur (Dianne *et al.*, 2011, 2014). Although the changes might appear less spectacular, it is likely that parasites might spend more time in their intermediate hosts as acanthella parasites, given that the changes induced by cystacanth stages might lead to a rapid predation of their host. Moreover, acanthella stages have been shown to induce other modifications, such as a decrease in gammarids general activity and food consumption (Dianne *et al.*, 2014), that could also lead to alterations in their ecological roles. The impact of acanthella parasites on their hosts clearly requires more attention, as well as its relation with environmental parameters.

Finally, many traits that are altered by parasites, either at the acanthella or at the cystacanth stage, are not believed to directly affect the probability of predation of gammarids. However, they might have other ecological consequences. For instance, the shredder role of gammarids, important in the recycling of organic matter within rivers, was shown to be reduced by acanthocephalan parasites (Labaude *et al.*, 2016). Furthermore, the composition of macroinvertebrate community might also depend on the presence of acanthocephalan parasites. For instance, some acanthocephalans are known to mediate intraguild predation between several species of gammarids (MacNeil *et al.*, 2003b), such that certain species were shown to co-occur more in the field in their presence (MacNeil & Dick, 2011). Thus, when investigating the impact of environment on the ecological role of parasites, through their impact on their hosts, it might be unproductive to limit the research to what is considered as “manipulation”. Moreover, the effects of the environment might be indirect (see Fig. 2 and Fig. 3). For instance, there is evidence that the degree of manipulation of acanthocephalan parasites could depend on their infection intensity (Franceschi *et al.*, 2008). In another host-parasite system, the importance of the effect of *Ligula intestinalis* (Cestoda) on the behavior of its intermediate fish host was found to be higher for larger parasites (Brown *et al.*, 2001b). Such parameters as the infection intensity or the development rate of parasites can themselves depend on many factors (e.g. temperature, size of the intermediate host, competition with other parasites; Benesh and Valtonen, 2007b; Steinauer and Nickol, 2003). Because of the complexity due to the interaction of numerous factors, further studies should adopt more integrative approaches, and consider multiple components of the systems with all their interactions. Long term studies and field studies might be particularly appropriate in this respect.

3. Effect of the environment: beyond the intermediate host

Manipulative parasites are largely recognized as mostly altering the phenotype of their intermediate host. As a consequence, parasites at this stage received a large attention. The effect of environment was accordingly investigated only in the relationship between parasites and their gammarid hosts in my thesis. However, gammarids constitute only one step in the life of the parasites. First, it is possible that environment affect the life-history of parasites, including their impact on gammarids, at earlier stages of their life. Second, it cannot be discarded that environmental factors experienced by parasites in gammarids, or earlier, might have consequences at latter developmental stages, and thus could not be evidenced in my experiments.

In my experiments, the temperature experienced by gammarids during the development of their *P. laevis* parasites did not lead to any noticeable impact on the extent of manipulation (Article 4). However, Franceschi et al. (2010a) documented a seasonal difference in the timing of behavioral alterations of gammarids infected by the same acanthocephalan species. In her study, gammarids and parasite eggs were collected at two different seasons. However, subsequent experimental infections and maintenance of gammarids during the development of parasites were conducted under similar conditions. Thus, the differences observed in manipulation between the two seasons might not be linked to direct environmental conditions (although the authors acknowledged that laboratory temperature could be slightly different between the two experiments). Rather, it is possible that the difference might be linked to the conditions experienced by either gammarids or parasites in the field.

One hypothesis is that environmental conditions experienced by the acanthor larvae or by their mothers might be determinant for their manipulative ability. Acanthocephalan parasites reproduce in the intestine of their definitive hosts, and eggs are released in the river along with host feces (Crompton & Nickol, 1985). Because of their microscopic size, no information is known about the time spent by the eggs in the river, although the infectivity of *P. laevis* eggs extracted from adults and maintained in the laboratory in water rapidly decreases within a few weeks (A. Bauer, personal communication ; although certain studies recorded a survival of eggs during several months at low temperature, e.g. DeGiusti, 1949; Hynes and Nicholas, 1963). Thus, it is probable that parasites successfully infecting gammarids spent a short time in the river, although it cannot be discarded that environmental conditions experienced in the river might have an impact on their development and manipulative ability. On the other hand, the conditions experienced by mothers might also influence

the life of their offspring. Acanthocephalan parasites present a sexual reproduction. After internal fertilization, eggs are released into the digestive tract of the definitive host. As highlighted by Parshad and Crompton (1982), the term “egg” actually refers to a fully-formed acanthor larva enclosed in several envelopes. Thus, the eggs that are released are already complex, as described by Parshad and Crompton (1982), measuring several dozens of micrometers (reviewed in Crompton and Nickol, 1985 ; see also Fig. 6A). Moreover, an accumulation of lipids and proteins was reported during the growth phase of the oocytes of several acanthocephalan species (Guraya, 1969; Anantaraman & Subramoniam, 1975; Parshad & Guraya, 1977), suggesting that the maternal investment in eggs might be important. Adult acanthocephalan parasites are known to obtain their nutrients directly from the content of the host intestine rather than from their tissues (Edmonds, 1965), thus directly depending on the diet of their hosts. Accordingly, several studies conducted on *Moniliformis moniliformis*, an acanthocephalan parasite infecting rats, showed that the composition of the diet of their host influenced the growth of the parasite, but also its reproduction, in particular in terms of number and size of ovaries (Nesheim *et al.*, 1977; Parshad *et al.*, 1980; Crompton *et al.*, 1982). The feeding behavior of fish depends on temperature (Kennedy, 1972; see also Fig. 35) and seasonal variations have been documented in the foraging and feeding activity of fish (Penttinen & Holopainen, 1992; Lucas & Batley, 1996). Such results might explain the seasonal variation observed in the reproduction of the fish acanthocephalan parasite *Echinorhynchus salmonis* (Tedla & Fernando, 1970), as well as the seasonal abundance documented in several acanthocephalan species (VanCleave, 1916; Tedla & Fernando, 1970; Muzzall & Rabalais, 1975; Amin *et al.*, 1980). It also provides some arguments to propose that the conditions experienced by the definitive host, in particular in terms of diet, might affect the reproduction of their acanthocephalan parasites, possibly leading to consequences on their development and modulating their impact on their hosts. Such assumptions deserve to be tested.

Furthermore, understanding the effects of environmental conditions, including those experienced by parasites during their growth inside their gammarid hosts, require to take into account the whole life cycle of parasites. Indeed, as discussed in chapter V.2, measuring the extent of the modification on one behavior might not reflect its consequences in terms of increased probability of transmission. Thus, further studies need to rely on predation tests to investigate such crucial parameter. Moreover, although many effects of environmental conditions on parasites were already documented in my experiments, such as modifications in their development time, infection intensity or cystacanth size, the conditions experienced by parasites during their growth could also have consequences latter in their life. For instance, several studies suggest that the volume of the cystacanth, influenced by the diet of the host (see chapter III), could influence the success of their establishment and their survival in the definitive host (Steinauer & Nickol, 2003), as well as their adult

size (Poulin *et al.*, 2003). Understanding the effect of environment on the whole life cycle of the parasite would be interesting and mandatory to draw accurate predictions about the whole consequences of global changes.

4. Investigating other environmental factors

The experiments presented in this thesis focus on the impact of two abiotic parameters, the level of resources and the temperature. These parameters were chosen mostly for two reasons. First, they are believed to be important factors modulating infections. Indeed, numerous studies documented that temperature is one of the main factor affecting the interaction between parasites and their hosts (e.g. Gillooly *et al.*, 2001; Harvell *et al.*, 2002; Barber *et al.*, 2016). Moreover, given that parasites extract resources from their hosts, it can be assumed that the food resources of hosts could directly affect their parasites (e.g. Pulkkinen & Ebert, 2004; Logan *et al.*, 2005; Seppälä *et al.*, 2008b). Second, these two parameters present strong variations within a year, and are also very susceptible to be modified due to global change. However, many other environmental parameters are likely to play a role in the relation between manipulative parasites and their hosts. For instance, the light intensity was shown to influence manipulation (Benesh *et al.*, 2005; Perrot-Minnot *et al.*, 2012). Albeit a priori non sensitive to global change, circadian variations were documented in the extent of manipulation by acanthocephalan parasites on gammarids (Lagrue *et al.*, 2007). It would therefore be interesting to test whether the duration of the day might play a role. Pollutants have also been shown to induce modifications in the behavior of gammarids that resemble those induced by acanthocephalan parasites (De Lange *et al.*, 2006; Guler & Ford, 2010). Further investigations of the effects of pollutants, and their interaction with parasites, are thus required. Predator cues are also known to be determinant in the expression of certain behaviors of gammarids, as well as their manipulation by parasites (Baldauf *et al.*, 2007; Kaldonski *et al.*, 2007; Perrot-Minnot *et al.*, 2007; Kullmann *et al.*, 2008). It would be interesting to investigate further the exact components, and their specificity, leading to such modifications.

Although my thesis largely focused on abiotic parameters, one major result concerns the effect of aggregation of gammarids, hence a component of the biotic environment, on parasite-induced alteration of their shredding efficiency (Article 6). Moreover, the behavior of uninfected gammarid might itself depend on the social conditions experienced by individuals. Indeed, in a short experiment, uninfected male gammarids were maintained for two weeks in glass dishes either alone (in six cm diameter glass dishes) or by groups of either three or ten individuals (in 10 cm diameter glass dishes). After this acclimatization time, their behavior in terms of use of refuges was tested (following the protocol described in Article 4). Interestingly, the behavior of gammarids was found to depend on the conditions of aggregation in which they were maintained prior to be tested (Fig. 36, Wilcoxon, $Chi2 = 8.97$, d.f. = 2, $P = 0.011$). In particular, gammarids maintained in individual glass dishes spent more time

in refuges than those maintained in large groups (Steel-Dwass post-hoc test, P = 0.0075). This confirms the hypothesis made in Article 6 that maintaining individuals in isolated conditions might constitute a stress, modifying their behavior. Moreover, this result could also go in the same direction as the hypothesis made in Article 3 about the mechanisms resulting in the manipulation of gammarids by parasites. As suggested, acanthocephalan parasites could decrease the level of anxiety of their hosts, leading them to adopt more risky behaviors such as spending less time in refuges. Here, along with article 6, the results seem to confirm that certain supposedly stressful conditions might indeed alter the same behaviors of gammarids that are modified by parasites. In most experimental studies, including mine, individuals are maintained and tested in isolated conditions. However, the results could differ when maintaining individuals in groups, representing more realistic conditions. The effect of biotic conditions on manipulation, such as the aggregative habit of gammarids, thus also deserves more attention.

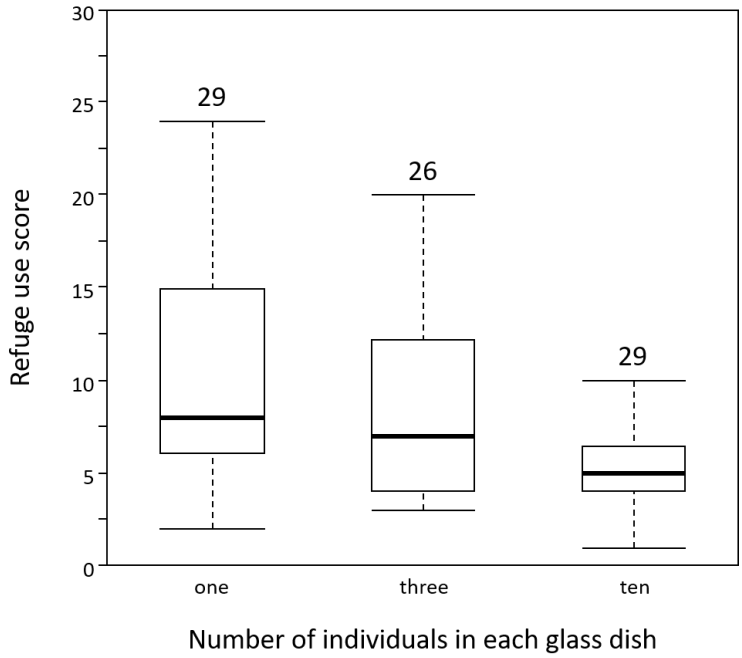

Figure 36. Scores of refuge use for uninfected gammarids maintained, prior to the test, either in individual glass dishes, or per groups of three or ten individuals. Only males were tested. Scores range from 0 (individuals always outside the refuge) to 30 (individuals always inside the refuge). Thick lines represent the medians, the boxes represent the upper and lower quartiles and dotted lines represent the upper and lower deciles. Sample sizes are given above each plot.

5. Understanding the mechanisms of manipulation: a necessary step

Although the effects of environmental factors on manipulation can be investigated without regard to the mechanisms underlying such behavioral alterations, the results of such experiments might confirm certain hypotheses (see for instance discussion in article 3). Conversely, a better understanding of the mechanisms might also shed a new light on the potential effects of environment. Many studies documented that monoamines, in particular serotonin, might play a major role in the modifications induced by acanthocephalan parasites on their intermediate hosts. Indeed, modifications in the serotonergic activity was found in gammarids infected by several species of acanthocephalan parasites, such as *Polymorphus paradoxus* (Maynard *et al.*, 1996), *P. laevis* (Tain *et al.*, 2006, 2007) and *P. tereticollis* (Tain *et al.*, 2006). Other manipulative parasites were also shown to induce such modifications in their intermediate hosts, such as the trematode *Microphallus papillorobustus* on gammarids (Helluy & Thomas, 2003), the cestode *Schistocephalus solidus* on sticklebacks (Øverli *et al.*, 2001), or the trematode *Euhaplorchis californiensis* on killifish (Shaw *et al.*, 2009; Shaw & Øverli, 2012). Moreover, Ponton et al. (2006) found a higher expression of a protein involved in the synthesis of serotonin in *Gammarus insensibilis* parasitized with *P. minutus* or *M. papillorobustus*. Consistent with the ideas discussed above, serotonin was pointed out to play a role in the regulation of fear and anxiety in invertebrates (Curran & Chalasani, 2012; see discussion in Article 3). Moreover, injections of serotonin in *G. pulex* gammarids was found to mimic infection by *P. laevis*, inducing similar modifications in several behaviors such as phototaxis or activity (Perrot-Minnot *et al.*, 2014, see also Helluy & Holmes, 1990).

Considering serotonin as the key factor responsible for behavioral changes induced by acanthocephalan parasites in gammarids might help explaining certain results obtained in this thesis and in other publications. First, the monoamine metabolism of invertebrates was shown to depend on acclimatization conditions, such that several days at high temperature increased serotonin level while cold temperature decreased it (Stefano & Catapane, 1977; Stefano *et al.*, 1977). Such effect might explain the overall positive relationship between phototaxis and temperature observed after two weeks of acclimatization of gammarids (Article 3). In contrast, it is possible that longer acclimatization time might lead to a stabilization in serotonin levels, with differences only remaining between uninfected and infected individuals due to the effect of the parasite. Such hypothesis could explain the absence of difference in manipulation, in terms of use of refuges, depending on the temperature

experienced by the gammarids during the development of their parasites (Article 4). Moreover, although parasites usually induce a significant decrease in the use of refuges of their hosts (e.g. Kaldonski et al., 2007; Perrot-Minnot et al., 2007), this effect was shown to be weaker than the effect on gammarids phototaxis, at least in the absence of predator clues (Perrot-Minnot *et al.*, 2014), and injections of serotonin did not lead to significant effect on gammarids sheltering behavior (Perrot-Minnot *et al.*, 2014). It is likely that even a small effect of the temperature experienced by gammarids during the development of their parasites would have been detected on gammarids use of refuges, considering the large sample size of the experiment (Article 4). However, it is possible that the non-significant effect of a two weeks acclimatization at different temperatures on this behavior was due to such considerations, i.e. a weaker effect of parasites on this trait (Article 3). This would be clarified by investigating the effect of temperature and the acclimatization duration on the two behaviors, as well as on the levels of serotonin of infected and uninfected gammarids.

The time of development of acanthocephalan parasites is known to be largely dependent on temperature (Olson & Pratt, 1971; Tokeson & Holmes, 1982), and large differences were found between the two temperatures experienced by hosts during parasite development (Article 4). However, contrary to the correlations found by Franceschi et al. (2010a), the differences in the development time of parasites were not linked to any change in their manipulation in my experiments. Rather, her results might derive from two phenomena. First, in her experiment, the stage of parasites was verified only once a week. However, my experiments highlighted that the difference of manipulation can be very important between one day and eight day old cystacanths (Article 4). Thus, the higher manipulation reported in parasites taking more time to develop in her study could derive from the fact that parasites were detected at later stages, thus leading to stronger manipulation. However, Franceschi et al. (2010a) also documented seasonal variations in manipulation. Such results might be expected given that serotonin levels are likely to present seasonal variations (Hiripi & Salánki, 1973; Catarsi *et al.*, 1990). Such effects might also explain the seasonal variations in manipulation found by Benesh et al. (2009a) in isopods. Similarly, variations observed in behavioral changes during the day (e.g. Lagrue et al., 2007) might be simply due to circadian variations in the serotonin levels. Indeed, serotonin is known to present variations during the day in invertebrates (Pandey & Habibulla, 1982; Escamilla-Chimal *et al.*, 2001) that are believed to be involved in the regulation of their circadian rhythms (Cymborowski, 1970, 1998). Such hypothesis is comforted by the effect of light properties on gammarids behavior (Benesh *et al.*, 2005; Perrot-Minnot *et al.*, 2012). The effect of different day durations, mimicking exposure to different seasons, are likely to induce variations in manipulation and deserves to be investigated.

6. Conclusion: the key role of parasites in ecology

In recent years, there has been an increasing awareness that parasites might play major roles in ecosystems, and parasites largely found their place in ecology. Since the beginning of my thesis, several articles were published highlighting the ecological importance of parasites (e.g. Dunne et al., 2013; Poulin et al., 2014; Wood and Johnson, 2015), including those manipulating the phenotype of their hosts (e.g. Hatcher et al., 2014). In accordance, there is a growing recognition from conservation biologists that not only endangered host species should be conserved, but also their parasites (Spencer & Zuk, 2016). Understanding the impact of environment on ecosystem functioning has become one of the major challenges in the present context of global change. The effect of such factors on parasites, from their life history traits to their effects on their hosts, also draw substantial attention from scientists in the last few years (e.g. Altman et al., 2016; Barber et al., 2016; Morley and Lewis, 2014; Sheath et al., 2016). Moreover, there is a growing recognition that the effects of environment on ecosystem functioning are complex, such that investigations should focus on combined and interactive effects of multiple factors (e.g. Griffiths et al., 2015; Moe et al., 2013; Rosenblatt and Schmitz, 2014). This is particularly true in host-parasite systems that themselves already rely on complex interactions (Marcogliese, 2016). In particular, one of my articles, concerning the additive effects of temperature and parasitism on the ecological role of gammarids (Article 6), illustrates the growing recognition of the need to include parasites in ecological considerations. Further studies should continue in this direction, with considerations of multiple environmental factors and all the consequences of parasites on their hosts, including manipulation, and their consequences within ecosystems. Finally, and beyond the ecological responses at short terms, evolutionary responses of host-parasite systems in a changing world might also deserve more attention in future years (Chaianunporn & Hovestadt, 2015).

References

Åbjörnsson K, Dahl J, Nyström P, Brönmark C (2000) Influence of predator and dietary chemical cues on the behaviour and shredding efficiency of *Gammarus pulex*. *Aquatic Ecology*, **34**, 379–387.

Adamo SA (2002) Modulating the modulators: Parasites, neuromodulators and host behavioral change. *Brain, Behavior and Evolution*, **60**, 370–377.

Alonso A, De Lange HJ, Peeters ETHM (2010) Contrasting sensitivities to toxicants of the freshwater amphipods *Gammarus pulex* and *G. fossarum*. *Ecotoxicology*, **19**, 133–140.

Altman KA, Paull SH, Johnson PTJ, Golembieski MN, Stephens JP, LaFonte BE, Raffel TR (2016) Host and parasite thermal acclimation responses depend on the stage of infection. *Journal of Animal Ecology*, **85**, 1014–1024.

Amin OM, Burns LA, Redlin MJ (1980) The ecology of *Acanthocephalus parksidei* Amin, 1975 (Acanthocephala: Echinorhynchidae) in its isopod intermediate host. *Proceedings of the Helminthological Society of Washington*, **47**, 37–46.

