High food quality increases infection of *Gammarus pulex* (Crustacea: Amphipoda) by the acanthocephalan parasite *Pomphorhynchus laevis*

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ABSTRACT

Parasitism is an important process in ecosystems, but has been largely neglected in ecosystem research. However, parasites are involved in most trophic links in food webs with, in turn, a major role in community structure and ecosystem processes. Several studies have shown that higher nutrient availability in ecosystems tends to increase the prevalence of parasites. Yet, most of these studies focused on resource availability, whereas studies investigating resource quality remain scarce. In this study, we tested the impact of the quality of host food resources on infection by parasites, as well as on the consequences for the host. Three resources were used to individually feed *Gammarus pulex* (Crustacea: Amphipoda) experimentally infected or not infected with the acanthocephalan species *Pomphorhynchus laevis*: microbially conditioned leaf litter without phosphorus input (standard resource); microbially conditioned leaf litter enriched in phosphorus; and microbially conditioned leaf litter without phosphorus input but complemented with additional inputs of benthic diatoms rich in both phosphorus and eicosapentaenoic acid. During the 110 day experiment, infection rate, parasite load, host survival, and parasite-mediated behavioral traits implicated in trophic transmission were measured (refuge use, geotaxis and locomotor activity). The resources of higher quality, regardless of the infection status, reduced gammarid mortality and increased gammarid growth. In addition, higher quality resources increased the proportion of infected gammarids, and led to more cases of multi-infections. While slightly modifying the geotaxis behavior of uninfected gammarids, resource quality did not modulate the impact of parasites on host behavior. Finally, for most parameters, consumption of algal resources had a greater impact than did phosphorus-enriched leaf litter. Therefore, manipulation of resource quality significantly affected host–parasite relationships, which stressed the need for future research to investigate in natura the relationships between resource availability, resource quality and parasite prevalence.

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1. Introduction

Parasitism is one of the most important processes in ecosystems but has been largely neglected in terms of research (Lafferty et al., 2008). Parasites, ranging from bacteria and protozoans to metazoans such as crustaceans or nematodes, use their hosts as sources of resources and habitats. Recent studies suggested that parasites represent at least half of the species on earth, and could be involved in 75% of the trophic links in food webs (Dobson et al., 2008). To date, numerous studies have investigated the often complex life cycles of parasites and the physiological mechanisms involved in host manipulation. Less is known about the impacts of environmental stressors on parasites success and, in turn, on the impacts of parasites on food webs and ecosystem processes (Moore, 2002; Labaude et al., 2015a).

In all ecosystems, the hosts of parasites are simultaneously exposed to several environmental stressors, either from natural

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or anthropogenic origins, but data on the interactive impacts of these stressors and parasites are lacking. Among the different stressors that hosts might be exposed to, resources availability is one of the most common (Power, 1992). Several studies have investigated the impact of resource quantity on host–parasite interactions (Wright and Gompper, 2005; Civitello et al., 2015). Some authors also suggested that current increases in parasitic and infectious diseases result from increased nutrient loading in the environment (Johnson et al., 2010). Yet, in numerous ecosystems, resource quality has been shown to be more important than resource quantity for understanding the physiology of organisms, food web structure, and ecosystem functioning (Elser et al., 1996; Frost and Elser, 2002; Marcarelli et al., 2011). Although investigating resource quality parameters instead of quantifying resource availability might be essential for understanding host–parasite interactions, data on this topic remain scarce.