Anantaraman S, Subramoniam T (1975) Oogenesis in *Acanthosentis oligospinus* n.sp., an acanthoephalan parasite of the fish, *Macrones gulio*. *Proceedings of the Indian Academy of Sciences*, **82**, 139–145.

Andersson KG, Bronmark C, Herrmann J, Malmqvist B, Otto C, Sjorstrom P (1986) Presence of sculpins (*Cottus gobio*) reduces drift and activity of *Gammarus pulex* (Amphipoda). *Hydrobiologia*, **133**, 209–215.

Andreï J, Pain-Devin S, Felten V et al. (2015) Silver nanoparticles impact the functional role of *Gammarus roeseli* (Crustacea Amphipoda). *Environmental Pollution*, **208**, 608–618.

Babin A, Biard C, Moret Y (2010) Dietary supplementation with carotenoids improves immunity without increasing its cost in a crustacean. *American Naturalist*, **176**, 234–241.

Babin A, Saciat C, Teixeira M, Troussard JP, Motreuil S, Moreau J, Moret Y (2015) Limiting immunopathology: Interaction between carotenoids and enzymatic antioxidant defences. *Developmental and Comparative Immunology*, **49**, 278–281.

Bacela-Spychalska K, Rigaud T, Wattier R (2014) A co-invasive microsporidian parasite that reduces the predatory behaviour of its host *Dikerogammarus villosus* (Crustacea, Amphipoda). *Parasitology*, **141**, 254–258.

Baldauf SA, Thünken T, Frommen JG, Bakker TCM, Heupel O, Kullmann H (2007) Infection with an acanthocephalan manipulates an amphipod's reaction to a fish predator's odours. *International Journal for Parasitology*, **37**, 61–65.

Barber I, Berkhout BW, Zalina I (2016) Thermal change and the dynamics of multi-host parasite life cycles in aquatic ecosystems. *Integrative and Comparative Biology*, **56**, 561–572.

Bates AE, Leiterer F, Wiedeback ML, Poulin R (2011) Parasitized snails take the heat: a case of host manipulation? *Oecologia*, **167**, 613–621.

Bauer A, Trouvé S, Grégoire A, Bollache L, Cézilly F (2000) Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on the behaviour of native and invader gammarid species. *International Journal for Parasitology*, **30**, 1453–1457.

Bauer A, Haine ER, Perrot-Minnot M-J, Rigaud T (2005) The acanthocephalan parasite *Polymorphus minutus* alters the geotactic and clinging behaviours of two sympatric amphipod hosts: the native *Gammarus pulex* and the invasive *Gammarus roeseli*. *Journal of Zoology*, **267**, 39–43.

Bechmann RK, Taban IC, Westerlund S et al. (2011) Effects of ocean acidification on early life stages of shrimp (*Pandalus borealis*) and mussel (*Mytilus edulis*). *Journal of Toxicology and Environmental Health. Part A*, **74**, 424–438.

Beckage NE, Riddiford LM (1983) Growth and development of the endoparasitic wasp *Apanteles congregatus*: dependence on host nutritional status and parasite load. *Physiological Entomology*, **8**, 231–241.

Benesh DP, Valtonen ET (2007a) Effects of *Acanthocephalus lucii* (Acanthocephala) on intermediate host survival and growth: implications for exploitation strategies. *The Journal of Parasitology*, **93**, 735–741.

Benesh DP, Valtonen ET (2007b) Proximate factors affecting the larval life history of *Acanthocephalus lucii* (Acanthocephala). *The Journal of Parasitology*, **93**, 742–749.

Benesh DP, Duclos LM, Nickol BB (2005) The behavioral response of amphipods harboring *Corynosoma constrictum* (Acanthocephala) to various components of light. *The Journal of Parasitology*, **91**, 731–736.

Benesh DP, Kitchen J, Pulkkinen K, Hakala I, Valtonen ET (2008) The effect of *Echinorhynchus borealis* (Acanthocephala) infection on the anti-predator behavior of a benthic amphipod. *The Journal of Parasitology*, **94**, 542–545.

Benesh DP, Hasu T, Seppälä O, Valtonen ET (2009a) Seasonal changes in host phenotype manipulation by an acanthocephalan: time to be transmitted? *Parasitology*, **136**, 219–230.

Benesh DP, Seppälä O, Valtonen ET (2009b) Acanthocephalan size and sex affect the modification of intermediate host colouration. *Parasitology*, **136**, 847–854.

Bentley AC, Hurd H (1993) *Pomphorhynchus laevis* (Acanthocephala): Elevation of haemolymph protein concentrations in the intermediate host, *Gammarus pulex* (Crustacea: Amphipoda). *Parasitology*, **107**, 193–198.

Berdoy M, Webster JP, Macdonald DW (2000) Fatal attraction in rats infected with *Toxoplasma gondii*. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 1591–1594.

Bethel WM, Holmes JC (1973) Altered evasive behavior and responses to light in amphipods harboring acanthocephalan cystacanths. *The Journal of Parasitology*, **59**, 945–956.

Bethel WM, Holmes JC (1974) Correlation of development of altered evasive behavior in *Gammarus lacustris* (Amphipoda) harboring cystacanths of *Polymorphus paradoxus* (Acanthocephala) with the infectivity to the definitive host. *The Journal of Parasitology*, **60**, 272–274.

Bethel WM, Holmes JC (1977) Increased vulnerability of amphipods to predation owing to altered

behavior induced by larval acanthocephalans. *Canadian Journal of Zoology*, **55**, 110–115.

Blaxter JHS, Hallers-Tjabbes CCT (1992) The effect of pollutants on sensory systems and behaviour of aquatic animals. *Netherlands Journal of Aquatic Ecology*, **26**, 43–58.

Bollache L, Gambade G, Cézilly F (2000) The influence of micro-habitat segregation on size assortative pairing in *Gammarus pulex* (L.) (Crustacea, Amphipoda). *Archiv für Hydrobiologie*, **147**, 547–558.

Bollache L, Gambade G, Cézilly F (2001) The effects of two acanthocephalan parasites, *Pomphorhynchus laevis* and *Polymorphus minutus*, on pairing success in male *Gammarus pulex* (Crustacea: Amphipoda). *Behavioral Ecology and Sociobiology*, **49**, 296–303.

Bollache L, Rigaud T, Cézilly F (2002) Effects of two acanthocephalan parasites on the fecundity and pairing status of female *Gammarus pulex* (Crustacea: Amphipoda). *Journal of Invertebrate Pathology*, **79**, 102–110.

Bollache L, Devin S, Wattier R, Chovet M, Beisel J-N, Moreteau JC, Rigaud T (2004) Rapid range extension of the Ponto-Caspian amphipod *Dikerogammarus villosus* in France: potential consequences. *Archiv für Hydrobiologie*, **160**, 57–66.

Bollache L, Dick JTA, Farnsworth KD, Montgomery WI (2008) Comparison of the functional responses of invasive and native amphipods. *Biology Letters*, **4**, 166–169.

Boulet, Reno, Libon, Neel J (2005) Douvologie. In: *La rubrique scientifique T.3* (ed Glénat), pp. 25–26.

Brett JR (1971) Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). *American Zoologist*, **113**, 99–113.

Brockton V, Smith VJ (2008) Crustin expression following bacterial injection and temperature change in the shore crab, *Carcinus maenas*. *Developmental and Comparative Immunology*, **32**, 1027–1033.

Brown AF (1989) Seasonal dynamics of the acanthocephalan *Pomphorhynchus laevis* (Muller, 1776) in its intermediate and preferred definitive hosts. *Journal of Fish Biology*, **34**, 183–194.

Brown AF, Pascoe D (1989) Parasitism and host sensitivity to cadmium: an acanthocephalan infection of the freshwater amphipod *Gammarus pulex*. *Journal of Applied Ecology*, **26**, 473–487.

Brown AF, Thompson DBA (1986) Parasite manipulation of host behaviour: acanthocephalans and shrimps in the laboratory. *Journal of Biological Education*, **20**, 121–127.

Brown SP, Renaud F, Guégan J, Thomas F (2001a) Evolution of trophic transmission in parasites: the need to reach a mating place? *Journal of Evolutionary Biology*, **14**, 815–820.

Brown SP, Loot G, Grenfell BT, Guégan JF (2001b) Host manipulation by *Ligula intestinalis*: accident or adaptation? *Parasitology*, **123**, 519–529.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1771–1789.

Bundschuh M, Zubrod JP, Kosol S, Maltby L, Stang C, Duester L, Schulz R (2011) Fungal composition on leaves explains pollutant-mediated indirect effects on amphipod feeding. *Aquatic Toxicology*,

104, 32–37.

Byström P, Andersson J, Kiessling A, Eriksson LO (2006) Size and temperature dependent foraging capacities and metabolism: Consequences for winter starvation mortality in fish. *Oikos*, **115**, 43–52.

Caddigan SC, Barkauskas RT, Sparkes TC (2014) Intra-population variation in behavior modification by the acanthocephalan *Acanthocephalus dirus*: are differences mediated by host condition? *Parasitology Research*, **113**, 4307–4311.

Catalán TP, Wozniak A, Niemeyer HM, Kalergis AM, Bozinovic F (2012) Interplay between thermal and immune ecology: effect of environmental temperature on insect immune response and energetic costs after an immune challenge. *Journal of Insect Physiology*, **58**, 310–317.

Catarsi S, Garcia-Gil M, Traina G, Brunelli M (1990) Seasonal variation of serotonin content and nonassociative learning of swim induction in the leech *Hirudo medicinalis*. *Journal of Comparative Physiology A*, **167**, 469–474.

Cerenius L, Söderhäll K (2004) The prophenoloxidase-activating system in invertebrates. *Immunological Reviews*, **198**, 116–126.

Cézilly F, Perrot-Minnot M-J (2010) Interpreting multidimensionality in parasite-induced phenotypic alterations: panselectionism versus parsimony. *Oikos*, **119**, 1224–1229.

Cézilly F, Grégoire A, Bertin A (2000) Conflict between co-occuring manipulative parasites; an experimental study of the joint influence of two acanthocephalan parasites on the behaviour of *Gammarus pulex*. *Parasitology*, **120**, 625–630.

Cézilly F, Thomas F, Médoc V, Perrot-Minnot M-J (2010) Host-manipulation by parasites with complex life cycles: adaptive or not? *Trends in Parasitology*, **26**, 311–317.

Cézilly F, Favrat A, Perrot-Minnot M-J (2013) Multidimensionality in parasite-induced phenotypic alterations: ultimate versus proximate aspects. *The Journal of Experimental Biology*, **216**, 27–35.

Cézilly F, Perrot-Minnot M-J, Rigaud T (2014) Cooperation and conflict in host manipulation: interactions among macro-parasites and micro-organisms. *Frontiers in Microbiology*, **5**, 248.

Chaianunporn T, Hovestadt T (2015) Evolutionary responses to climate change in parasitic systems. *Global Change Biology*, **21**, 2905–2916.

Chen H-Y, Chen Y-LL (1999) Temperature preferendum of postlarval black tiger shrimp (*Penaeus monodon*). *Marine and Freshwater Research*, **50**, 67–70.

Chen M, Yang H, Delaporte M, Zhao S (2007) Immune condition of *Chlamys farreri* in response to acute temperature challenge. *Aquaculture*, **271**, 479–487.

Cheng W, Chen J-C (2000) Effects of pH, temperature and salinity on immune parameters of the freshwater prawn *Macrobrachium rosenbergii*. *Fish & Shellfish Immunology*, **10**, 387–391.

Cheng W, Chen SM, Wang FI, Hsu PI, Liu CH, Chen J-C (2003) Effects of temperature, pH, salinity and ammonia on the phagocytic activity and clearance efficiency of giant freshwater prawn *Macrobrachium rosenbergii* to *Lactococcus garvieae*. *Aquaculture*, **219**, 111–121.

Cheng W, Hsiao IS, Hsu C-H, Chen J-C (2004) Change in water temperature on the immune response of Taiwan abalone *Haliotis diversicolor* supertexta and its susceptibility to *Vibrio parahaemolyticus*. *Fish & Shellfish Immunology*, **17**, 235–243.

Cheng W, Wang L-U, Chen J-C (2005) Effect of water temperature on the immune response of white shrimp *Litopenaeus vannamei* to *Vibrio alginolyticus*. *Aquaculture*, **250**, 592–601.

Chrisholm JRS, Smith VJ (1994) Variation of antibacterial activity in the haemocytes of the shore crab, *Carcinus maenas*, with temperature. *Journal of the Marine Biological Association of the UK*, **74**, 979–982.

Cliff N (1996) *Ordinal methods for behavioral data analysis*. Lawrence Erlbaum Associates, Inc.

Coats J, Poulin R, Nakagawa S (2010) The consequences of parasitic infections for host behavioural correlations and repeatability. *Behaviour*, **147**, 367–382.

Cohen J (1988) *Statistical power analysis for the behavioral sciences*. Erlbaum, Hillsdale: NJ.

Constable D, Birkby NJ (2016) The impact of the invasive amphipod *Dikerogammarus haemobaphes* on leaf litter processing in UK rivers. *Aquatic Ecology*, **50**, 273–281.

Cornet S (2011) Density-dependent effects on parasite growth and parasite-induced host immunodepression in the larval helminth *Pomphorhynchus laevis*. *Parasitology*, **138**, 257–265.

Cornet S, Sorci G (2010) Parasite virulence when the infection reduces the host immune response. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 1929–1935.

Cornet S, Biard C, Moret Y (2007) Is there a role for antioxidant carotenoids in limiting self-harming immune response in invertebrates? *Biology Letters*, **3**, 284–288.

Cornet S, Franceschi N, Bauer A, Rigaud T, Moret Y (2009a) Immune depression induced by acanthocephalan parasites in their intermediate crustacean host: Consequences for the risk of super-infection and links with host behavioural manipulation. *International Journal for Parasitology*, **39**, 221–229.

Cornet S, Biard C, Moret Y (2009b) Variation in immune defence among populations of *Gammarus pulex* (Crustacea: Amphipoda). *Oecologia*, **159**, 257–269.

Cornet S, Sorci G, Moret Y (2010) Biological invasion and parasitism: invaders do not suffer from physiological alterations of the acanthocephalan *Pomphorhynchus laevis*. *Parasitology*, **137**, 137–147.

Coulaud R, Geffard O, Xuereb B et al. (2011) In situ feeding assay with *Gammarus fossarum* (Crustacea): Modelling the influence of confounding factors to improve water quality biomonitoring. *Water Research*, **45**, 6417–6429.

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters*, **11**, 1304–1315.

Crompton DWT (1970) *An ecological approach to acanthocephalan physiology*. Cambridge University Press.

Crompton DWT, Nickol BB (1985) *Biology of the Acanthocephala*. Cambridge University Press.

Crompton DWT, Singhvi A, Keymer A (1982) Effects of host dietary fructose on experimentally stunted *Moniliformis* (Acanthocephala). *International Journal for Parasitology*, **12**, 117–121.

Curran KP, Chalasani SH (2012) Serotonin circuits and anxiety: What can invertebrates teach us? *Invertebrate Neuroscience*, **12**, 81–92.

Curtis LA (1987) Vertical distribution of an estuarine snail altered by a parasite. *Science*, **235**, 1509–1511.

Cymborowski B (1970) The assumed participation of 5-Hydroxytryptamine in regulation of the circadian rhythm of locomotor activity in *Acheta domesticus* L. *Comparative and General Pharmacology*, **1**, 316–322.

Cymborowski B (1998) Serotonin modulates a photic response in circadian locomotor rhythmicity of adults of the blow fly, *Calliphora vicina*. *Physiological Entomology*, **23**, 25–32.

Darling ES, Côté IM (2008) Quantifying the evidence for ecological synergies. *Ecology Letters*, **11**, 1278–1286.

Dawkins R (1982) *The Extended Phenotype*. Oxford University Press.

Dedourge-Geffard O, Palais F, Biagianti-Risbourg S, Geffard O, Geffard A (2009) Effects of metals on feeding rate and digestive enzymes in *Gammarus fossarum*: An in situ experiment. *Chemosphere*, **77**, 1569–1576.

Degani G, Bromley HJ, Ortal R, Netzer Y, Harari N (1987) Diets of rainbow trout (*Salmo gairdneri*) in a thermally constant stream. *Vie Milieu*, **37**, 99–103.

DeGiusti D (1949) The life cycle of *Leptorhynchoides thecatus* (Linton), an acanthocephalan of fish. *The Journal of Parasitology*, **35**, 437–460.

Devin S, Beisel J-N (2008) Geographic patterns in freshwater gammarid invasions: An analysis at the pan-European scale. *Aquatic Sciences*, **70**, 100–106.

Dezfuli BS, Zanini N, Reggiani G, Rossi R (1991) *Echinogammarus stammen* (Amphipoda) as an intermediate host for *Pomphorhynchus laevis* (Acanthocephala) parasite of fishes from the river Brenta. *Bolletino di Zoologia*, **58**, 267–271.

Dezfuli BS, Giari L, Poulin R (2001) Costs of intraspecific and interspecific host sharing in acanthocephalan cystacanths. *Parasitology*, **122**, 483–489.

Dezfuli BS, Maynard BJ, Wellnitz TA (2003) Activity levels and predator detection by amphipods infected with an acanthocephalan parasite, *Pomphorhynchus laevis*. *Folia Parasitologica*, **50**, 129–134.

Dezfuli BS, Simoni E, Duclos LM, Rossetti E (2008a) Crustacean - acanthocephalan interaction and host cell-mediated immunity: parasite encapsulation and melanization. *Folia Parasitologica*, **55**, 53–59.

Dezfuli BS, Lui A, Giovinazzo G, Giari L (2008b) Effect of Acanthocephala infection on the reproductive potential of crustacean intermediate hosts. *Journal of Invertebrate Pathology*, **98**, 116–119.

Dianne L, Rigaud T, Léger E, Motreuil S, Bauer A, Perrot-Minnot M-J (2010) Intraspecific conflict over

host manipulation between different larval stages of an acanthocephalan parasite. *Journal of Evolutionary Biology*, **23**, 2648–2655.

Dianne L, Perrot-Minnot M-J, Bauer A, Gaillard M, Léger E, Rigaud T, Elsa L (2011) Protection first then facilitation: a manipulative parasite modulates the vulnerability to predation of its intermediate host according to its own developmental stage. *Evolution*, **65**, 2692–2698.

Dianne L, Bollache L, Lagrue C, Franceschi N, Rigaud T (2012) Larval size in acanthocephalan parasites: influence of intraspecific competition and effects on intermediate host behavioural changes. *Parasites & Vectors*, **5**, 166.

Dianne L, Perrot-Minnot M-J, Bauer A, Guvenatam A, Rigaud T (2014) Parasite-induced alteration of plastic response to predation threat: increased refuge use but lower food intake in *Gammarus pulex* infected with the acanothocephalan *Pomphorhynchus laevis*. *International Journal for Parasitology*, **44**, 211–216.

Dick JTA, Armstrong M, Clarke HC et al. (2010) Parasitism may enhance rather than reduce the predatory impact of an invader. *Biology Letters*, **6**, 636–638.

Dittmar J, Janssen H, Kuske A, Kurtz J, Scharsack JP (2013) Heat and immunity: an experimental heat wave alters immune functions in three-spined sticklebacks (*Gasterosteus aculeatus*). *The Journal of Animal Ecology*, **83**, 744–757.

Dobson AP, Lafferty KD, Kuris AM, Hechinger RF, Jetz W (2008) Homage to Linnaeus: how many parasites? How many hosts? *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 11482–11489.

Dolinsky ZS, Hardy CA, Burright RG, Donovick PJ (1985) The progression of behavioral and pathological effects of the parasite *Toxocara canis* in the mouse. *Physiology and Behavior*, **35**, 33–42.

Van Duin EHS, Blom G, Los FJ et al. (2001) Modeling underwater light climate in relation to sedimentation, resuspension, water quality and autotrophic growth. *Hydrobiologia*, **444**, 25–42.

Dukes JS, Mooney HA (1999) Does global change increase the success of biological invaders? *Trends in Ecology and Evolution*, **14**, 135–139.

Dunn AM, Dick JTA (1998) Parasitism and epibiosis in native and non-native gammarids in freshwater in Ireland. *Ecography*, **21**, 593–598.

Dunn AM, Hogg JC, Hatcher MJ (2006) Transmission and burden and the impact of temperature on two species of vertically transmitted microsporidia. *International Journal for Parasitology*, **36**, 409–414.

Dunn AM, Torchin ME, Hatcher MJ et al. (2012) Indirect effects of parasites in invasions. *Functional Ecology*, **26**, 1262–1274.

Dunne JA, Lafferty KD, Dobson AP et al. (2013) Parasites affect food web structure primarily through increased diversity and complexity. *PLoS Biology*, **11**, e1001579.

Durieux R, Rigaud T, Médoc V (2012) Parasite-induced suppression of aggregation under predation risk in a freshwater amphipod. Sociality of infected amphipods. *Behavioural Processes*, **91**, 207–213.

Edmonds SJ (1965) Some experiments on the nutrition of *Moniliformis dubius* Meyer (Acanthocephala). *Parasitology*, **55**, 337–344.

Eedy RI, Giberson DJ (2007) Macroinvertebrate distribution in a reach of a north temperate eastern Canadian river: Relative importance of detritus, substrate and flow. *Fundamental and Applied Limnology / Archiv für Hydrobiologie*, **169**, 101–114.

Elliott JM (1976) The energetics of feeding, metabolism and growth of brown trout (*Salmo trutta* L.) in relation to body weight, water temperature and ration size. *Journal of Animal Ecology*, **45**, 923–948.

Emde S, Rueckert S, Palm HW, Klimpel S (2012) Invasive Ponto-Caspian amphipods and fish increase the distribution range of the acanthocephalan *Pomphorhynchus tereticollis* in the River Rhine. *PLoS ONE*, **7**, e53218.

Englund G, Öhlund G, Hein CL, Diehl S (2011) Temperature dependence of the functional response. *Ecology Letters*, **14**, 914–921.

Escamilla-Chimal EG, Van Herp F, Fanjul-Moles ML (2001) Daily variations in crustacean hyperglycaemic hormone and serotonin immunoreactivity during the development of crayfish. *The Journal of Experimental Biology*, **204**, 1073–1081.

Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ, Millero FJ (2004) Impact of anthropogenic CO2 on the CaCO3 system in the oceans. *Science*, **305**, 362–366.

Felten V, Charmantier G, Mons R et al. (2008) Physiological and behavioural responses of *Gammarus pulex* (Crustacea: Amphipoda) exposed to cadmium. *Aquatic Toxicology*, **86**, 413–425.

Fenton A, Rands SA (2006) The impact of parasite manipulation and predator foraging behavior on predator-prey communities. *Ecology*, **87**, 2832–2841.

Fielding NJ, MacNeil C, Dick JTA, Elwood RW, Riddell GE, Dunn AM (2003) Effects of the acanthocephalan parasite *Echinorhynchus truttae* on the feeding ecology of *Gammarus pulex* (Crustacea: Amphipoda). *Journal of Zoology*, **261**, 321–325.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Foucreau N, Puijalon S, Hervant F, Piscart C (2013a) Effect of leaf litter characteristics on leaf conditioning and on consumption by *Gammarus pulex*. *Freshwater Biology*, **58**, 1672–1681.

Foucreau N, Piscart C, Puijalon S, Hervant F (2013b) Effect of climate-related change in vegetation on leaf litter consumption and energy storage by *Gammarus pulex* from continental or Mediterranean populations. *PLoS ONE*, **8**, e77242.

Foucreau N, Cottin D, Piscart C, Hervant F (2014) Physiological and metabolic responses to rising temperature in *Gammarus pulex* (Crustacea) populations living under continental or Mediterranean climates. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, **168**, 69–75.

Foucreau N, Piscart C, Puijalon S, Hervant F (2016) Effects of rising temperature on a functional process:

consumption and digestion of leaf litter by a freshwater shredder. *Fundamental and Applied Limnology / Archiv für Hydrobiologie*, **187**, 295–306.

Franceschi N, Rigaud T, Moret Y, Hervant F, Bollache L (2007) Behavioural and physiological effects of the trophically transmitted cestode parasite, *Cyathocephalus truncatus*, on its intermediate host, *Gammarus pulex*. *Parasitology*, **134**, 1839–47.

Franceschi N, Bauer A, Bollache L, Rigaud T (2008) The effects of parasite age and intensity on variability in acanthocephalan-induced behavioural manipulation. *International Journal for Parasitology*, **38**, 1161–1170.

Franceschi N, Cornet S, Bollache L, Dechaume-Moncharmont F-X, Bauer A, Motreuil S, Rigaud T (2010a) Variation between populations and local adaptation in acanthocephalan-induced parasite manipulation. *Evolution*, **64**, 2417–2430.

Franceschi N, Bollache L, Cornet S, Bauer A, Motreuil S, Rigaud T (2010b) Co-variation between the intensity of behavioural manipulation and parasite development time in an acanthocephalan–amphipod system. *Journal of Evolutionary Biology*, **23**, 2143–2150.

Fredensborg BL, Poulin R (2005) Larval helminths in intermediate hosts: does competition early in life determine the fitness of adult parasites? *International Journal for Parasitology*, **35**, 1061–1070.

Friberg N, Andersen TH, Hansen H, Iversen TM, Jacobsen D, Krojgaard L, Larsen SE (1994) The effect of brown trout (*Salmo Trutta* L.) on stream invertebrate drift, with special reference to *Gammarus pulex* L. *Hydrobiologia*, **294**, 105–110.

Fuller CA, Rock P, Philips T (2003) Behavior, color changes, and predation risk induced by Acanthocephalan parasitism in the Caribbean termite *Nasutitermes acajutlae*. *Caribbean Journal of Science*, **39**, 128–135.

Fuller T, Bensch S, Müller I et al. (2012) The ecology of emerging infectious diseases in migratory birds: An assessment of the role of climate change and priorities for future research. *EcoHealth*, **9**, 80–88.

García-Varela M, Pérez-Ponce de León G, de la Torre P, Cummings MP, Sarma SS, Laclette JP (2000) Phylogenetic relationships of Acanthocephala based on analysis of 18S ribosomal RNA gene sequences. *Journal of Molecular Evolution*, **50**, 532–40.

Gaworecki KM, Klaine SJ (2008) Behavioural and biochemical responses of hybrid striped bass during and after fluoxetine exposure. *Aquatic Toxicology*, **88**, 207–213.

Genchi C, Rinaldi L, Mortarino M, Genchi M, Cringoli G (2009) Climate and Dirofilaria infection in Europe. *Veterinary Parasitology*, **163**, 286–292.

Giles JR, Peterson AT, Busch JD et al. (2014) Invasive potential of cattle fever ticks in the southern United States. *Parasites & Vectors*, **7**, 189.

Gillooly JF, Brown JH, West GB, Savage VB, Charnov EL (2001) Effects of size and temperature on metabolic rate. *Science*, **293**, 2248–2251.

Gilman SE, Urban MC, Tewksbury J, Gilchrist GW, Holt RD (2010) A framework for community interactions under climate change. *Trends in Ecology and Evolution*, **25**, 325–331.

Gismondi E, Beisel J-N, Cossu-Leguille C (2012a) *Polymorphus minutus* affects antitoxic responses of *Gammarus roeseli* exposed to cadmium. *PLoS ONE*, **7**, e41475.

Gismondi E, Rigaud T, Beisel J-N, Cossu-Leguille C (2012b) Effect of multiple parasitic infections on the tolerance to pollutant contamination. *PLoS ONE*, **7**, e41950.

Gismondi E, Cossu-Leguille C, Beisel J-N (2012c) Acanthocephalan parasites: Help or burden in gammarid amphipods exposed to cadmium? *Ecotoxicology*, **21**, 1188–1193.

Glazier DS (2010) A unifying explanation for diverse metabolic scaling in animals and plants. *Biological Reviews of the Cambridge Philosophical Society*, **85**, 111–138.

Gomez-Jimenez S, Uglow RF, Gollas-Galvan T (2000) The effects of cooling and emersion on total haemocyte count and phenoloxidase activity of the spiny lobster *Panulirus interruptus*. *Fish & Shellfish Immunology*, **10**, 631–635.

Grabner DS, Weigand AM, Leese F, Winking C, Hering D, Tollrian R, Sures B (2015) Invaders, natives and their enemies: distribution patterns of amphipods and their microsporidian parasites in the Ruhr Metropolis, Germany. *Parasites & Vectors*, **8**, 419.

Grabowski M, Konopacka A, Jazdzewski K, Janowska E (2006) Invasions of alien gammarid species and retreat of natives in the Vistula Lagoon (Baltic Sea, Poland). *Helgoland Marine Research*, **60**, 90–97.

Griffiths JI, Warren PH, Childs DZ (2015) Multiple environmental changes interact to modify species dynamics and invasion rates. *Oikos*, **124**, 458–468.

Guinnee MA, Moore J (2004) The effect of parasitism on host fecundity is dependent on temperature in a cockroach-acanthocephalan system. *The Journal of Parasitology*, **90**, 673–677.

Guler Y, Ford AT (2010) Anti-depressants make amphipods see the light. *Aquatic Toxicology*, **99**, 397–404.

Gunn J, Crumley CL (1991) Global energy balance and regional hydrology: a Burgundian case study. *Earth surface processes and landforms*, **16**, 579–592.

Guraya SS (1969) Histochemical observations on the developing acanthocephalan oocyte. *Acta Embryologiae Experimentalis*, **2**, 147–155.

Hadeler KP, Freedman HI (1989) Predator-prey populations with parasitic infection. *Journal of Mathematical Biology*, **27**, 609–631.

Hafer N, Milinski M (2015) When parasites disagree: Evidence for parasite-induced sabotage of host manipulation. *Evolution*, **69**, 611–620.

Hafer N, Milinski M (2016) Inter- and intraspecific conflicts between parasites over host manipulation. *Proceedings of the Royal Society B: Biological Sciences*, **283**, 20152870.

Haine ER, Boucansaud K, Rigaud T (2005) Conflict between parasites with different transmission strategies infecting an amphipod host. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 2505–2510.

Hamilton CD, Lydersen C, Ims RA, Kovas KM (2015) Predictions replaced by facts: a keystone species'

behavioural responses to declining arctic sea-ice. *Biology Letters*, **11**, 20150803.

Hammerschmidt K, Koch K, Milinski M, Chubb JC, Parker GA (2009) When to go: Optimization of host switching in parasites with complex life cycles. *Evolution*, **63**, 1976–1986.

Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD (2002) Climate warming and disease risks for terrestrial and marine biota. *Science*, **296**, 2158–2162.

Hatcher MJ, Dick JTA, Dunn AM (2012) Diverse effects of parasites in ecosystems: Linking interdependent processes. *Frontiers in Ecology and the Environment*, **10**, 186–194.

Hatcher MJ, Dick JTA, Dunn AM (2014) Parasites that change predator or prey behaviour can have keystone effects on community composition. *Biology Letters*, **10**, 20130879.

Hawlena D, Strickland MS, Bradford MA, Schmitz OJ (2012) Fear of predation slows plant-litter decomposition. *Science*, **336**, 1434–1438.

Haye PA, Ojeda PF (1998) Metabolic and behavioral alterations in the crab *Hemigrapsus crenulatus* (Milne-Edwards 1837) induced by its acanthocephalan parasite *Profilicollis antarcticus* (Zdzitowiecki 1985). *Journal of Experimental Marine Biology and Ecology*, **228**, 73–82.

Heil M (2016) Host manipulation by parasites: cases, patterns, and remaining doubts. *Frontiers in Ecology and Evolution*, **4**, 80.

Helluy S, Holmes JC (1990) Serotonin, octopamine, and the clinging behavior induced by the parasite *Polymorphus paradoxus* (Acanthocephala) in *Gammarus lacustris* (Crustacea). *Canadian Journal of Zoology*, **68**, 1214–1220.

Helluy S, Thomas F (2003) Effects of *Microphallus papillorobustus* (Platyhelminthes: Trematoda) on serotonergic immunoreactivity and neuronal architecture in the brain of *Gammarus insensibilis* (Crustacea: Amphipoda). *Proceedings of the Royal Society B: Biological Sciences*, **270**, 563–568.

Hernandez AD, Sukhdeo MVK (2008) Parasite effects on isopod feeding rates can alter the host's functional role in a natural stream ecosystem. *International Journal for Parasitology*, **38**, 683–690.

Hernroth B, Nilsson H, Wiklander K, Jutfelt F, Baden S (2012) Simulated climate change causes immune suppression and protein damage in the crustacean *Nephrops norvegicus*. *Fish & shellfish immunology*, **33**, 1095–1101.

Hervant F, Mathieu J, Barré H, Simon K, Pinon C (1997) Comparative study on the behavioral, ventilatory, and respiratory responses of hypogean and epigean crustaceans to long-term starvation and subsequent feeding. *Comparative Biochemistry and Physiology*, **118A**, 1277–1283.

Hindsbo O (1972) Effects of *Polymorphus* (Acanthocephala) on colour and behaviour of *Gammarus lacustris*. *Nature*, **238**, 333.

Hiripi L, Salánki J (1973) Seasonal and activity-dependent changes of the serotonin level in the C.N.C and heart of the snail (*Helix pomatia* L.). *Comparative and General Pharmacology*, **4**, 285–292.

Hoback WW, Barnhart MC (1996) Lethal limits and sublethal effects of hypoxia on the amphipod *Gammarus pseudolimnaeus*. *Journal of the North American Benthological Society*, **15**, 117–126.

Hudson PJ, Greenman J (1998) Competition mediated by parasites: biological and theoretical progress. *Trends in Ecology & Evolution*, **13**, 387–390.

Hudson PJ, Dobson AP, Lafferty KD (2006) Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology and Evolution*, **21**, 381–385.

Hughes DP, Brodeur J, Thomas F (2012) *Host manipulation by parasites*. Oxford University Press.

Hume KD, Elwood RW, Dick JTA, Morrison J (2005) Sexual dimorphism in amphipods: The role of male posterior gnathopods revealed in *Gammarus pulex*. *Behavioral Ecology and Sociobiology*, **58**, 264–269.

Hurd H (2003) Manipulation of medically important insect vectors by their parasites. *Annual Review of Entomology*, **48**, 141–161.

Hynes HBN (1954) The ecology of *Gammarus duebeni* Lilljeborg and its occurrence in fresh water in Western Britain. *Journal of Animal Ecology*, **23**, 38–84.

Hynes HBN, Nicholas WL (1958) The resistance of *Gammarus* Spp. to Infection by *Polymorphus Minutus* (Goeze, 1782) (Acanthocephala). *Annals of Tropical Medicine & Parasitology*, **52**, 376–383.

Hynes HBN, Nicholas WL (1963) The importance of the acanthocephalan *Polymorphus minutus* as a parasite of domestic ducks in the United Kingdom. *Journal of Helminthology*, **37**, 185–198.

Ikeh EI, Anosike JC, Okon E (1992) Acanthocephalan infection in man in northern Nigeria. *Journal of Helminthology*, **66**, 241–242.

Ingwell LL, Eigenbrode SD, Bosque-Pérez NA (2012) Plant viruses alter insect behavior to enhance their spread. *Scientific Reports*, **2**, 1–6.

Issartel J, Hervant F, Voituron Y, Renault D, Vernon P (2005) Behavioural, ventilatory and respiratory responses of epigean and hypogean crustaceans to different temperatures. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, **141**, 1–7.

Jacquin L, Mori Q, Pause M, Steffen M, Medoc V (2014) Non-specific manipulation of gammarid behaviour by *P. minutus* parasite enhances their predation by definitive bird hosts. *PLoS ONE*, **9**, e101684.

Johansson MW, Keyser P, Sritunyalucksana K, Söderhäll K (2000) Crustacean haemocytes and haematopoiesis. *Aquaculture*, **191**, 45–52.

Joly DO, Messier F (2004) The distribution of *Echinococcus granulosus* in moose: Evidence for parasite-induced vulnerability to predation by wolves? *Oecologia*, **140**, 586–590.

Jordán F (2009) Keystone species and food webs. *Philisophical Transactions Royal Society B*, **364**, 1733–1741.

Kaldonski N, Perrot-Minnot M-J, Cézilly F (2007) Differential influence of two acanthocephalan parasites on the antipredator behaviour of their common intermediate host. *Animal Behaviour*, **74**, 1311–1317.

Kaldonski N, Perrot-Minnot M-J, Motreuil S, Cézilly F (2008) Infection with acanthocephalans increases the vulnerability of *Gammarus pulex* (Crustacea Amphipoda) to non-host invertebrate predators.

Parasitology, **135**, 627–632.

Kaldonski N, Perrot-Minnot M-J, Dodet R, Martinaud G, Cézilly F (2009) Carotenoid-based colour of acanthocephalan cystacanths plays no role in host manipulation. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 169–76.

Karaman GS, Pinkster S (1977a) Freshwater *Gammarus* species from Europe, North Africa and adjacent regions of Asia (Crustacea-Amphipoda) Part I. *Gammarus pulex*-group and related species. *Bijdragen tot de Dierkunde*, **47**, 1–97.

Karaman GS, Pinkster S (1977b) Freshwater *Gammarus* species from Europe, North Africa and adjacent regions of Asia (Crustacea- Amphipoda) Part II. *Gammarus roeseli*-group and related species. *Bijdragen tot de Dierkunde*, **47**, 165–196.

Karvonen A, Rintamäki P, Jokela J, Valtonen ET (2010) Increasing water temperature and disease risks in aquatic systems: climate change increases the risk of some, but not all, diseases. *International Journal for Parasitology*, **40**, 1483–1488.

Kaushik M, Knowles SCL, Webster JP (2014) What makes a feline fatal in *Toxoplasma gondii*'s fatal feline attraction? Infected rats choose wild cats. *Integrative and Comparative Biology*, **54**, 118–128.

Kelly AE, Goulden ML (2008) Rapid shifts in plant distribution with recent climate change. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 11823–11826.

Kelly DW, Dick JTA, Montgomery WI (2002) The functional role of *Gammarus* (Crustacea, Amphipoda): shredders, predators, or both? *Hydrobiologia*, **485**, 199–203.

Kelly DW, Bailey RJ, MacNeil C, Dick JTA, McDonald RA (2006) Invasion by the amphipod *Gammarus pulex* alters community composition of native freshwater macroinvertebrates. *Diversity and Distributions*, **12**, 525–534.

Kennedy CR (1972) The effects of temperature and other factors upon the establishment and survival of *Pomphorhynchus laevis* (Acanthocephala) in goldfish, *Carassius auratus*. *Parasitology*, **65**, 283–294.

Kennedy CR (2006) *Ecology of the Acanthocephala*. Cambridge University Press.

Kennedy CR, Broughton PF, Hine PM (1978) The status of brown and rainbow trout, *Salmo trutta* and *S . gairdneri* as hosts of the acanthocephalan, *Pomphorhynchus laevis*. *Journal of Fish Biology*, **13**, 265–275.

Khalil M, Furness DN, Zholobenko V, Hoole D (2014) Effect of tapeworm parasitisation on cadmium toxicity in the bioindicator copepod, *Cyclops strenuus*. *Ecological Indicators*, **37**, 21–26.

Khan RA (1990) Parasitism in marine fish after chronic exposure to petroleum hydrocarbons in the laboratory and to the exxon valdez oil spill. *Bulletin of Environmental Contamination and Toxicology*, **44**, 759–763.

Kidawa A, Potocka M, Janecki T (2010) The effects of temperature on the behaviour of the Antarctic sea star *Odontaster validus*. *Polish Polar Research*, **31**, 273–284.

Kivivuori L (1994) Temperature selection behaviour of cold- and warm-acclimated crayfish [*Astacus astacus* (L.)]. *Journal of Thermal Biology*, **19**, 291–297.

Kivivuori L, Lagerspetz KYH (1990) Temperature selection behaviour of the isopod *Saduria entomon* (L.). *Journal of Thermal Biology*, **15**, 83–86.

Klaphake A, Scheumann W, Schliep R (2001) *Biodiversity and International Water Policy*. Institute for Management in Environmental Planning, Technical University of Berlin, Germany.

Klein SL (2003) Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. *Physiology & Behavior*, **79**, 441–449.

Klein SL (2005) Parasite manipulation of host behavior: mechanisms, ecology, and future directions. *Behavioural Processes*, **68**, 219–221.