Several parameters are generally considered for describing quality. The elemental content of resources, generally expressed as resource carbon (C): nitrogen (N): phosphorus (P) ratios, have regularly been shown to impact the life history traits of consumers, especially of herbivores (Elser et al., 2001; Schade et al., 2003) or detritivores (Danger et al., 2013; González et al., 2014; Fuller et al., 2015). This approach, called "Ecological Stoichiometry" (Sterner and Elser, 2002), specifically investigates the consequences of elemental imbalances between the requirements of consumers and elements available in resources. Given that parasites rely on their hosts for resource acquisition, optimal elemental composition of resources for an infected host might fulfill not only the requirements of the parasites, but also the minimal requirements of the host for staying alive (Aalto et al., 2015; Bernot and Poulain, 2018). In a recent opinion paper, Bernot and Poulain (2018) highlighted the use of ecological stoichiometry as a framework for studying feedback of parasites on the environment as well as the effect of the environment on parasites and diseases. Indeed, elemental requirements of infected hosts might differ from those of non-parasitized ones (Frost et al., 2008a) and, in turn, alter the response of infected organisms to the elemental quality of resources (Frost et al., 2008b; Aalto and Pulkkinen, 2013). For example, Frost et al. (2008b) showed that *Daphnia* fed phosphorus-rich resources had higher infection rates and parasite loads compared with those fed phosphorus-poor resources, this observation being explained by phosphorus availability limiting the growth rate of the parasites. In addition, an indicator of resource quality is its lipid profile, especially polyunsaturated fatty acids (PUFAs; Arts et al., 2009). Indeed, some fatty acids are considered to be essential for consumers, because they cannot be synthesized or at least not in sufficient amounts to fulfill the requirements of consumers (Arts et al., 2009). The availability of long-chain PUFA in resources has been regularly reported as controlling the growth and/or reproduction of consumers, especially in aquatic ecosystems where such lipids are mainly found in some algal taxa (e.g. diatoms) and protozoans (Muller-Navarra et al., 2004; Bec et al., 2006). Moreover, some long-chain PUFAs such as arachidonic acid and eicosapentaenoic acid serve as biosynthetic precursors of eicosanoids, which are signaling molecules in many physiological processes, mostly related to reproduction and immunity (Stanley-Samuelson, 2006). This lead Schlotz et al. (2013) to hypothesize that a diet supplemented with PUFA would lead to more pronounced immune responses to parasites, resulting in increased resistance to infection.

Among the numerous invertebrate species inhabiting freshwater ecosystems, the crustacean *Gammarus pulex* (Crustacea: Amphipoda) is an interesting biological model for studying responses of host–parasite interactions to changes in resource quality. This species is generally found in small headwater streams, and is particularly sensitive to environmental changes (Maltby

et al., 2002). It is also a host for many parasitic species, from bacteria to macro-parasites such as helminths (Grabner, 2017). In particular, several acanthocephalan species such as *Pomphorhynchus laevis* use gammarids as intermediate hosts (Crompton and Nickol, 1985; Kennedy, 2006). These trophically transmitted parasites are well known for inducing phenotypic modifications in their intermediate hosts, making them more vulnerable to predation by definitive host species (Bakker et al., 1997). In this way, parasites increase the probability of completing their life cycle. Research has shown that the presence of acanthocephalan parasites alters the response of gammarids to contaminant exposure, increasing their mortality (Brown and Pascoe, 1989) and decreasing their antitoxic defenses (Marcogliese and Pietrock, 2011; Gismondi et al., 2012). A recent study showed that increased temperature and parasite infection additively reduced leaf litter consumption by gammarids (Labaude et al., 2017a). Finally, the only study to our knowledge that has investigated the effect of resource quality was that by Labaude et al. (2015b), who tested the influence of diet protein content on the parasite load and host behavioral modifications. They showed that healthier, well fed gammarids exhibited higher infection intensity without any significant alteration in host behavior.

Following the river continuum concept (RCC; Vannote et al., 1980), headwater streams, where *G. pulex* can be found, are generally described as small streams flowing in forested areas (or at least with a large canopy cover), at least in temperate watersheds from the northern hemisphere. Their functioning is generally related to allochthonous inputs of terrestrial detritus such as leaf litter, dead wood and soil leachates, and they are the main energy and nutrient source at the base of food webs in such streams. However, due to their small size and the anthropogenized surfaces that they drain, these streams are generally altered both physically and chemically, especially when in agricultural settings (Moore and Palmer, 2005). For example, headwater streams that are generally naturally oligotrophic can receive large inputs of nutrients such as nitrogen and/or phosphorus (Carpenter et al., 1998; Sims et al., 1998; Peterson et al., 2001). Moreover, a large proportion of these streams have been exposed to, or are currently impacted by, morphological changes as well as degradation of the riparian corridors (Naiman et al., 2010), inducing, in turn, an increase in instream algal production and a reduction in the relative contribution of allochthonous detritus inputs compared with autochthonous primary production (Feio et al., 2010). Thus, headwater stream perturbations can not only lead to the higher elemental quality of resources for detritivorous invertebrates, given that microbial decomposers are able to immobilize dissolved nutrients in their biomass (Cross et al., 2003), but also enable these invertebrates to access larger amounts of algae, providing them with essential compounds for their growth and survival (Crenier et al., 2017; Rollin et al., 2018).