Knudsen R, Gabler HM, Kuris AM, Amundsen PA (2001) Selective predation on parasitized prey - a comparison between two helminth species with different life-history strategies. *The Journal of Parasitology*, **87**, 941–945.

Koella JC, Sørensen FL, Anderson RA (1998) The malaria parasite, *Plasmodium falciparum*, increases the frequency of multiple feeding of its mosquito vector, *Anopheles gambiae*. *Proceedings of the Royal Society B: Biological Sciences*, **265**, 763–768.

Koella JC, Rieu L, Paul REL (2002) Stage-specific manipulation of a mosquito's host-seeking behavior by the malaria parasite *Plasmodium gallinaceum*. *Behavioral Ecology*, **13**, 816–820.

Kolpin DW, Furlong ET, Meyer MT, Michael Thurman E, Zaugg SD, Barber LB, Buxton HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. *Environmental Science & Technology*, **36**, 1202–1211.

Köster M, Krause C, Paffenhöfer G-A (2008) Time-series measurements of oxygen consumption of copepod nauplii. *Marine Ecology Progress Series*, **353**, 157–164.

Krull WH, Mapes CR (1952) Studies on the biology of *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 (Trematoda: Dicrocoeliidae), including its relation to the intermediate host, *Cionella lubrica* (Müller). III. Observations on the slimeballs of *Dicrocoelium dendriticum*. *The Cornell Veterinarian*, **42**, 253–276.

Kullmann H, Thünken T, Baldauf SA, Bakker TCM, Frommen JG (2008) Fish odour triggers conspecific attraction behaviour in an aquatic invertebrate. *Biology Letters*, **4**, 458–460.

Labaude S, Rigaud T, Cézilly F (2015a) Host manipulation in the face of environmental changes: Ecological consequences. *International Journal for Parasitology: Parasites and Wildlife*, **4**, 442–451.

Labaude S, Cézilly F, Tercier X, Rigaud T (2015b) Influence of host nutritional condition on post-infection traits in the association between the manipulative acanthocephalan *Pomphorhynchus laevis* and the amphipod *Gammarus pulex*. *Parasites & Vectors*, **8**, 403.

Labaude S, Rigaud T, Cézilly F (2016) Additive effects of temperature and infection with an acanthocephalan parasite on the shredding activity of *Gammarus fossarum* (Crustacea: Amphipoda): the importance of social context. *Global Change Biology*, in press.

Lackie J (1972) The effect of temperature on the development of *Moniliformis dubius* (Acanthocephala) in the intermediate host, *Periplaneta americana*. *Parasitology*, **65**, 371–377.

Lafferty KD, Kuris AM (1999) How environmental stress affects the impacts of parasites. *Limnology and Oceanography*, **44**, 925–931.

Lafferty KD, Kuris AM (2012) Ecological consequences of manipulative parasites. In: *Host manipulation by parasites* (eds Hughes DP, Brodeur J, Thomas F), pp. 158–171. Oxford University Press.

Lafferty KD, Morris K (1996) Altered behaviour of parasitised killfish increases succeptability to predation by bird final hosts. *Ecology*, **77**, 1390–1397.

Lafferty KD, Shaw JC (2013) Comparing mechanisms of host manipulation across host and parasite taxa. *The Journal of Experimental Biology*, **216**, 56–66.

Lagrue C, Poulin R (2010) Manipulative parasites in the world of veterinary science: implications for epidemiology and pathology. *Veterinary Journal*, **184**, 9–13.

Lagrue C, Kaldonski N, Perrot-Minnot M-J, Motreuil S, Bollache L (2007) Modification of hosts' behavior by a parasite: Field evidence for adaptive manipulation. *Ecology*, **88**, 2839–2847.

Lagrue C, Wattier R, Galipaud M et al. (2014) Confrontation of cryptic diversity and mate discrimination within *Gammarus pulex* and *Gammarus fossarum* species complexes. *Freshwater Biology*, **59**, 2555–2570.

Lagrue C, Heaphy K, Presswell B, Poulin R (2016) Strong association between parasitism and phenotypic variation in a supralittoral amphipod. *Marine Ecology Progress Series*, **553**, 111–123.

De Lange HJ, Noordoven W, Murk AJ, Lürling M, Peeters ETHM (2006) Behavioural responses of *Gammarus pulex* (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. *Aquatic Toxicology*, **78**, 209–216.

Le Lann C, Lodi M, Ellers J (2014) Thermal change alters the outcome of behavioural interactions between antagonistic partners. *Ecological Entomology*, **39**, 578–588.

Lebarbenchon C, Brown SP, Poulin R, Gauthier-Clerc M, Thomas F (2008) Evolution of pathogens in a man-made world. *Molecular Ecology*, **17**, 475–484.

van Leeuwenhoek A (1693) 75ste Missive, geschreven aan de Koninglijke Societeyt in London. In: *Derde Vervolg der Brieven, geschreven aan de Koninglijke Societeit tot London*, pp. 508–530. Krooneveld.

Lefèvre T, Lebarbenchon C, Gauthier-Clerc M, Missé D, Poulin R, Thomas F (2009) The ecological significance of manipulative parasites. *Trends in Ecology & Evolution*, **24**, 41–48.

Lenoir J, Gégout JC, Marquet PA, de Ruffray P, Brisse H (2008) A significant upward shift in plant species optimum elevation during the 20th century. *Science*, **320**, 1768–1771.

Lepetz V, Massot M, Schmeller DS, Clobert J (2009) Biodiversity monitoring: Some proposals to adequately study species' responses to climate change. *Biodiversity and Conservation*, **18**, 3185–3203.

Lettini SE, Sukhdeo MVK (2010) The energetic cost of parasitism in isopods. *Ecoscience*, **17**, 1–8.

Lewis SE, Hodel A, Sturdy T, Todd R, Weigl C (2012) Impact of acanthocephalan parasites on aggregation behavior of amphipods (*Gammarus pseudolimnaeus*). *Behavioural Processes*, **91**, 159–163.

Logan A, Ruiz-González MX, Brown MJF (2005) The impact of host starvation on parasite development and population dynamics in an intestinal trypanosome parasite of bumble bees. *Parasitology*, **130**, 637–642.

Loiseau C, Harrigan RJ, Bichet C et al. (2013) Predictions of avian *Plasmodium* expansion under climate change. *Scientific Reports*, **3**, 1126.

Loos R, Gawlik BM, Locoro G, Rimaviciute E, Contini S, Bidoglio G (2009) EU-wide survey of polar organic persistent pollutants in European river waters. *Environmental Pollution*, **157**, 561–568.

Loot G, Aulagnier S, Lek S, Thomas F, Guégan J (2002) Experimental demonstration of a behavioural modification in a cyprinid fish, *Rutilus rutilus* (L.), induced by a parasite, *Ligula intestinalis* (L.). *Canadian Journal of Zoology*, **744**, 738–744.

Loreau M, Roy J, Tilman D, Thomas F, Guegan JF, Renaud F (2005) Linking ecosystem and parasite ecology. In: *Parasitism and Ecosystems*, pp. 13–22. Oxford University Press.

Lucas MC, Batley E (1996) Seasonal movements and behaviour of adult barbel *Barbus barbus*, a riverine cyprinid fish: implications for river management. *Journal of Applied Ecology*, **33**, 1345–1358.

Luong LT, Grear DA, Hudson PJ (2014) Manipulation of host-resource dynamics impacts transmission of trophic parasites. *International Journal for Parasitology*, **44**, 737–742.

Luque SP, Ferguson SH, Breed GA (2014) Spatial behaviour of a keystone Arctic marine predator and implications of climate warming in Hudson Bay. *Journal of Experimental Marine Biology and Ecology*, **461**, 504–515.

Maazouzi C, Piscart C, Legier F, Hervant F (2011) Ecophysiological responses to temperature of the "killer shrimp" *Dikerogammarus villosus*: Is the invader really stronger than the native *Gammarus pulex*? *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, **159**, 268–274.

Mackenzie CL, Lynch SA, Culloty SC, Malham SK (2014) Future oceanic warming and acidification alter immune response and disease status in a commercial shellfish species, *Mytilus edulis* L. *PLoS ONE*, **9**, e99712.

MacLeod CD, Poulin R (2012) Host-parasite interactions: a litmus test for ocean acidification? *Trends in Parasitology*, **28**, 365–369.

MacLeod CD, Poulin R (2015) Differential tolerance to ocean acidification by parasites that share the same host. *International Journal for Parasitology*, **45**, 485–493.

Macnab V, Barber I (2012) Some (worms) like it hot: fish parasites grow faster in warmer water, and alter host thermal preferences. *Global Change Biology*, **18**, 1540–1548.

MacNeil C, Dick JTA (2011) Parasite-mediated intraguild predation as one of the drivers of co-existence and exclusion among invasive and native amphipods (Crustacea). *Hydrobiologia*, **665**, 247–256.

MacNeil C, Dick JTA, Elwood RW (1997) The trophic ecology of freshwater *Gammarus* Spp. (crustacea:amphipoda): problems and perspectives concerning the functional feeding group concept. *Biological Reviews*, **72**, 349–364.

MacNeil C, Dick JTA, Hatcher MJ, Dunn AM (2003a) Differential drift and parasitism in invading and native *Gammarus* spp. (Crustacea: Amphipoda). *Ecography*, **26**, 467–473.

MacNeil C, Fielding NJ, Dick JTA, Briffa M, Prenter J, Hatcher MJ, Dunn AM (2003b) An acanthocephalan parasite mediates intraguild predation between invasive and native freshwater amphipods (Crustacea). *Freshwater Biology*, **48**, 2085–2093.

MacNeil C, Fielding NJ, Hume KD, Dick JTA, Elwood RW, Hatcher MJ, Dunn AM (2003c) Parasite altered micro-distribution of *Gammarus pulex* (Crustacea: Amphipoda). *International Journal for Parasitology*, **33**, 57–64.

Madeira D, Mendonça V, Dias M et al. (2015) Physiological, cellular and biochemical thermal stress response of intertidal shrimps with different vertical distributions: *Palaemon elegans* and *Palaemon serratus*. *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology*, **183**, 107–115.

Maltby L, Clayton SA, Wood RM, McLoughlin N (2002) Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: robustness, responsiveness, and relevance. *Environmental Toxicology and Chemistry*, **21**, 361–368.

Mapes CR (1951) Studies on the biology of *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 (Trematoda: Dicrocoeliidae), including its relation to the intermediate host, *Cionella lubrica* (Müller). I. A study of *Dicrocoelium dendriticum* and *Dicrocoelium* infection. *The Cornell Veterinarian*, **41**, 382–432.

Mapes CR, Krull WH (1951) Studies on the biology of *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 (Trematoda: Dicrocoeliidae), including its relation to the intermediate host, *Cionella lubrica* (Müller). II. Collection of the snail, *Cionella lubrica*, and its maintenance in. *The Cornell Veterinarian*, **41**, 433–444.

Marcogliese DJ (2001) Implications of climate change for parasitism of animals in the aquatic environment. *Canadian Journal of Zoology*, **79**, 1331–1352.

Marcogliese DJ (2004) Parasites: small players with crucial roles in the ecological theater. *EcoHealth*, **1**, 151–164.

Marcogliese DJ (2016) The distribution and abundance of parasites in aquatic ecosystems in a changing climate: more than just temperature. *Integrative and Comparative Biology*, **56**, 611–619.

Van Maren M (1979) The amphipod *Gammarus fossarum* Koch (Crustacea) as intermediate host for some helminth parasites, with notes on their occurrence in the final host. *Bijdragen tot de Dierkunde*, **4**, 97–110.

Marriott DR, Collins ML, Paris RM et al. (1989) Behavioural modifications and increased predation risk of *Gammarus pulex* infected with *Polymorphus minutus*. *Journal of Biological Education*, **23**, 135–141.

Matozzo V, Gallo C, Marin MG (2011) Effects of temperature on cellular and biochemical parameters in the crab *Carcinus aestuarii* (Crustacea, Decapoda). *Marine Environmental Research*, **71**, 351–356.

Maure F, Brodeur J, Ponlet N, Doyon J, Firlej A, Elguero E, Thomas F (2011) The cost of a bodyguard. *Biology Letters*, **7**, 843–846.

Maure F, Brodeur J, Hughes DP, Thomas F (2013) How much energy should manipulative parasites leave to their hosts to ensure altered behaviours? *The Journal of Experimental Biology*, **216**, 43–46.

Mayer G, Maas A, Waloszek D (2012) Mouthpart morphology of three sympatric native and nonnative gammaridean species: *Gammarus pulex*, *G. fossarum*, and *Echinogammarus berilloni* (Crustacea: Amphipoda). *International Journal of Zoology*, **2012**, 1–23.

Maynard BJ, DeMartini L, Wright WG (1996) *Gammarus lacustris* harboring *Polymorphus paradoxus* show altered patterns of serotonin-like immunoreactivity. *The Journal of Parasitology*, **82**, 663–666.

Maynard BJ, Wellnitz TA, Zanini N, Wright WG, Dezfuli BS (1998) Parasite-altered behavior in a crustacean intermediate host: field and laboratory studies. *The Journal of Parasitology*, **84**, 1102–1106.

McAuliffe K (2016) *This is your brain on parasites: How tiny creatures manipulate our behavior and shape society* (ed Houghton Mifflin Harcourt).

McCahon CP, Pascoe D (1988) Increased sensitivity to cadmium of the freshwater amphipod *Gammarus pulex* (L.) during the reproductive period. *Aquatic Toxicology*, **13**, 183–193.

McCahon CP, Brown AF, Pascoe D (1988) The effect of the acanthocephalan *Pomphorhynchus laevis* (Müller 1776) on the acute toxicity of cadmium to its intermediate host, the amphipod *Gammarus pulex* (L.). *Archives of Environmental Contamination and Toxicology*, **17**, 239–243.

McCahon CP, Maund SJ, Poulton MJ (1991) The effect of the acanthocephalan parasite (*Pomphorhynchus laevis*) on the drift of its intermediate host (*Gammarus pulex*). *Freshwater Biology*, **25**, 507–513.

McCallum H, Dobson AP (1995) Detecting disease and parasite threats to endangered species and ecosystems. *Trends in Ecology and Evolution*, **10**, 190–194.

McCurdy DG, Forbes MR, Boates JS (1999) Evidence that the parasitic nematode *Skrjabinoclava* manipulates host *Corophium* behavior to increase transmission to the sandpiper, *Calidris pusilla*. *Behavioral Ecology*, **10**, 351–357.

Médoc V, Beisel J-N (2011) When trophically-transmitted parasites combine predation enhancement with predation suppression to optimize their transmission. *Oikos*, **120**, 1452–1458.

Médoc V, Bollache L, Beisel J-N (2006) Host manipulation of a freshwater crustacean (*Gammarus roeseli*) by an acanthocephalan parasite (*Polymorphus minutus*) in a biological invasion context. *International Journal for Parasitology*, **36**, 1351–1358.

Médoc V, Rigaud T, Bollache L, Beisel J-N (2009) A manipulative parasite increasing an antipredator

response decreases its vulnerability to a nonhost predator. *Animal Behaviour*, **77**, 1235–1241.

Médoc V, Rigaud T, Motreuil S, Perrot-Minnot M-J, Bollache L (2011a) Paratenic hosts as regular transmission route in the acanthocephalan *Pomphorhynchus laevis*: potential implications for food webs. *Die Naturwissenschaften*, **98**, 825–835.

Médoc V, Piscart C, Maazouzi C, Simon L, Beisel J-N (2011b) Parasite-induced changes in the diet of a freshwater amphipod: field and laboratory evidence. *Parasitology*, **138**, 537–546.

Médoc V, Albert H, Spataro T (2015) Functional response comparisons among freshwater amphipods: ratio-dependence and higher predation for *Gammarus pulex* compared to the non-natives *Dikerogammarus villosus* and *Echinogammarus berilloni*. *Biological Invasions*, **17**, 3625–3637.

Mikheev VN, Pasternak AF, Taskinen J, Valtonen ET (2010) Parasite-induced aggression and impaired contest ability in a fish host. *Parasites & Vectors*, **3**, 17.

Mills LS, Soulé ME, Doak DF (1993) The keystone-species concept in ecology and conservation. *BioScience*, **43**, 219–224.

Miura O, Chiba S (2007) Effects of trematode double infection on the shell size and distribution of snail hosts. *Parasitology International*, **56**, 19–22.

Miura O, Kuris AM, Torchin ME, Hechinger RF, Chiba S (2006) Parasites alter host phenotype and may create a new ecological niche for snail hosts. *Proceedings. Biological sciences / The Royal Society*, **273**, 1323–1328.

Moe SJ, De Schamphelaere K, Clements WH, Sorensen MT, Van den Brink PJ, Liess M (2013) Combined and interactive effects of global climate change and toxicants on populations and communities. *Environmental Toxicology and Chemistry*, **32**, 49–61.

Moenickes S, Schneider A-K, Mühle L, Rohe L, Richter O, Suhling F (2011) From population-level effects to individual response: modelling temperature dependence in *Gammarus pulex*. *Journal of Experimental Biology*, **214**, 3678–3687.

Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment*, **6**, 485–492.

Moore J (1983) Responses of an avian predator and its isopod prey to an acanthocephalan parasite. *Ecology*, **64**, 1000–1015.

Moore J (1984) Altered behavioral responses in intermediate hosts - an acanthocephalan parasite strategy. *The American Naturalist*, **123**, 572–577.

Moore J (1995) The behavior of parasitized animals. *Bioscience*, **45**, 89–96.

Moore J (2002a) Parasites and host behaviour. *Trends in Ecology & Evolution*, **17**, 585–586.

Moore J (2002b) *Parasites and the behavior of animals*. Oxford University Press.

Moore J, Gotelli NJ (1990) A phylogenetic perspective on the evolution of altered host behaviours: a critical look at the manipulation hypothesis. In: *Parasitism and host behaviour* (eds Barnard CJ, Behnke JM), pp. 193–233. Taylor & Francis, London.

Moore S, Shrestha S, Tomlinson KW, Vuong H (2012) Predicting the effect of climate change on African trypanosomiasis: integrating epidemiology with parasite and vector biology. *Journal of The Royal Society Interface*, **9**, 817–830.

Mordecai EA, Paaijmans KP, Johnson LR et al. (2013) Optimal temperature for malaria transmission is dramatically lower than previously predicted. *Ecology Letters*, **16**, 22–30.

Moret Y, Schmid-Hempel P (2009) Immune responses of bumblebee workers as a function of individual and colony age: Senescence versus plastic adjustment of the immune function. *Oikos*, **118**, 371–378.

Morley NJ (2011) Thermodynamics of cercarial survival and metabolism in a changing climate. *Parasitology*, **138**, 1442–1452.

Morley NJ, Lewis JW (2014) Temperature stress and parasitism of endothermic hosts under climate change. *Trends in Parasitology*, **30**, 221–227.

Moss BR (2010) *Ecology of Freshwaters: a view for the twenty-first century, 4th Edition*. Wiley-Blackwell.

Le Moullac G, Haffner P (2000) Environmental factors affecting immune responses in Crustacea. *Aquaculture*, **191**, 121–131.

Mouritsen KN, Jensen KT (1997) Parasite transmission between soft-bottom invertebrates: Temperature mediated infection rates and mortality in *Corophium volutator*. *Marine Ecology Progress Series*, **151**, 123–134.

Mouritsen KN, Jensen T (2006) The effect of *Sacculina carcini* infections on the fouling, burying behaviour and condition of the shore crab, *Carcinus maenas*. *Marine Biology Research*, **2**, 270–275.

Mouritsen KN, Poulin R (2003) Parasite-induced trophic facilitation exploited by a non-host predator: a manipulator's nightmare. *International Journal for Parasitology*, **33**, 1043–1050.

Mouritsen KN, Poulin R (2005) Parasites boosts biodiversity and changes animal community structure by trait-mediated indirect effects. *Oikos*, **108**, 344–350.

Mouritsen KN, Tompkins DM, Poulin R (2005) Climate warming may cause a parasite-induced collapse in coastal amphipod populations. *Oecologia*, **146**, 476–483.

Muzzall PM, Rabalais FC (1975) Studies on *Acanthocephalus jacksoni* Bullock, 1962 (Acanthocephala: Echinorhynchidae). I. Seasonal Periodicity and New Host Records. *Proceedings of the Helminthological Society of Washington*, **42**, 31–34.

Nakagawa S, Cuthill IC (2007) Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological reviews of the Cambridge Philosophical Society*, **82**, 591–605.

Nesheim MC, Crompton DWT, Arnold S, Barnard D (1977) Dietary relations between *Moniliformis* (Acanthocephala) and laboratory rats. *Proceedings of the Royal Society B: Biological Sciences*, **197**, 363–383.

Ness JH, Foster SA (1999) Parasite-associated phenotype modifications in threespine stickleback.

Oikos, **85**, 127–134.

Noguchi K, Gel YR, Brunner E, Konietschke F (2012) nparLD: an R software package for the nonparametric analysis of longitudinal data in factorial experiments. *Journal of Statistical Software*, **50**, 1–23.

O'Brien JJ, van Wyk P (1985) Effects of crustacean parasitic castrators (epicaridean isopods and rhizocephalan barnacles) on growth of crustacean hosts. In: *Crustacean issues 3. Factors in adult growth* (ed Wenner AM), pp. 191–218. Balkema, Rotterdam.

Okaka CE (1989) Studies on the development of the oncosphere and procercoid of *Cyathocephalus truncatus* (Cestoda) in the intermediate host, *Gammarus pulex*. *Zoologica Scripta*, **18**, 205–209.

Olson RE, Pratt I (1971) The life cycle and larval development of *Echinorhynchus lageniformis* Ekbaum, 1938 (Acanthocephala: Echinorhynchidae). *The Journal of Parasitology*, **57**, 143–149.

Øverli Ø, Páll M, Borg B, Jobling M, Winberg S (2001) Effects of *Schistocephalus solidus* infection on brain monoaminergic activity in female three-spined sticklebacks *Gasterosteus aculeatus*. *Proceedings of the Royal Society B-Biological Sciences*, **268**, 1411–1415.

Paaijmans KP, Blanford S, Bell AS, Blanford JI, Read AF, Thomas MB (2010) Influence of climate on malaria transmission depends on daily temperature variation. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 15135–15139.

Pan LQ, Hu FW, Jing FT, Liu HJ (2008) The effect of different acclimation temperatures on the prophenoloxidase system and other defence parameters in *Litopenaeus vannamei*. *Fish and Shellfish Immunology*, **25**, 137–142.

Pandey A, Habibulla M (1982) Circadian rhythms of serotonin and the electrical activity of the frontal ganglion of the cockroach, *Periplaneta americana*. *Experientia*, **38**, 946–948.

Parker GA, Chubb JC, Ball MA, Roberts GN (2003) Evolution of complex life cycles in helminth parasites. *Nature*, **425**, 480–484.

Parker GA, Ball MA, Chubb JC (2015a) Evolution of complex life cycles in trophically transmitted helminths. I. Host incorporation and trophic ascent. *Journal of Evolutionary Biology*, **28**, 267–291.

Parker GA, Ball MA, Chubb JC (2015b) Evolution of complex life cycles in trophically transmitted helminths. II. How do life-history stages adapt to their hosts? *Journal of Evolutionary Biology*, **28**, 292–304.

Parshad VR, Crompton DWT (1982) Aspects of acanthocephalan reproduction. *Advances in Parasitology*, **19**, 73–138.

Parshad VR, Guraya SS (1977) Morphological and histochemical observations on the ovarian balls of *Centrorhynchus corvi* (Acanthocephala). *Parasitology*, **74**, 243–253.

Parshad VR, Crompton DWT, Nesheim MC (1980) The growth of *Moniliformis* (Acanthocephala) in rats fed on various monosaccharides and disaccharides. *Proceedings of the Royal Society B: Biological Sciences*, **209**, 299–315.

Pascoe D, Kedwards TJ, Blockwell SJ, Taylor EJ (1995) *Gammarus pulex* (L.) feeding bioassay - effects of

parasitism. *Bulletin of Environmental Contamination and Toxicology*, **55**, 629–632.

Pascual C, Sánchez A, Sánchez A, Vargas-Albores F, LeMoullac G, Rosas C (2003) Haemolymph metabolic variables and immune response in *Litopenaeus setiferus* adult males: the effect of an extreme temperature. *Aquaculture*, **218**, 637–650.

Pellan L, Médoc V, Renault D, Spataro T, Piscart C (2015) Feeding choice and predation pressure of two invasive gammarids, *Gammarus tigrinus* and *Dikerogammarus villosus*, under increasing temperature. *Hydrobiologia*, in press.

Penttinen OP, Holopainen IJ (1992) Seasonal feeding activity and ontogenetic dietary shifts in crucian carp, *Carassius carassius*. *Environmental Biology of Fishes*, **33**, 215–221.

Perrot-Minnot M-J (2004) Larval morphology, genetic divergence, and contrasting levels of host manipulation between forms of *Pomphorhynchus laevis* (Acanthocephala). *International Journal for Parasitology*, **34**, 45–54.

Perrot-Minnot M-J, Cézilly F (2013) Investigating candidate neuromodulatory systems underlying parasitic manipulation: concepts, limitations and prospects. *Journal of Experimental Biology*, **216**, 134–141.

Perrot-Minnot M-J, Kaldonski N, Cézilly F (2007) Increased susceptibility to predation and altered anti-predator behaviour in an acanthocephalan-infected amphipod. *International Journal for Parasitology*, **37**, 645–651.

Perrot-Minnot M-J, Gaillard M, Dodet R, Cézilly F (2011) Interspecific differences in carotenoid content and sensitivity to UVB radiation in three acanthocephalan parasites exploiting a common intermediate host. *International Journal for Parasitology*, **41**, 173–81.

Perrot-Minnot M-J, Maddaleno M, Balourdet A, Cézilly F (2012) Host manipulation revisited: no evidence for a causal link between altered photophobia and increased trophic transmission of amphipods infected with acanthocephalans. *Functional Ecology*, **26**, 1007–1014.

Perrot-Minnot M-J, Sanchez-Thirion K, Cézilly F (2014) Multidimensionality in host manipulation mimicked by serotonin injection. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20141915.

Perrot-Minnot M-J, Maddaleno M, Cézilly F (2015) Parasite-induced inversion of geotaxis in a freshwater amphipod: a role for anaerobic metabolism? *Functional Ecology*, **30**, 780–788.

Pincebourde S, Sanford E, Helmuth B (2008) Body temperature during low tide alters the feeding performance of a top intertidal predator. *Limnology and Oceanography*, **53**, 1562–1573.

Piscart C, Genoel R, Doledec S, Chauvet E, Marmonier P (2009) Effects of intense agricultural practices on heterotrophic processes in streams. *Environmental Pollution*, **157**, 1011–1018.

Piscart C, Bergerot B, Laffaille P, Marmonier P (2010) Are amphipod invaders a threat to regional biodiversity? *Biological Invasions*, **12**, 853–863.

Piscart C, Roussel J-M, Dick JTA, Grosbois G, Marmonier P (2011) Effects of coexistence on habitat use and trophic ecology of interacting native and invasive amphipods. *Freshwater Biology*, **56**, 325–334.

Plaistow SJ, Troussard J-P, Cézilly F (2001) The effect of the acanthocephalan parasite *Pomphorhynchus laevis* on the lipid and glycogen content of its intermediate host *Gammarus pulex*. *International Journal for Parasitology*, **31**, 346–351.

Pöckl M, Humpesch UH (1990) Intra- and inter-specific variations in egg survival and brood development time for Austrian populations of *Gammarus fossarum* and *G. roeseli* (Crustacea: Amphipoda). *Freshwater Biology*, **23**, 441–455.

Pöckl M, Webb BW, Sutcliffe DW (2003) Life history and reproductive capacity of *Gammarus fossarum* and *G. roeseli* (Crustacea: Amphipoda) under naturally fluctuating water temperatures: a simulation study. *Freshwater Biology*, **48**, 53–66.

Policar T, Stejskal V, Kristan J, Podhorec P, Svinger V, Blaha M (2012) The effect of fish size and stocking density on the weaning success of pond-cultured pikeperch *Sander lucioperca* L. juveniles. *Aquaculture International*, **21**, 869–882.

Ponton F, Biron DG, Joly C, Helluy S, Duneau D, Thomas F (2005) Ecology of parasitically modified populations: a case study from a gammarid-trematode system. *Marine Ecology Progress Series*, **299**, 205–215.

Ponton F, Lefèvre T, Lebarbenchon C et al. (2006) Do distantly related parasites rely on the same proximate factors to alter the behaviour of their hosts? *Proceedings of the Royal Society B: Biological Sciences*, **273**, 2869–2877.

Poulin R (1993) Age-dependent effects of parasites on anti-predator responses in two New Zealand freshwater fish. *Oecologia*, **96**, 431–438.

Poulin R (1995) "Adaptive" changes in the behaviour of parasitized animals: a critical review. *International Journal for Parasitology*, **25**, 1371–1383.

Poulin R (2003) Information about transmission opportunities triggers a life-history switch in a parasite. *Evolution*, **57**, 2899–2903.

Poulin R (2006) Variation in infection parameters among populations within parasite species: intrinsic properties versus local factors. *International Journal for Parasitology*, **36**, 877–885.

Poulin R (2010) Parasite manipulation of host behavior: an update and frequently asked questions. *Advances in the Study of Behavior*, **41**, 151–186.

Poulin R, Morand S (2000) The diversity of parasites. *The Quarterly Review of Biology*, **75**, 277–293.

Poulin R, Mouritsen KN (2006) Climate change, parasitism and the structure of intertidal ecosystems. *Journal of Helminthology*, **80**, 183–191.

Poulin R, Thomas F (1999) Phenotypic variability induced by parasites: extent and evolutionary implications. *Parasitology Today*, **15**, 28–32.

Poulin R, Wise M, Moore J (2003) A comparative analysis of adult body size and its correlates in acanthocephalan parasites. *International Journal for Parasitology*, **33**, 799–805.

Poulin R, Blasco-Costa I, Randhawa HS (2014) Integrating parasitology and marine ecology: Seven challenges towards greater synergy. *Journal of Sea Research*, **113**, 3–10.

Prenter J, MacNeil C, Dick JTA, Dunn AM (2004) Roles of parasites in animal invasions. *Trends in Ecology and Evolution*, **19**, 385–390.

Pulkkinen K, Ebert D (2004) Host starvation decreases parasite load and mean host size in experimental populations. *Ecology*, **85**, 823–833.

Raffel TR, Rohr JR, Kiesecker JM, Hudson PJ (2006) Negative effects of changing temperature on amphibian immunity under field conditions. *Functional Ecology*, **20**, 819–828.

Reisinger LS, Petersen I, Hing JS, Davila RL, Lodge DM (2015) Infection with a trematode parasite differentially alters competitive interactions and antipredator behaviour in native and invasive crayfish. *Freshwater Biology*, **60**, 1581–1595.

Rigaud T, Moret Y (2003) Differential phenoloxidase activity between native and invasive gammarids infected by local acanthocephalans: differential immunosuppression? *Parasitology*, **127**, 571–577.

Rosenblatt AE, Schmitz OJ (2014) Interactive effects of multiple climate change variables on trophic interactions: a meta-analysis. *Climate Change Reponses*, **1**, 1–10.

Rosi-marshall EJ, Wallace JB (2002) Invertebrate food webs along a stream resource gradient. *Freshwater Biology*, **47**, 129–141.

Roux C, Roux AL (1967) Température et métabolisme respiratoire d'espèces sympatriques de gammares du groupe pulex (Crustacés, Amphipodes). *Annales de Limnologie*, **3**, 3–16.

Rowell DP (2005) A scenario of European climate change for the late twenty-first century: Seasonal means and interannual variability. *Climate Dynamics*, **25**, 837–849.

Rumpus AE, Kennedy CR (1974) The effect of the acanthocephalan *Pomphorhynchus laevis* upon the respiration of its intermediate host, *Gammarus pulex*. *Parasitology*, **68**, 271–284.

Sadd BM, Siva-Jothy MT (2006) Self-harm caused by an insect's innate immunity. *Proceedings. Biological sciences / The Royal Society*, **273**, 2571–2574.

Salminen J, Tan Anh B, van Gestel CAM (2001) Indirect effects of zinc on soil microbes via a keystone enchytraeid species. *Environmental Toxicology and Chemistry*, **20**, 1167–1174.

Sánchez MI, Georgiev BB, Green AJ (2007) Avian cestodes affect the behaviour of their intermediate host *Artemia parthenogenetica*: an experimental study. *Behavioural Processes*, **74**, 293–299.

Sánchez MI, Ponton F, Schmidt-Rhaesa A, Hughes DP, Misse D, Thomas F (2008) Two steps to suicide in crickets harbouring hairworms. *Animal Behaviour*, **76**, 1621–1624.

Sanford E (1999) Regulation of keystone predation by small changes in ocean temperature. *Science*, **283**, 2095–2097.

Sargent LW, Baldridge AK, Vega-Ross M, Towle KM, Lodge DM (2014) A trematode parasite alters growth, feeding behavior, and demographic success of invasive rusty crayfish (*Orconectes rusticus*). *Oecologia*, **175**, 947–958.

Sato T, Watanabe K, Kanaiwa M, Niizuma Y, Harada Y, Lafferty KD (2011) Nematomorph parasites drive energy flow through a riparian ecosystem. *Ecology*, **92**, 201–207.

Sato T, Egusa T, Fukushima K et al. (2012) Nematomorph parasites indirectly alter the food web and ecosystem function of streams through behavioural manipulation of their cricket hosts. *Ecology Letters*, **15**, 786–793.

Scheef G, Sures B, Taraschewski H (2000) Cadmium accumulation in *Moniliformis moniliformis* (Acanthocephala) from experimentally infected rats. *Parasitology Research*, **86**, 688–691.

Schmidlin L, von Fumetti S, Nagel P (2015a) Temperature effects on the feeding and electron transport system (ETS) activity of *Gammarus fossarum*. *Aquatic Ecology*, **49**, 71–80.

Schmidlin L, von Fumetti S, Nagel P (2015b) Effects of increased temperatures on *Gammarus fossarum* under the influence of copper sulphate. *Ecotoxicology*, **24**, 433–444.

Schmidt GD (1971) Acanthocephalan infections of man, with two new records. *The Journal of Parasitology*, **57**, 582–584.

Schoebel CN, Tellenbach C, Spaak P, Wolinska J (2011) Temperature effects on parasite prevalence in a natural hybrid complex. *Biology Letters*, **7**, 108–11.

Scott GR, Sloman KA (2004) The effects of environmental pollutants on complex fish behaviour: Integrating behavioural and physiological indicators of toxicity. *Aquatic Toxicology*, **68**, 369–392.

Seppälä O, Karvonen A, Tellervo Valtonen E (2004) Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke-fish interaction. *Animal Behaviour*, **68**, 257–263.

Seppälä O, Valtonen ET, Benesh DP (2008a) Host manipulation by parasites in the world of dead-end predators: adaptation to enhance transmission? *Proceedings of the Royal Society B: Biological Sciences*, **275**, 1611–1615.

Seppälä O, Liljeroos K, Karvonen A, Jokela J (2008b) Host condition as a constraint for parasite reproduction. *Oikos*, **117**, 749–753.

Shaw JC, Øverli Ø (2012) Brain-encysting trematodes and altered monoamine activity in naturally infected killifish *Fundulus parvipinnis*. *Journal of Fish Biology*, **81**, 2213–2222.

Shaw JC, Korzan WJ, Carpenter RE, Kuris AM, Lafferty KD, Summers CH, Øverli Ø (2009) Parasite manipulation of brain monoamines in California killifish (*Fundulus parvipinnis*) by the trematode *Euhaplorchis californiensis*. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 1137–1146.

Sheath DJ, Andreou D, Britton JR (2016) Interactions of warming and exposure affect susceptibility to parasite infection in a temperate fish species. *Parasitology*, **143**, 1340–1346.

Shik JZ, Kaspari M, Yanoviak SP (2011) Preliminary assessment of metabolic costs of the nematode *Myrmeconema neotropicum* on its host, the tropical ant *Cephalotes atratus*. *Journal of Parasitology*, **97**, 958–959.

von Siebold CT (1853) Ueber *Leukochloridium paradoxum*. *Zeitschrift für wissenschaftliche Zoologie*, **4**, 425–437.

Sielaff M, Schmidt H, Struck TH, Rosenkranz D, Mark Welch DB, Hankeln T, Herlyn H (2016) Phylogeny of Syndermata (syn. Rotifera): Mitochondrial gene order verifies epizoic Seisonidea as sister to

endoparasitic Acanthocephala within monophyletic Hemirotifera. *Molecular Phylogenetics and Evolution*, **96**, 79–92.

Siva-Jothy MT, Moret Y, Rolff J (2005) Insect immunity: an evolutionary ecology perspective. *Advances in Insect Physiology*, **32**, 1–48.

Söderhäll K, Cerenius L (1992) Crustacean immunity. *Annual Review of Fish Diseases*, **2**, 3–23.

Söderhäll K, Cerenius L (1998) Role of the prophenoloxidase-activating system in invertebrate immunity. *Current Opinion in Immunology*, **10**, 23–28.

Sornom P, Felten V, Médoc V, Sroda S, Rousselle P, Beisel J-N (2010) Effect of gender on physiological and behavioural responses of *Gammarus roeseli* (Crustacea Amphipoda) to salinity and temperature. *Environmental Pollution*, **158**, 1288–1295.

Sparkes TC, Weil KA, Renwick DT, Talkington JA (2006) Development-related effects of an acanthocephalan parasite on pairing success of its intermediate host. *Animal Behaviour*, **71**, 439–448.

Spencer HG, Zuk M (2016) For host's sake: the pluses of parasite preservation. *Trends in Ecology & Evolution*, **31**, 341–343.

Stefano GB, Catapane EJ (1977) The effects of temperature acclimation on monoamine metabolism. *The Journal of Pharmacology and Experimental Therapeutics*, **203**, 449–456.

Stefano GB, Hirtpi L, Catapane EJ (1977) The effects of short and long term temperature stress on serotonin, dopamine and norepinephrine concentrations in molluscan ganglia. *Journal of Thermal Biology*, **3**, 79–83.

Steinauer ML, Nickol BB (2003) Effect of cystacanth body size on adult success. *The Journal of Parasitology*, **89**, 251–254.

Stensgaard AS, Utzinger J, Vounatsou P et al. (2013) Large-scale determinants of intestinal schistosomiasis and intermediate host snail distribution across Africa: Does climate matter? *Acta Tropica*, **128**, 378–390.

Sternberg ED, Thomas MB (2014) Local adaptation to temperature and the implications for vector-borne diseases. *Trends in Parasitology*, **30**, 115–122.

Stone CF, Moore J (2014) Parasite-induced alteration of odour responses in an amphipod-acanthocephalan system. *International Journal for Parasitology*, **44**, 969–975.

Studer A, Poulin R (2013) Differential effects of temperature variability on the transmission of a marine parasite. *Marine Biology*, **160**, 2763–2773.

Sures B, Taraschewski H (1995) Cadmium concentrations in two adult acanthocephalans, *Pomphorhynchus laevis* and *Acanthocephalus lucii*, as compared with their fish hosts and cadmium and lead levels in larvae of *A. lucii* as compared with their crustacean host. *Parasitology Research*, **81**, 494–497.

Sures B, Siddall R, Taraschewski H (1999) Parasites as accumulation indicators of heavy metal pollution. *Parasitology Today*, **15**, 16–21.

Sutcliffe DW (1992) Reproduction in *Gammarus* (Crustacea, Amphipoda): basic processes. *Freshwater Forum*, **2**, 102–128.

Tada I, Otsuji Y, Kamiya H, Mimori T, Sakaguchi Y, Makizumi S (1983) The first case of a Human infected with an Acanthocephalan parasite, *Bolbosoma sp*. *The Journal of Parasitology*, **69**, 205–208.

Tain L, Perrot-Minnot M-J, Cézilly F (2006) Altered host behaviour and brain serotonergic activity caused by acanthocephalans: evidence for specificity. *Proceedings. Biological sciences / The Royal Society*, **273**, 3039–45.

Tain L, Perrot-Minnot M-J, Cézilly F (2007) Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on brain serotonergic activity in two congeneric host species. *Biology Letters*, **3**, 68–71.

Taraschewski H (2000) Host-parasite interactions in Acanthocephala: a morphological approach. *Advances in Parasitology*, **46**, 1–179.

Tedla S, Fernando CH (1970) Some remarks on the ecology of *Echinorhynchus salmonis* Muller 1784. *Canadian Journal of Zoology*, **48**, 137–132.

Thomas MB, Blanford S (2003) Thermal biology in insect-parasite interactions. *Trends in Ecology and Evolution*, **18**, 344–350.

Thomas F, Poulin R (1998) Manipulation of a mollusc by a trophically transmitted parasite: convergent evolution or phylogenetic inheritance? *Parasitology*, **116**, 431–436.

Thomas F, Renaud F, Rousset F, Cézilly F, De Meeûs T (1995) Differential mortality of two closely related host species induced by one parasite. *Proceedings of the Royal Society B: Biological Sciences*, **260**, 349–352.

Thomas F, Renaud F, De Meeûs T, Poulin R (1998) Manipulation of host behaviour by parasites: ecosystem engineering in the intertidal zone? *Proceedings of the Royal Society B: Biological Sciences*, **265**, 1091–1096.

Thomas F, Poulin R, De Meeûs T, Guégan J, Renaud F (1999) Parasites and ecosystem engineering: what roles could they play? *Oikos*, **84**, 167–171.

Thomas F, Poulin R, Guégan J, Michalakis Y, Renaud F (2000a) Are there pros as well as cons to being parasitized? *Parasitology Today*, **16**, 533–536.

Thomas F, Gudner E, Renaud F (2000b) Differential parasite (Trematoda) encapsulation in *Gammarus aequicauda* (Amphipoda). *The Journal of parasitology*, **86**, 650–654.

Thomas F, Brown SP, Sukhdeo MVK, Renaud F (2002a) Understanding parasite strategies: a state-dependent approach? *Trends in Parasitology*, **18**, 387–390.

Thomas F, Fauchier J, Lafferty KD (2002b) Conflict of interest between a nematode and a trematode in an amphipod host: test of the “sabotage” hypothesis. *Behavioral Ecology and Sociobiology*, **51**, 296–301.

Thomas F, Renaud F, Guégan J (2005a) *Parasitism and Ecosystems*. Oxford University Press.

Thomas F, Adamo SA, Moore J (2005b) Parasitic manipulation: where are we and where should we go?

Behavioural Processes, **68**, 185–199.

Thomas F, Brodeur J, Maure F, Franceschi N, Blanchet S, Rigaud T (2011) Intraspecific variability in host manipulation by parasites. *Infection, Genetics and Evolution*, **11**, 262–269.

Thünken T, Baldauf SA, Bersau N, Bakker TCM, Kullmann H, Frommen JG (2010) Impact of olfactory non-host predator cues on aggregation behaviour and activity in *Polymorphus minutus* infected *Gammarus pulex*. *Hydrobiologia*, **654**, 137–145.

Tierney AJ, Atema J (1986) Effects of acidification on the behavioral response of crayfishes (*Orconectes virilis* and *Procambarus acutus*) to chemical stimuli. *Aquatic Toxicology*, **9**, 1–11.

Tokeson JPE, Holmes JC (1982) The effects of temperature and oxygen on the development of *Polymorphus marilis* (Acanthocephala) in *Gammarus lacustris* (Amphipoda). *Journal of Parasitology*, **68**, 112–119.

Tompkins DM, Veltman CJ (2015) Behaviour-manipulating parasites as adjuncts to vertebrate pest control. *Ecological Modelling*, **302**, 1–8.

Torchin ME, Mitchell CE (2004) Parasites, pathogens, and invasions by plants and animals. *Frontiers in Ecology and the Environment*, **2**, 183–190.

Torchin ME, Lafferty KD, Kuris AM (2002) Parasites and marine invasions. *Parasitology*, **124**, S137–S151.

Torchin ME, Byers JE, Huspeni TC (2005) Differential parasitism of native and introduced snails: Replacement of a parasite fauna. *Biological Invasions*, **7**, 885–894.

Tylianakis JM, Didham RK, Bascompte J, Wardle DA (2008) Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, **11**, 1351–1363.

VanCleave HJ (1916) Seasonal distribution of some acanthocephala from fresh-water hosts. *The Journal of Parasitology*, **2**, 106–110.

Vazquez L, Alpuche J, Maldonado G, Agundis C, Pereyra-Morales A, Zenteno E (2009) Immunity mechanisms in crustaceans. *Innate Immunity*, **15**, 179–188.

Voigt MOC (1991) Community structure of the helminth parasite fauna of gammarids (Crustacea: Amphipoda) in Kiel bay, western Baltic Sea. *Meeresforschung*, **33**, 266–274.

Vyas A, Kim S-K, Giacomini N, Boothroyd JC, Sapolsky RM (2007) Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 6442–6447.

Walther GR, Roques A, Hulme PE et al. (2009) Alien species in a warmer world: risks and opportunities. *Trends in Ecology and Evolution*, **24**, 686–693.

Weber M, Wey-Fabrizius AR, Podsiadlowski L et al. (2013) Phylogenetic analyses of endoparasitic Acanthocephala based on mitochondrial genomes suggest secondary loss of sensory organs. *Molecular Phylogenetics and Evolution*, **66**, 182–189.

Webster JP (1994) The effect of *Toxoplasma gondii* and other parasites on activity levels in wild and hybrid *Rattus norvegicus*. *Parasitology*, **109**, 583–589.

Webster JP, Brunton CFA, Macdonald DW (1994) Effect of *Toxoplasma gondii* upon neophobic behaviour in wild brown rats, *Rattus norvegicus*. *Parasitology*, **109**, 37–43.

Wellnitz TA, Giari L, Maynard BJ, Dezfuli BS (2003) A parasite spatially structures its host population. *Oikos*, **100**, 263–268.

Werren JH, Baldo L, Clark ME (2008) Wolbachia: master manipulators of invertebrate biology. *Nature Reviews. Microbiology*, **6**, 741–751.

Westram AM, Baumgartner C, Keller I, Jokela J (2011) Are cryptic host species also cryptic to parasites? Host specificity and geographical distribution of acanthocephalan parasites infecting freshwater *Gammarus*. *Infection, Genetics and Evolution*, **11**, 1083–1090.

White N, Sutherst RW, Hall N, Whish-wilson P (2003) The vulnerability of the Australian beef industry to impacts of the cattle tick (*Boophilus microplus*) under climate change. *Climatic Change*, **61**, 157–190.

Wood CL, Johnson PT (2015) A world without parasites: exploring the hidden ecology of infection. *Frontiers in Ecology and the Environment*, **13**, 425–434.

Zala SM, Penn DJ (2004) Abnormal behaviours induced by chemical pollution: A review of the evidence and new challenges. *Animal Behaviour*, **68**, 649–664.

Zamani A, Talebi A, Fathipour Y, Baniameri V (2006) Temperature-dependent functional response of two aphid parasitoids, *Aphidius colemani* and *Aphidius matricariae* (Hymenoptera: Aphidiidae), on the cotton aphid. *Journal of Pest Science*, **79**, 183–188.

Zohar S, Holmes JC (1998) Pairing success of male *Gammarus lacustris* infected by two acanthocephalans: a comparative study. *Behavioral Ecology*, **9**, 206–211.

Résumé étendu

Introduction

Selon certaines estimations, les parasites pourraient constituer la moitié de toute la biodiversité sur Terre. Ces organismes qui utilisent d'autres êtres vivants pour y puiser ressources et habitats sont très variés. En effet, le mode de vie parasitaire est apparu de nombreuses fois au cours de l'évolution dans tous les grands groupes du vivant, depuis les plus simples tels que certains virus et bactéries, jusqu'à des animaux complexes tels des helminthes ou crustacés. Beaucoup de parasites, qualifiés d'hétéroxènes, ont un cycle de vie qui inclut plusieurs espèces d'hôtes successives. Un tel cycle complexe s'accompagne obligatoirement d'une étape critique dans la vie du parasite : sa transmission entre les différents hôtes.

Chez de nombreux parasites, la transmission se fait de manière trophique. L'hôte intermédiaire, dans lequel le parasite effectue son développement larvaire, doit être mangé par l'hôte définitif, dans lequel le parasite se reproduit, pour que la transmission se fasse. De ce fait, le succès de la transmission du parasite dépend de la probabilité de prédation entre ses deux hôtes.

Cependant, de nombreuses espèces de parasites qualifiés de « manipulateurs » sont capables d'induire, chez leurs hôtes intermédiaires, des modifications de phénotype. Certains de ces changements, notamment dans l'apparence ou le comportement de leurs hôtes, peuvent directement affecter la probabilité de prédation de l'hôte intermédiaire par l'hôte définitif, et donc le succès de transmission du parasite. D'une part, les parasites ayant atteint le stade transmissible à l'hôte définitif peuvent altérer les stratégies anti-prédatrices de leurs hôtes, par exemple en supprimant leur aversion à l'odeur de prédateur ou en induisant des comportements qui les rendent plus repérables. En conséquence, les individus infectés subissent une plus forte prédation que les individus sains, illustrant la « facilitation trophique » induite par les parasites. D'autre part, un renforcement des comportements anti-prédateurs a été documenté chez des hôtes infectés par des parasites n'ayant pas encore atteint le stade transmissible, aboutissant à une « protection » de leur hôte pendant le développement larvaire du parasite.

Les parasites sont largement reconnus pour jouer de nombreux rôles dans les écosystèmes. De par leurs liens intriqués dans les réseaux trophiques, via l'altération des probabilités de prédation entres leurs hôtes, les parasites manipulateurs sont d'autant plus susceptibles d'avoir des impacts conséquents dans les écosystèmes. De fait, de nombreuses études aussi bien théoriques

qu'empiriques ont pu souligner l'impact écologique des parasites manipulateurs, capables notamment d'affecter les dynamiques des populations de leurs hôtes ainsi que, par répercussion, celles d'espèces qui leurs sont liées, ou encore de modifier les habitats et les réseaux trophiques.

A l'heure actuelle, les changements globaux liés à des causes anthropiques menacent la stabilité de nombreux écosystèmes, et il est devenu de première importance de comprendre et anticiper l'impact de tels changements sur les acteurs de ces écosystèmes. Une des étapes indispensables est de comprendre comment les variables environnementales, à la fois biotiques et abiotiques, peuvent influencer les espèces et leurs interactions. Considérant le rôle prépondérant des parasites manipulateurs au sein des écosystèmes, il est très probable que l'impact de changements environnementaux sur ces parasites et sur l'impact qu'ils ont sur leurs hôtes pourrait avoir des répercussions à large échelle. Cependant, l'interaction entre l'environnement et l'impact des parasites, notamment en termes de manipulation, a été jusqu'à présent peu étudié.

Le but de ma thèse est de combler cette lacune en apportant une meilleure compréhension de la façon dont l'environnement, essentiellement abiotique, peut affecter les parasites manipulateurs et l'impact qu'ils ont sur leurs hôtes.

Modèles biologiques

Les espèces de parasites considérées comme manipulatrices sont nombreuses et variées. Parmi les plus connues, les trématodes *Leucochloridium paradoxum* et *Dicrocoelium dendriticum* font figure d'exemples historiques. Dès le 19ème siècle, l'effet impressionnant de *L. paradoxum* sur les antennes de son hôte intermédiaire escargot a attiré l'attention des naturalistes de l'époque. En effet, il a été suggéré que la modification d'apparence résultante des antennes pouvait attirer les oiseaux, qui constituent eux-mêmes l'hôte définitif du parasite. Le trématode *D. dendriticum* est quant à lui connu pour induire un comportement aberrent chez son hôte fourmi, le poussant à escalader les brins d'herbe et augmentant par là sa probabilité de se faire manger par l'hôte définitif du parasite, des grands herbivores. D'autres parasites, tels que le protozoaire *Toxoplasma gondii* connu pour supprimer l'aversion innée des rats à l'odeur de chat et la transformer en attraction, sont également particulièrement documentés, notamment lorsqu'ils posent problème en santé humaine comme c'est le cas avec *T. gondii* qui provoque la toxoplasmose.

Les parasites acanthocéphales, un groupe monophylétique qui fait partie des syndermates (encore appelés rotifères), se sont cependant imposés comme un groupe modèle dans l'étude de la manipulation parasitaire, et ce dès les années 70 avec les travaux des scientifiques Bethel et Holmes,

en dépit de l'absence de menace qu'ils posent sur les humains ou les élevages. Avec un peu plus d'un millier d'espèces connues, ce groupe s'illustre par son uniformité. En effet, tous les acanthocéphales ont un cycle de vie relativement similaire. Leur hôte intermédiaire, généralement une espèce d'arthropode, s'infecte par consommation des œufs du parasite. La larve grandit ensuite, passant du stade acanthelle non infectieux au stade cystacanthe, infectieux pour l'hôte définitif. Plusieurs espèces animales vertébrées constituent des hôtes définitifs pour les acanthocéphales, dont la transmission de fait de manière trophique, c'est-à-dire par prédation de l'hôte intermédiaire par l'hôte définitif. Les parasites adultes se reproduisent ensuite de manière sexuée dans le tractus digestif de leur hôte, permettant aux œufs d'être libérés directement dans le milieu.

Les acanthocéphales présentent également une grande homogénéité dans leur morphologie, avec notamment la présence remarquable d'un proboscis rétractile couvert de crochets incurvés leur permettant de s'ancrer dans la paroi intestinale de leur hôte définitif. Cependant, le caractère le plus exceptionnel du groupe réside dans leur capacité à manipuler le comportement de leurs hôtes intermédiaires, particularité reconnue chez toutes les espèces étudiées et qui est de ce fait considérée comme un caractère d'origine ancestrale. Cependant, la manipulation reste variée au sein du groupe, avec des altérations de différents traits comportementaux des hôtes intermédiaires qui pourraient notamment être spécifiques à l'hôte définitif de l'espèce de parasite. Par exemple, des acanthocéphales de poissons au stade cystacanthe sont connus pour induire, chez leur hôte intermédiaire crustacé, une augmentation de l'attraction à la lumière sans modification de ses préférences géotactiques. L'inverse est observé chez un parasite d'oiseaux infectant le même hôte crustacé. De plus, plusieurs études ont également mis en évidence une altération inverse du comportement des hôtes intermédiaire au stade acanthelle, non infectieux pour l'hôte définitif, avec un renforcement des comportements anti-prédateurs de l'hôte aboutissant à une réduction des risques de prédation.

Un autre intérêt majeur à l'utilisation d'acanthocéphales comme modèles de la manipulation parasitaire réside dans leurs hôtes intermédiaires. En effet, parmi les nombreuses espèces pouvant abriter des acanthocéphales, les gammares constituent un groupe de crustacés amphipodes de grande importance écologique. Présents en grande densité dans une large gamme d'habitats aquatiques, ils sont notamment répandus dans les rivières européennes où ils jouent plusieurs rôles d'importance majeure. Tout d'abord, ils représentent une ressource alimentaire quantitativement importante pour les prédateurs aquatiques. Ensuite, les gammares constituent eux-mêmes des prédateurs pour de nombreux macro-invertébrés, avec une pression de prédation suffisamment importante pour moduler la composition des communautés d'invertébrés d'eau douce. Enfin, les gammares sont également

considérés comme des détritivores, jouant un rôle central dans la décomposition de la matière organique et donc dans le maintien de la qualité de l'eau.

Plusieurs espèces de parasites acanthocéphales ont pour hôte intermédiaire une ou plusieurs espèces de gammares. En conséquence, de nombreuses altérations ont déjà été documentées sur plusieurs traits. En termes de comportement, les acanthocéphales sont susceptibles d'induire des modifications variées chez leurs hôtes gammares, tels des altérations de phototaxie, de géotaxie, d'utilisation des refuges, d'agrégation, ou encore de réaction à l'odeur de prédateur. Les capacités reproductives du gammare sont également altérées, avec notamment une diminution de la fécondité des femelles ou du succès d'appariement des mâles. De nombreuses modifications d'ordre physiologique sont également répertoriées, avec des altérations du système immunitaire des gammares, de leurs réserves énergétiques, ou encore de leur alimentation.

Toutes ces altérations ne sont pas nécessairement la conséquence d'une manipulation, impliquant un bénéfice pour le parasite. Certaines modifications peuvent en effet simplement résulter d'un effet pathologique du parasite. Néanmoins, l'ensemble de ces changements provoque des modifications du rôle des gammares dans les écosystèmes. Bien que l'environnement, et notamment les paramètres abiotiques soumis à des modifications d'origine anthropique, soit susceptible d'affecter à la fois les parasites et les gammares, mais également l'impact des parasites sur leurs hôtes et donc sur leur rôle dans les écosystèmes, l'impact de l'environnement sur l'interaction entre acanthocéphales et gammares demeurent mal connu.

Au cours de ce travail, trois espèces de parasites acanthocéphales ont été utilisées dans des expériences visant à mieux comprendre cet impact : les parasites de poisson *Pomphorhynchus laevis* et *Pomphorhynchus tereticollis* et, dans une moindre mesure, le parasite d'oiseau *Polymorphus minutus*. Tandis que les deux premiers sont particulièrement connus pour altérer la phototaxie et l'utilisation de refuges des gammares, *P. minutus* provoque des modifications de leur géotaxie. Des infections expérimentales ont permis de contrôler les conditions abiotiques, notamment en termes de ressources disponibles et température, lors du développement des parasites dans les gammares. Des tests de comportements ont ensuite permis de mesurer les effets de telles variables sur la manipulation comportementale résultante. De plus, des gammares naturellement infectés ont également été utilisés pour mieux comprendre l'impact proximal de la température.

Impact des ressources

Les parasites puisent directement les ressources de leurs hôtes pour se développer. Bien que les mécanismes de la manipulation comportementale ne soient pas totalement élucidés, les comportements modifiés sont probablement coûteux à produire pour l'hôte, mais également à induire pour le parasite. De ce fait, la quantité et la qualité des ressources alimentaires disponibles pour l'hôte pourrait impacter l'interaction entre l'hôte et son parasite, et notamment en termes de manipulation. D'une part, un hôte disposant de ressources limitées pourrait ne plus être capable d'exprimer les comportements modifiés. Au contraire, le parasite pourrait adopter une stratégie visant à augmenter et/ou accélérer les modifications induites chez son hôte si celui-ci est en mauvaise condition corporelle, garantissant sa transmission avant la mort de son hôte.

Pour mieux comprendre l'impact des ressources alimentaires des gammares sur l'interaction qu'ils ont avec leurs parasites, notamment en termes de manipulation comportementale, une infection expérimentale a été utilisée. Des mâles *Gammarus pulex* ont été exposés à des œufs de parasites de l'espèce *P. laevis* provenant de deux populations différentes. A l'issu de cette exposition, les individus ont été répartis en deux traitements. La moitié des individus était nourrie avec un régime standard, composé de feuilles d'orme conditionnées et de larves de chironomes. L'autre partie des individus recevait un régime pauvre en protéines, ne comprenant pas les larves de chironomes. Les individus infectés, ainsi que des individus contrôles non exposés aux parasites, ont ainsi été maintenus durant tout le développement des parasites. La mortalité a été suivie tout le long de l'expérience.

Une fois le stade cystacanthe atteint, le comportement des gammares a été mesuré. D'une part, la proportion de temps passé sous un refuge a été mesurée à trois reprises pour chaque individu : un jour, 10 jours et 20 jours après le passage du parasite au stage cystacanthe. Pour ce faire, les gammares ont été individuellement placés dans des boîtes contenant un refuge à une extrémité, et la position de chaque individu (dans ou hors du refuge) a été relevée toutes les trois minutes pendant 90 minutes. D'autre part, le métabolisme des gammares a été estimé en mesurant leur consommation d'oxygène, 13 jours après la détection du stade cystacanthe, grâce à un dispositif non invasif de mesure de la concentration d'oxygène dans l'eau basé sur de la fluorescence. Au cours de chaque test, des individus contrôles, non infectés, ont également été utilisés. Tous les individus utilisés au cours de l'expérience ont été mesurés et disséqués. La charge parasitaire dans chaque gammare a été comptée, et la taille des parasites ayant atteint le stade cystacanthe a également été mesurée.

Au cours de cette expérience, les analyses statistiques montrent que le régime alimentaire des gammares n'a pas eu d'impact sur leur probabilité d'infection, mais que la charge parasitaire était réduite, pour une des deux populations de parasites, lorsque l'hôte était nourri avec un régime pauvre

en protéines. Une différence non significative était également constatée dans la taille des parasites, le régime standard permettant d'atteindre globalement des tailles plus importantes à charge parasitaire égale.

La survie des gammares était réduite lorsqu'ils étaient infectés par des parasites, mais également lorsqu'ils avaient un régime pauvre en protéines. Le métabolisme des gammares était également affecté par ces deux paramètres, avec une consommation d'oxygène plus importante pour les gammares infectés comparés aux gammares contrôles, ainsi qu'une diminution de la consommation d'oxygène pour les gammares nourris avec un régime pauvre en protéines.

En dépit des différences en termes de survie et de métabolisme liées au régime alimentaire, suggérant un effet du traitement sur la condition corporelle des gammares, aucun effet du régime alimentaire n'a pu être mis en évidence sur le comportement d'utilisation des refuges. Tandis que l'utilisation des refuges a augmenté au cours du temps pour les gammares non infectés, les gammares infectés présentaient une utilisation plus forte des refuges au premier test, qui a rapidement diminué au cours du temps.

Les résultats suggèrent que les ressources alimentaires de l'hôte n'ont pas d'impact sur l'intensité et le timing de la manipulation comportementale, quand bien même ils induisent des modifications notables de la condition corporelle de l'hôte. Contrairement à ce qui a été suggéré dans d'autres études, il est donc possible que le parasite ne puisse pas moduler la manipulation en réponse à une stratégie dépendant de la condition de l'hôte. Cependant, une certaine variabilité dans la manipulation a tout de même été notée dans plusieurs études, suggérant que d'autres paramètres pourraient moduler cette manipulation. En particulier, la température est connue pour impacter de nombreux traits de la relation entre les hôtes et leurs parasites, notamment chez les acanthocéphales, et pourrait donc également moduler l'intensité de la manipulation.

Impact de la température

La température constitue un paramètre majeur impactant les systèmes hôtes-parasites de multiples façons. En particulier, les parasites acanthocéphales se développement plus rapidement à haute température, et leur succès d'infection de leur hôte définitif dépend également de ce trait. En parallèle, la température affecte également les gammares, modifiant leur métabolisme, leur croissance ou encore leur activité. Cependant, l'impact de la température sur l'interaction entre les gammares et les acanthocéphales, notamment en termes de manipulation, reste à comprendre. Deux expériences ont de ce fait été conduites. La première, utilisant des gammares naturellement infectés,

vise à mieux comprendre l'effet à court terme de la température. La deuxième, utilisant des gammares infectés en laboratoire, permet de comprendre l'impact de la température ressentie par les gammares durant le développement des parasites. Une troisième expérience vise à explorer l'impact de la température sur le système immunitaire des gammares, un paramètre potentiellement important dans le succès d'infection des parasites.

Infection naturelle avec P. tereticollis et P. minutus

Pour comprendre l'effet proximal, à court terme, de la température sur la manipulation parasitaire, des gammares de l'espèce *Gammarus fossarum* naturellement infectés par deux espèces de parasites ont été utilisés. D'une part, des gammares contenant des cystacanthes du parasite de poisson *P. tereticollis* ont été collectés. Du fait de différences dans les comportements manipulés, des gammares contenant des cystacanthes du parasite d'oiseau *P. minutus* ont également été étudiés. Tous les traitements et tests ont été conduits en parallèle sur des individus contrôles, non infectés.

Après sélection des individus infectés ou sain par inspection visuelle (la présence de parasites, leur espèce ainsi que leur stade étant visibles par transparence), les gammares infectés *par P. tereticollis* et leurs contrôles ont été placés à trois températures différentes, respectivement 10, 14 et 18°C, tandis que ceux infectés par *P. minutus* et leur contrôles ont été divisés en deux groupes (14 et 18°C). Après 12 jours d'acclimatation, le comportement des individus a été testé aux mêmes températures.

Deux tests de comportements ont été menés pour les gammares infectés par *P. tereticollis* et leurs contrôles, correspondant aux comportements connus pour être altérés par le parasite. D'une part, la phototaxie des individus a été testée grâce à un dispositif comprenant des tubes en verre horizontaux dont une moitié était transparente et l'autre opaque. Après l'introduction d'un individu par tube, la position de chaque individu était relevée toutes les 30 secondes pendant cinq minutes, aboutissant à un score du temps passé à la lumière et à l'obscurité. De plus, la proportion de temps passée dans un refuge a également été mesurée dans un dispositif similaire à celui décrit plus haut. La position de chaque individu (dans ou hors du refuge) a été relevée toutes les deux minutes pendant 30 minutes.

Le parasite *P. minutus* est particulièrement connu pour affecter la géotaxie de son hôte gammare. De ce fait, ce paramètre a été mesuré par la position des gammares dans la colonne d'eau. Chaque gammare infecté ou sain était placé dans un cylindre vertical artificiellement divisé dans la hauteur en cinq zones égales. Un filet, disposé dans chaque cylindre, permettait aux gammares de

s'accrocher aux parois. La position de chaque individu dans la hauteur d'eau a été relevée toutes les 30 secondes pendant cinq minutes, aboutissant à un score d'autant plus élevé que les gammares passaient du temps près de la surface.

Les résultats de cette expérience mettent en avant un effet de la température sur les comportements des gammares en termes de phototaxie. En effet, une augmentation des scores de phototaxie, donc une augmentation de l'attraction à la lumière, a été mise en évidence à la fois chez les gammares sains et les gammares infectés par *P. tereticollis*. Cependant, tandis que le score continuait d'augmenter entre 14 et 18°C chez les individus infectés, il demeurait stable entre ces deux températures pour les individus contrôles. De fait, la différence de comportement entre individus sains et infectés, et donc potentiellement l'efficacité de la manipulation, était plus importante à haute température. Le comportement des gammares en termes d'utilisation des refuges était quant à lui beaucoup moins affecté par la température, avec tout de même une légère tendance dans le même sens et une différence entre individus sains et infectés qui n'était significative qu'à haute température. Enfin, le comportement de géotaxie des gammares, qu'ils soient sains ou infectés, n'était pas différent entre les deux températures testées.

Ces résultats suggèrent que certaines conditions environnementales proximales pourraient affecter la manipulation, en impactant de manière différente le comportement d'individus sains et d'individus manipulés par des parasites. Il semble que certains paramètres tels que la phototaxie puissent être sensibles à de telles conditions, tandis que d'autres, tels que la géotaxie, semblent complètement indépendants des conditions de température. De telles différences pourraient être dues aux mécanismes sous-tendant les modifications comportementales. En effet, il a été suggéré que les modifications de phototaxie et d'utilisation des refuges pourraient être liées à des modifications des concentrations de sérotonine chez les gammares. Or, ce neurotransmetteur est sensible à la température, ce qui pourrait expliquer les différences observées. Au contraire, les modifications de géotaxie pourraient découler d'un autre mécanisme, possiblement lié à un métabolisme anaérobique et à de l'hypoxie.

Infection expérimentale avec P. laevis

L'expérience précédente permet de mettre en évidence un effet de la température à court terme. Cependant, l'effet des conditions environnementales subies par l'hôte durant le développement du parasite pourrait aussi s'avérer important, tel que suggéré par plusieurs études. Pour mieux comprendre cet impact, des infections expérimentales ont de nouveau été utilisées.

Des individus *G. pulex* mâles ont été exposés à des œufs de parasite de l'espèce *P. laevis*. D'autres individus ont été gardés comme contrôles. Les gammares ont ensuite été répartis en deux températures, 14°C et 17°C, où ils ont été maintenus durant tout le développement des parasites. La mortalité des gammares était suivie quotidiennement. Les gammares ont été mesurés le jour de leur mort ou en fin d'expérience et tous les individus ont été disséqués pour déterminer leur charge en parasites.

Une fois le stade cystacanthe des parasites atteint, visible à travers la cuticule des gammares par observation sous une loupe binoculaire, des tests de comportement ont été effectués sur les individus, aux températures correspondant aux conditions de maintien. De même que la première expérience, des tests de temps passé sous un refuge ont été menés le lendemain du passage au stade cystacanthe, puis huit jours et 16 jours plus tard. Au cours de ces tests, les gammares étaient placés individuellement dans des boîtes contenant un refuge, et leur position (dans ou hors du refuge) a été relevée toutes les deux minutes pendant une heure. L'activité globale des gammares a également été mesurée, à l'aide d'une arène artificiellement divisée en plusieurs zones. Un score d'activité a été assigné à chaque individu en fonction du nombre de zones traversées pendant cinq minutes. Enfin, l'effet de la température sur un trait propre au parasite a également été mesuré : la vitesse à laquelle les cystacanthes déployaient leur proboscis. Pour cela, les gammares ont été disséqués et les cystacanthes ont rapidement été plongés dans une solution contenant de la bile de poisson. Chaque cystacanthe a ensuite été observé à moindre luminosité sous une loupe binoculaire, toutes les cinq minutes, afin de déterminer le début du déploiement du proboscis.

La température est un paramètre connu pour fortement modifier le temps de développement des parasites. De ce fait, il était attendu que les parasites se développent plus vite dans les gammares maintenus à 17°C que dans les gammares maintenus à 14°C, fait qui a été confirmé dans cette expérience. En conséquence, deux biais étaient susceptibles d'affecter les résultats. D'une part, le comportement des hôtes était testé au même stade parasitaire et non au même âge absolu des parasites. Ainsi, les gammares testés à 14°C abritaient des parasites plus âgés en temps absolu et ayant passé plus de temps dans leur hôte. D'autre part, tous les gammares étant simultanément exposés aux œufs de parasites, les individus maintenus à 14°C avaient passé plus de temps dans des conditions de laboratoire au moment des tests comportementaux comparés aux individus maintenus à 17°C. Pour écarter ces biais, des contrôles supplémentaires ont été effectués. D'une part, le comportement de gammares maintenus à 14°C a été mesuré en même temps que celui des gammares maintenus à 17°C. Bien que n'étant pas au même stade (les parasites ayant atteint le stade cystacanthe seulement à 17°C), ce contrôle a permis de tester l'effet de l'âge absolu des parasites. D'autre part, une autre infection expérimentale a été conduite sur deux groupes de gammares, l'un ayant au préalable été

maintenu au laboratoire. Placés ensuite dans les mêmes conditions, la comparaison de ces deux groupes a permis d'étudier l'effet du temps passé par les gammares en laboratoire sur leur comportement.

Les données issues de la première infection de l'expérience montrent que la survie des gammares était affectée par la température, avec une plus forte mortalité à 17°C qu'à 14°C, ainsi que par la présence de parasites, les gammares infectés survivant moins que les gammares contrôles. La charge parasitaire des gammares était également affectée par la température, avec plus de parasites se développant par gammares à haute température, ainsi qu'une tendance à un meilleur succès d'infection.

Au contraire, aucun effet de la température n'a été mis en évidence sur le comportement des gammares en termes d'utilisation de refuges, que ce soit chez les individus sains ou infectés. Comme observé dans le premier chapitre, les individus non infectés ont augmenté leur utilisation de refuge au cours du temps, alors qu'une forte diminution a été observée chez les individus parasités. Au contraire, l'activité globale des gammares était affectée par la température, avec une activité globalement plus importante à 17°C comparé à 14°C. De plus, l'effet du parasitisme sur l'activité des gammares était différent selon la température, avec une activité plus importante pour les gammares infectés comparés aux gammares sains à 14°C, et une tendance à un effet inverse à 17°C.

La vitesse de déploiement du proboscis des cystacanthes était également dépendante de la température, étant plus rapide à 17°C qu'à 14°C.

Le comportement mesuré chez des gammares infectés par des acanthelles était différent de celui mesuré chez des gammares infectés par des cystacanthes du même âge absolu, mais similaire à celui des individus contrôles, à la fois pour le comportement de refuge et pour l'activité. Ce résultat écarte donc un effet de l'âge absolu des parasites sur le comportement des gammares, les seules différences étant dues au stade du parasite.

De même, le temps passé par les gammares en laboratoire n'a influencé aucun des paramètres de l'infection, en termes de temps de développement des parasites, de succès d'infection et de charge parasitaire. Le temps passé dans les refuges n'était pas non plus lié au temps passé par les gammares en laboratoire, ni la rapidité du déploiement du proboscis des parasites. En revanche, un effet a été noté sur l'activité globale des gammares, les individus ayant passé un temps prolongé au laboratoire étant globalement moins actifs.

Cette expérience montre que la température ressentie par les gammares durant le développement de leurs parasites est susceptible de modifier de nombreux paramètres de

l'interaction entre hôtes et parasites. En particulier, plusieurs paramètres liés à la physiologie des gammares, tels que la survie ou l'activité, étaient modifiés. De même, les parasites étaient également directement affectés par la température, avec un développement plus rapide à haute température, un déploiement plus rapide de leur proboscis et une charge parasitaire plus élevée. En dépit de tous ces effets, ni le timing ni l'intensité de la manipulation, en termes d'utilisation des refuges, n'étaient affectés par la température.

Ce résultat suggère que la manipulation comportementale, en termes d'utilisation de refuges, pourrait ne pas être plastique, ainsi que déjà suggéré dans le premier chapitre. Tout du moins, ce trait ne semble lié ni au métabolisme des gammares, ni à celui des parasites. Un tel résultat soulève des questions à la fois sur les mécanismes, seulement en partie élucidés, par lesquels les parasites acanthocéphales manipulent leurs hôtes, ainsi que sur les raisons de variations observées dans de précédentes études, notamment suivant la saison.

Impact sur le système immunitaire des gammares

Les résultats de l'expérience précédente suggèrent que la température pourrait influencer la charge parasitaire et éventuellement le succès d'infection des acanthocéphales parasitant les gammares. De plus, la température est également connue pour fortement affecter le développement des parasites dans le gammare, de même que leur succès d'infection de l'hôte définitif. Il est couramment admis que ces effets découlent essentiellement de l'impact de la température sur le métabolisme du parasite. Cependant, tous les paramètres cités pourraient résulter de l'interaction entre le parasite et son hôte. De ce fait, il est possible que l'effet de la température sur le système immunitaire des gammares puisse en partie moduler ces paramètres. Pour s'en assurer, une expérience a été menée afin de mesurer l'efficacité du système immunitaire de gammares acclimatés à différentes températures.

Des individus mâles *G. pulex*, non infectés, ont été acclimatés pendant trois semaines à différentes températures. Des mesures régulières des températures de maintien renseignent sur la température moyenne exacte ressentie par chaque groupe (respectivement 8.8, 11.1, 14.2 et 17.0°C). A l'issu de l'acclimatation, les individus ont été séparés en deux groupes, chacun subissant des mesures différentes. D'une part, la résistance à une infection bactérienne a été mesurée. Pour cela, une injection d'une solution contenant des bactéries *Escherichia coli* a été effectuée sur les individus, qui ont ensuite été replacés à leur température de maintien. Sept heures plus tard, l'hémolymphe de ces gammares a été extraite, mise en solution puis étalée sur des boîtes de Pétri. Après croissance des bactéries, le comptage des colonies a permis d'estimer la quantité de bactéries encore vivantes, et donc la capacité des gammares à se débarrasser des bactéries. La température pouvant affecter en

elle-même la survie des bactéries, la même opération a été répétée en utilisant des tubes remplis de PBS à la place des gammares.

D'autres gammares, non injectés de bactéries, ont été utilisés pour mesurer les autres paramètres immunitaires. Leur hémolymphe a été prélevée et séparée en deux. D'une part, la concentration en hémocytes, des cellules du système immunitaire, a été estimée pour chaque individu. D'autre part, une partie de l'hémolymphe a été immédiatement congelée dans de l'azote liquide, permettant de stopper toute réaction enzymatique. L'activité enzymatique de la phénoloxidase, ainsi que l'activité enzymatique incluant son précurseur, la prophénoloxidase, ont été mesurées par spectrométrie.

Les résultats suggèrent que la température d'acclimatation pourrait affecter certains paramètres immunitaires. Comme attendu, les bactéries *in vitro* ont montré une réaction à la température, avec une augmentation linéaire de la quantité de bactéries avec la température. Cependant, le pattern des bactéries survivantes était différent après passage dans l'hémolymphe de gammares vivants. En effet, les résultats montrent une relation convexe entre la température et le nombre de colonies comptées, suggérant une augmentation de la capacité des gammares à lutter contre les bactéries entre 8.8 et 14.2°C, suivi d'une diminution à plus hautes températures.

Tandis que la température a également impacté les autres paramètres immunitaires de manière globale, et ce avec un effet quadratique, il semble que ce résultat ait été principalement dû à l'effet de la température sur l'activité phenoloxidase, ainsi que sur la concentration d'hémocytes, tandis que l'activité enzymatique totale semblait indépendante de la température. Contrairement à l'efficacité de la résistance aux bactéries, l'activité enzymatique et la concentration en hémocytes étaient plus importantes aux températures extrêmes.

Ces résultats suggèrent que le système immunitaire des gammares puisse effectivement être lié à la température. Si de tels effets avaient déjà été mis en évidence chez des crustacés, aucun consensus n'a jamais pu être établi, l'effet de la température variant notamment selon les espèces et le temps d'acclimatation. Contrairement à d'autres études, le temps d'acclimatation choisi dans cette expérience était relativement long, et les conséquences observées ne découlent donc probablement pas d'un effet de choc thermique, mais plutôt de la plasticité du système immunitaire des gammares en réponse à la température. Les plupart des paramètres immunitaires étant plus performants à haute température, ces résultats suggèrent que le meilleur succès des parasites, en termes de charge parasitaire ou succès d'infection, à haute température n'est probablement pas dû à un système immunitaire de leur hôte moins performant.

Conséquences écologiques sur les gammares

Les expériences conduites dans les chapitres précédents suggèrent que certains paramètres environnementaux pourraient affecter l'interaction entre les acanthocéphales et leurs hôtes gammares. De tels effets sont susceptibles d'aboutir à des conséquences écologiques.

Modifications des préférences thermiques des gammares

Du fait de l'impact de la température sur la relation entre gammares et parasites, des stratégies peuvent exister chez les deux protagonistes. D'une part, le gammare pourrait choisir des températures suffisamment basses pour ralentir la croissance de ses parasites, lui permettant de continuer sa reproduction. D'autre part, le parasite pourrait manipuler les préférences thermiques des gammares vers des températures plus élevées qui découleraient d'un compromis entre vitesse de croissance et survie du gammare, celle-ci étant diminuée à haute température. Ainsi, les gammares sains et infectés pourraient présenter des préférences thermiques différentes, de telle manière que l'effet réel de la température du milieu pourrait dépendre du choix des gammares en termes de micro-habitats.

Afin de comparer les préférences thermiques de gammares sains et infectés, deux groupes de gammares ont été utilisés. D'une part, des gammares contrôles ont été comparés à des gammares naturellement infectés par des parasites au stade cystacanthe, c'est-à-dire ayant fini leur croissance dans leurs hôtes intermédiaires. D'autre part, des gammares contrôles ont été comparés à des gammares expérimentalement infectés, permettant de les tester alors que les parasites n'étaient qu'au stade acanthelle, c'est-à-dire toujours en croissance dans leur hôte.

Le dispositif expérimental utilisé consistait en une gouttière longue de 3,5 mètres dans laquelle un gradient thermique était créé grâce à un système d'échange thermique à contre-courant. Le dispositif était artificiellement divisé en 35 zones permettant de repérer la position des individus, du point le plus froid au point le plus chaud. Les individus étaient testés séparément, et la position de chaque individu au sein du dispositif a été relevée toutes les deux minutes pendant 30 minutes pour le premier groupe, et toutes les 15 secondes pendant 20 minutes pour le second groupe (ceux-ci montrant une forte activité lors des tests préliminaires).

Dans les deux groupes, les résultats montrent une différence de préférence thermique entre gammares sains et infectés, avec une préférence plus froide pour les gammares infectés comparés aux gammares contrôles. Cette différence n'était cependant significative que pour les gammares du premier groupe. Il semble donc que les gammares modifient leurs préférences thermiques lorsqu'ils

sont infectés, passant plus de temps dans des habitats plus froids, et ce quel que soit le stade de leur parasite.

Altération du rôle détritivore des gammares

Les effets de la température documentés au cours des chapitres précédents suggèrent que de nombreux traits peuvent être modifiés dans l'interaction entre gammares et parasites. Les gammares sont connus pour jouer plusieurs rôles écologiques de première importance. S'il est connu que les parasites, notamment les acanthocéphales, sont susceptibles d'altérer ces rôles, l'effet de la température sur le rôle des gammares, infectés ou non, reste à déterminer. En particulier, les gammares sont considérés comme des détritivore de première importance dans les rivières, où ils consomment les feuilles mortes, participant ainsi à leur recyclage et au maintien de la qualité de l'eau.

Afin de comprendre l'impact combiné de la température et du parasitisme sur le rôle détritivore des gammares, des tests de consommation ont été menés sur des gammares sains ou naturellement infectés par le parasite *P. tereticollis* au stade cystacanthe. Les individus ont été acclimatés pendant 10 jours à trois températures (10, 14 ou 18°C), et les tests ont eu lieu à ces mêmes températures. Deux tests ont été conduits. D'une part, la consommation individuelle des gammares a été testée. Pour cela, les individus ont été placés dans des cristallisoirs séparés, et une quantité de feuilles mortes conditionnées de masse sèche déterminée leur a été donnée. Après 24 heures de consommation, les feuilles ont de nouveau été pesées pour en déterminer la quantité consommée. D'autre part, les gammares vivant en grande densité, la consommation a également été testée dans des conditions plus fidèles aux conditions naturelles. Les individus ont été placés par groupes de dix, soit tous infectés, soit tous sains, dans ces cristallisoirs plus gros et enrichis de cailloux. De même que précédemment, des morceaux de feuilles préalablement pesées leur ont été fournies, et une deuxième pesée a permis de déterminer la quantité consommée en 48 heures. Les quantités de nourriture consommées ont été exprimées en fonction de la taille des gammares, les plus gros gammares consommant globalement plus que les petits individus.

Comme attendu, la consommation individuelle des gammares a été altérée à la fois par la température et par le parasitisme. En effet, une réduction de la consommation de feuilles a été observée chez les gammares infectés comparés aux gammares sains, tandis que la consommation a augmenté entre 10 et 14°C, avec une diminution globale à 18°C. La consommation mesurée en groupe était légèrement différente, avec une absence de diminution à haute température.

De manière générale, aucune interaction de la température et du parasitisme n'a pu être mise en évidence, suggérant un effet additif de ces deux paramètres. Cependant, la possibilité des gammares de s'agréger semble être un paramètre pouvant moduler leur consommation de nourriture. Ce résultat pourrait être dû au stress. En effet, les gammares forment des populations de haute densité. Il est possible que l'isolement constitue un facteur stressant qui, additionné au stress d'une température trop élevée, aboutirait à la diminution de consommation observée à 18°C chez les gammares testés individuellement.

Ces résultats soulignent également l'importance de considérer, dans les études, des paramètres multiples et proches de la réalité biologique. En effet, la plupart des études se focalisent sur des individus sains uniquement, et une grande part d'entre elles étudie des individus isolés. Or, les résultats d'individus en groupe ou d'individus infectés présentent une grande différence avec ceux d'individus sains et testés seuls. Bien que ces résultats suggèrent que des modifications de température, notamment liés aux changements globaux, pourraient modifier le rôle détritivore des gammares, la multitude de paramètres à prendre en compte, de même que l'effet de la température sur chacun de ces paramètres, rend des prévisions particulièrement compliquées.

Impact sur le rôle des gammares en tant que proies

En dehors de leur rôle détritivore, les gammares constituent également à la fois des prédateurs majeurs pour de nombreuses espèces d'invertébrés ainsi que des proies quantitativement importantes pour plusieurs espèces aquatiques. Du fait de la facilitation de prédation engendrée par la manipulation comportementale des gammares par les parasites, ce dernier rôle est particulièrement susceptible d'être modifié par ces parasites à transmission trophique. Il est alors possible que l'environnement puisse également moduler l'impact des parasites sur le rôle des gammares en tant que proie.

Des expériences de prédation de gammares sains et infectés, menéee à différentes températures, pourraient permettre de mettre en évidence un tel effet. Une expérience préliminaire a été menée dans cette optique, n'utilisant que des proies non infectées et permettant donc de s'assurer d'un effet de la température sur la prédation de gammares par des poissons.

Plusieurs poissons rouges, *Carassius auratus*, ont été acclimatés à deux températures, 14°C et 17°C, pendant au minimum une semaine. Cette espèce n'est pas présente dans le milieu naturel du gammare, qui est donc naïf à son odeur, ce qui permet de mesurer la prédation sans biais liés à l'évitement de l'odeur du prédateur. Des gammares sains ont été acclimatés aux températures

similaires. Les tests se sont déroulés, à ces mêmes températures, dans des aquariums permettant de tester les poissons individuellement. Pour chaque test, 40 gammares étaient introduits. Des tests ont été menés en n'utilisant que des mâles, que des femelles, ou un mélange de gammares mâles et femelles à sexe-ratio équilibrée. Des refuges étaient disponibles dans l'aquarium de test, sous forme de briques percées, le comportement de refuge étant une composante essentielle du comportement anti-prédateur et faisant partie des comportements modifiés par le parasite. Les tests de prédations duraient 30 minutes, à l'issu desquelles les gammares restants étaient comptés et sexés.

Comme attendu, la consommation des gammares par les poissons était largement dépendante de la température, avec une consommation presque deux fois plus importante à 17°C qu'à 14°C. Lorsque les gammares étaient maintenus séparés selon leur sexe, une plus grande quantité de femelle était consommée, en valeur absolue, comparées aux mâles. En revanche, en présence des deux sexes, un biais de prédation a été mis en évidence vers les mâles.

Ces résultats confirment que le comportement de prédation des poissons est sujet à modifications selon la température. De plus, une expérience menée précédemment a également mis en évidence un effet de la température sur la manipulation comportementale des gammares par leurs parasites. En plus de modifications comportementales en l'absence de prédateur, les parasites provoquent également, chez leurs hôtes, des modifications de leurs réactions face aux prédateurs. De ce fait, les résultats mis en évidence peuvent difficilement prévoir l'issue de l'impact de la température sur le différentiel de prédation entre gammares sains et infectés, et des études supplémentaires sont nécessaires.

Conclusion et perspectives

Résultats principaux

Les paramètres environnementaux vécus par les gammares durant le développement des parasites, le niveau de ressources de l'hôte et la température, se sont tous deux avérés déterminants pour de nombreux traits de l'hôte et de ses parasites. En particulier, le métabolisme de l'hôte était altéré par un régime alimentaire pauvre en protéine, et était réduit à basse température. Les parasites étaient eux plus nombreux à haute température, se sont développés plus rapidement et ont déployé leur proboscis plus vite. Cependant, en dépit des nombreux effets constatés de ces deux paramètres, la manipulation parasitaire du comportement de refuge des gammares n'était pas affectée par ces conditions abiotiques. Ces résultats suggèrent que la manipulation, tout du moins sur ce trait, pourrait

ne dépendre ni de l'état métabolique du parasite, ni de celui de l'hôte, et pourrait ne pas présenter de plasticité.

Cependant, la température vécue, sur un laps de temps plus restreint, par des gammares déjà infectée par des cystacanthes, a mené à des modifications de l'intensité de la manipulation. Cet effet était notamment remarqué sur le comportement de phototaxie. De plus, une telle exposition à différentes températures a également eu un effet sur un rôle primordial des gammares, leur consommation de feuilles, affectant donc leur rôle détritivore.

Effets de l'environnement au-delà de la facilitation de prédation

La majorité des expériences de cette thèse se sont attachées à étudier l'effet de l'environnement sur l'utilisation des refuges des gammares. Ce choix était basé sur le fait que ce comportement est connu pour être modifié par plusieurs espèces d'acanthocéphales, et qu'il constitue un comportement anti-prédateur important, modifiant la probabilité des gammares de subir la prédation. Cependant, les changements induits par les parasites sur leurs hôtes sont multidimensionnels, plusieurs traits étant simultanément altérés. De ce fait, d'autres comportements méritent également d'être étudiés au regard de l'effet de l'environnement, par exemple la phototaxie, ou le comportement d'agrégation.

Bien que souvent ignorées dans les études portant sur la manipulation parasitaire, plusieurs études ont montré que les acanthocéphales induisent également des modifications de comportement au stade acanthelle, non infectieux. Le parasite passant un temps conséquent à ce stade dans l'hôte intermédiaire, ce stade mériterait plus d'attention de la part des scientifiques, notamment en relation avec les paramètres environnementaux.

Enfin, de nombreux traits sont altérés par les parasites, que ce soit au stade acanthelle ou au stade cystacanthe, sans que ceux-ci aient un impact direct sur la probabilité de prédation des gammares. Cependant, de telles modifications peuvent également avoir des conséquences écologiques importantes, ainsi qu'il a été montré avec l'altération du rôle détritivore des gammares. Par exemple, certains parasites acanthocéphales sont connus pour altérer la prédation de certaines espèces de gammares sur d'autres espèces, affectant leurs proportions respectives sur le terrain. De plus, les effets de l'environnement sont susceptibles d'affecter la manipulation de manière indirecte, modifiant par exemple l'intensité de l'infection, un paramètre connu pour moduler l'intensité de la manipulation. De ce fait, restreindre les études sur l'effet de l'environnement aux seules modifications induites par manipulation ne permet pas de tirer des conclusions complètes. Du fait de la complexité d'un tel système, des études à long terme seraient particulièrement adaptées.

Effets de l'environnement au-delà de l'hôte intermédiaire

Les parasites manipulateurs sont largement reconnus pour modifier essentiellement le phénotype de leurs hôtes intermédiaires. De ce fait, une attention particulière a été portée sur ces stades, y compris au cours de cette thèse avec l'étude des effets de l'environnement sur l'interaction entre les parasites et les gammares. Cependant, les gammares ne constituent qu'une étape dans le cycle de vie des parasites. Il est ainsi possible que l'environnement affecte les traits d'histoire de vie des parasites, y compris dans leurs hôtes gammares, à des stades plus précoces de leur vie. De même, les paramètres environnementaux vécus par les parasites dans les gammares peuvent avoir des conséquences à d'autres stades de développement qui ne pouvaient pas être étudiés au cours de mes expériences.

Bien que dans mes expériences aucun effet de la température vécue par les gammares durant le développement de leurs parasites n'ait pu être mis en évidence sur la manipulation, d'autres études ont déjà documenté un effet de la saison pendant laquelle étaient collectés les individus sur la manipulation, en dépit de conditions en laboratoire similaires. De tels résultats suggèrent que les conditions vécues par les individus avant l'infection, que ce soient les gammares ou les parasites, pourraient affecter la manipulation.

Il est possible que les conditions vécues par les larves de parasites puissent être déterminantes sur leurs capacités de manipulation. Les acanthocéphales se reproduisent dans l'intestin de leurs hôtes définitifs et y pondent leurs œufs qui seront évacués dans le milieu. Certaines études suggèrent que les œufs soient capables de survivre un certain temps dans la rivière, où ils sont donc soumis aux conditions environnantes. De plus, les conditions vécues par les mères pourraient également être importantes. Les œufs produits sont en effet relativement complexes, et des accumulations de lipides et protéines ont été documentés lors du développement des oocytes. L'investissement maternel dans les œufs pourrait ainsi être conséquent. Les parasites adultes puisant leurs ressources directement dans l'intestin de leurs hôtes, il est donc possible que le régime alimentaire des hôtes puisse directement affecter les ressources des parasites, et donc l'investissement qu'ils sont capables de fournir pour leurs œufs, avec de potentielles conséquences dans le développement des parasites.

De plus, comprendre les effets des conditions environnementales, incluant celles vécues par les parasites durant leur développement dans des gammares, requière de prendre en compte le cycle de vie complet des parasites. En effet, des mesures de l'intensité de la manipulation de reflètent pas nécessairement les conséquences en termes d'augmentation de la probabilité de transmission. De plus, les conditions vécues par les parasites durant leur développement pourraient avoir des conséquences dans des stades futures de leur vie, qui n'ont pas été étudiés durant mes expériences. Par exemple, plusieurs études suggèrent que la taille des cystacanthes, influencée notamment par le

régime alimentaire de leurs hôtes, pourrait influencer leur succès d'établissement et leur survie dans l'hôte définitif. Il devient alors important de mesurer l'effet de l'environnement sur l'intégralité du cycle des parasites pour pouvoir en tirer les conclusions adéquates, notamment au regard des changements induits par les modifications climatiques.

Effets d'autres facteurs environnementaux

Les expériences de cette thèse se focalisent principalement sur deux facteurs abiotiques, le niveau de ressources alimentaires et la température. Ces paramètres ont notamment été sélectionnés en raison de leurs effets déjà documentés sur plusieurs aspects des infections. Cependant, de nombreux paramètres environnementaux sont susceptibles de modifier la relation entre les parasites manipulateurs et leurs hôtes. Par exemple, l'intensité lumineuse est connue pour influencer la manipulation. Des variations circadiennes ont également été observées. Certains polluants sont également connus pour induire des modifications dans le comportement des gammares qui ressemblent à celles induites par les parasites. Tous ces paramètres méritent donc une plus grande attention au regard des effets qu'ils peuvent avoir, seuls ou combinés, sur la manipulation.

En dehors de l'effet des conditions abiotiques, un résultat majeur des expériences de ma thèse concerne l'effet de l'agrégation des gammares sur l'efficacité détritivore des gammares. De plus, le comportement des gammares non infectés pourrait lui-même dépendre des conditions sociales vécues par les individus. Un tel effet a été mis en évidence dans une courte expérience, au cours de laquelle des gammares étaient maintenus pendant deux semaines dans des conditions différentes : soit individuellement, soit par groupes de trois ou dix individus. Après ce temps d'acclimatation, leur comportement en termes d'utilisation de refuges a été testé. De manière intéressante, les résultats montrent que ce comportement dépendait des conditions d'agrégation dans lesquelles étaient maintenus les gammares. En particulier, les gammares maintenus individuellement ont passé plus de temps à l'intérieur des refuges que ceux maintenus en larges groupes. Ce résultat suggère que l'utilisation des refuges pourrait dépendre du niveau de stress des gammares, potentiellement plus important lorsqu'ils sont écartés d'un groupe. Si ce résultat pourrait permettre de mieux comprendre les mécanismes de la manipulation, qui pourrait faire intervenir des niveaux différents de stress des gammares, il souligne également la nécessité d'étudier les effets de l'environnement biotique sur la manipulation, tels que l'agrégation des gammares.

Comprendre les mécanismes de la manipulation : une étape nécessaire

Même si les effets de l'environnement peuvent être étudiés sans tenir compte des mécanismes sous-jacents la manipulation, les résultats obtenus peuvent permettre de mieux les comprendre. A l'inverse, une meilleure compréhension des mécanismes peut également éclairer sur les effets de l'environnement. Beaucoup d'études suggèrent que les monoamines, et en particulier la sérotonine, jouent un rôle de première importance dans les modifications induites par certains acanthocéphales sur les gammares. En effets, des modifications de l'activité sérotoninergique ont été documentées chez des gammares infectés par des acanthocéphales, tandis que des injections de sérotonine chez des gammares non infectés permettent de mimer des modifications de comportement induites par les parasites.

Le rôle de la sérotonine dans la manipulation pourrait permettre de comprendre certains résultats observés. D'une part, le métabolisme des monoamines des invertébrés dépend des conditions d'acclimatation, notamment en température. Ainsi, une augmentation de température de quelques jours provoquerait une augmentation des niveaux de sérotonine qui pourrait expliquer l'augmentation de phototaxie trouvée dans une des présentes expériences. Il est également possible qu'une acclimatation plus longue, telle que celle vécue par les gammares durant le développement des parasites, mène à une stabilisation des niveaux de sérotonine expliquant l'absence d'effet de la température dans ces conditions. De plus, l'effet des parasites sur le comportement de refuge des hôtes est connu pour être plus faible que celui sur la phototaxie. Cette même différence est trouvée en réponse à la sérotonine, qui ne modifierait pas de manière significative le comportement de refuge. Ce résultat pourrait expliquer le fait que le comportement de refuge n'était pas significativement modifié par la température après une acclimatation de 12 jours, contrairement à la phototaxie. Pour confirmer ces hypothèses, il serait intéressant de mesurer les niveaux de sérotonine chez les gammares sains et infectés à l'issu d'une telle acclimatation.

Conclusion : le rôle clé des parasites en écologie

Au cours des dernières années, le rôle majeur des parasites dans les écosystèmes a été de mieux en mieux admis. Plusieurs articles sont parus récemment sur l'importance écologique des parasites, incluant ceux capable de manipuler le phénotype de leurs hôtes. En conséquence, on observe une reconnaissance montante de l'intérêt de préserver non seulement les espèces hôtes menacées, mais également leurs parasites. Comprendre l'impact de l'environnement sur le fonctionnement des écosystèmes est devenu un challenge majeur au regard des changements climatiques actuels. Les

effets de tels facteurs sur les parasites, depuis leurs traits d'histoire de vie jusqu'aux effets qu'ils ont sur leurs hôtes, ont également attiré l'attention des scientifiques au cours des dernières années. De plus, il est maintenant reconnu que les effets de l'environnement sur le fonctionnement des écosystèmes sont très complexes, et les études tendent désormais à prendre en considération les effets de facteurs multiples et combinés. Ce constat est particulièrement vrai en ce qui concerne les parasites manipulateurs, qui disposent déjà d'interactions complexes avec leurs hôtes et l'environnement. Les études futures devraient continuer dans ces directions, en considérant de multiples facteurs et tous les effets des parasites sur leurs hôtes, incluant la manipulation, ainsi que les conséquences pour les écosystèmes. Enfin, au-delà des réponses écologiques à court terme, la réponse évolutive des systèmes hôtes-parasites dans un environnement changeant requière également l'attention des scientifiques.

Summary

Many parasites with complex life cycle have developed the ability to alter the phenotype of their hosts. It is recognized that such changes in appearance and behavior are responsible for an increase in the probability of predation of their intermediate hosts by their definitive hosts. This phenomenon of parasite manipulation can have numerous consequences at the scale of the ecosystem, modifying the interactions between host populations and altering their ecological role. However, manipulative parasites received little attention from an ecological point of view. Thus, the effect of the environment on the interaction between these parasites and their hosts, in particular in terms of manipulation, is largely unknown. In this thesis, I studied the effect of the environment on the interaction between gammarids – ecologically important crustacean amphipods in rivers – and their acanthocephalan parasites. My experiments showed that the conditions of food resources and temperature experienced by gammarids during the development of their parasites influenced several infection parameters, but did not affect behavioral manipulation in terms of use of refuges. Nevertheless, while the geotaxis was not either effected by temperature, the impact of parasites on gammarids phototaxis was stronger at high temperature. Moreover, my studies showed that the cumulative effect of temperature and parasitism could alter the shredder role of gammarids. In a context of global changes, this work provides a better understanding of the importance of the impact of parasites on their hosts, allowing to make previsions on their subsequent ecological consequences.

Résumé

Beaucoup de parasites à cycle complexe ont développé la capacité d'altérer le phénotype de leurs hôtes. Il est reconnu que ces modifications d'apparence ou de comportement sont responsables d'une augmentation de la probabilité de prédation de l'hôte intermédiaire par l'hôte définitif. Ce phénomène de manipulation parasitaire peut avoir de nombreuses conséquences à l'échelle de l'écosystème, modifiant les interactions entre les populations d'hôtes et bouleversant leur rôle écologique. Cependant, les parasites manipulateurs sont peu étudiés sur le plan écologique, et l'effet de l'environnement sur l'interaction entre ces parasites et leurs hôtes, notamment en termes de manipulation, est encore largement inconnu. Au cours de cette thèse, j'ai étudié l'effet de l'environnement sur l'interaction entre les gammares, des crustacés amphipodes de grande importance écologique dans les rivières, et leurs parasites acanthocéphales. Mes travaux ont montré que les conditions de ressources alimentaires et de température subies par les hôtes durant le développement des parasites influençaient plusieurs paramètres de l'infection mais n'affectaient pas la manipulation en termes d'utilisation de refuges. Toutefois, tandis que la géotaxie ne dépendait pas non plus de la température, l'impact des parasites sur la phototaxie des gammares était plus fort à haute température. De plus, mes travaux ont montré que l'impact conjoint de la température et des parasites pouvait modifier le rôle détritivore des gammares. Dans un contexte de changements globaux, ces travaux permettent de mieux comprendre l'importance de l'impact des parasites sur leurs hôtes et en prévoir les conséquences écologiques.