In this study, we tested the effects of resource quality on the infection rate, parasite load, host survival, and parasite-mediated behavioral traits implied in trophic transmission (i.e. refuge use, geotaxis and locomotor activity of surviving amphipods). Three resources were used to feed individual *G. pulex* experimentally infected or not infected by *Pomphorhyncus laevis*: microbially conditioned leaf litter without phosphorus input (standard resource, S); microbially conditioned leaf litter enriched in phosphorus (P); and microbially conditioned leaf litter without phosphorus input but complemented with additional inputs of benthic diatoms rich in both phosphorus and eicosapentaenoic acid (EPA, D). As reported by Rollin et al. (2018), we assumed that the addition of algal resources to low quality leaf litter would have a greater impact compared with phosphorus-enriched leaf litter, given that algae contain both phosphorus and essential fatty acids. We hypothesized that higher quality resources, regardless of the infec-

tion status, would reduce *G. pulex* mortality (hypothesis 1) and increase their growth (hypothesis 2). Higher quality resources, by increasing the energetic status of hosts, were expected to increase parasite load (hypothesis 3), modify the behavior of infected gammarids (hypothesis 4) and increase parasite impacts on host behavioral changes (hypothesis 5).

2. Materials and methods

All chemicals used for culture media, leaf manipulation, supplementation and water analyses were purchased from Sigma–Aldrich (St. Quentin Fallavier, France).

2.1. Sampling and maintenance

Specimens of *G. pulex* were sampled in April 2015 by kick-sampling in a small tributary of the Suzon river (Burgundy, eastern France; 47°24′12.6″ N; 4°52′058.2″ E), an unpolluted forested stream. Given that *P. laevis* had not been found in this stream for over 20 years, the *G. pulex* population was assumed to be naïve for this parasite (Labaude et al., 2015b). During the sampling, only male *G. pulex* were collected because experimental parasite infection has been shown to be more efficient in males than in females (Franceschi et al., 2008). No noticeably infected gammarids were found. The animals were placed in plastic coolers and rapidly transported to the laboratory, where they were sorted by size to obtain animals of the same size for the experiment (mean initial size ± S.D.: 10.39 ± 0.11 mm). For logistical reasons, the experiments were carried out using water from La Mance stream (6°02′24.4″ E; 49°05′11 7″ N), a headwater forested stream, close to the laboratory, and presenting similar water physico-chemical characteristics compared with the *G. pulex* native streams: pH = 8.2, conductivity = 580 μS cm^{-1}, acid neutralizing capacity (ANC) = 4720 μeq L^{-1}, Cl$^-$ = 13.3 mg L^{-1}, NO$_3^-$ = 24.4 mg L^{-1}, SO$_4^{2-}$ = 31.7 mg L^{-1}, PO$_4^{3-}$ < 0.1 mg L^{-1}, Na$^+$ = 6.5 mg L^{-1}, Ca^{2+} = 100 mg L^{-1}, K$^+$ = 0.82 mg L^{-1}, and

Mg^{2+} = 5.76 mg L^{-1}. Individuals were kept in groups of 500 animals in aerated aquaria ($37 \times 55 \times 10$ cm) filled with La Mance water and acclimatized to laboratory conditions (15 °C ± 1; 12:12 h light:dark cycle) for 10 days before experimental infections. Sycamore maple (*Acer pseudoplatanus*) leaves were provided ad libitum as the food resource.

Parasite eggs were collected from five naturally parasitized chubs (*Squalius cephalus*) caught in the Vouges river (Burgundy, eastern France, 47°9′34.36″ N; 5°9′2.50″ E). Fish were anaesthetized, killed, and dissected within 24 h after collection. Female parasites were immediately taken from the intestines of the fish. Parasite eggs were obtained after dissection of 10 *P. laevis* gravid females. The taxonomy of parasites was verified by genetic analyses as described in Franceschi et al. (2008). Sampled eggs were pooled in La Mance water to obtain a concentrated but homogenous solution (1 mL). The number of eggs per microliter was counted using a microscope, with 10 replicates.

2.2. Experimental design

Six combinations were studied in a fully balanced 3×3 factorial design (Fig. 1). Three resource qualities (see Section 2.3.) were tested crosswise with the three parasite conditions related to the laboratory infestation procedure and subsequent egg exposure and/or infection status.

The three tested resource qualities were: (i) conditioned *A. pseudoplatanus* leaves without P addition (standard litter, S, low quality treatment); (ii) with P addition (P, medium quality treatment); or (iii) with diatom supplementation using agarose pellets (D; in this case P and PUFA were supplied by diatoms; high quality treatment; Rollin et al., 2018).

The three parasite conditions were: (a) unexposed gammarids (Ue); (b) exposed but uninfected gammarids (Ui); and (c) exposed and successfully infected gammarids (I). For statistical analysis Ui and I conditions were also regrouped as exposed condition (E) and then compared with Ue.
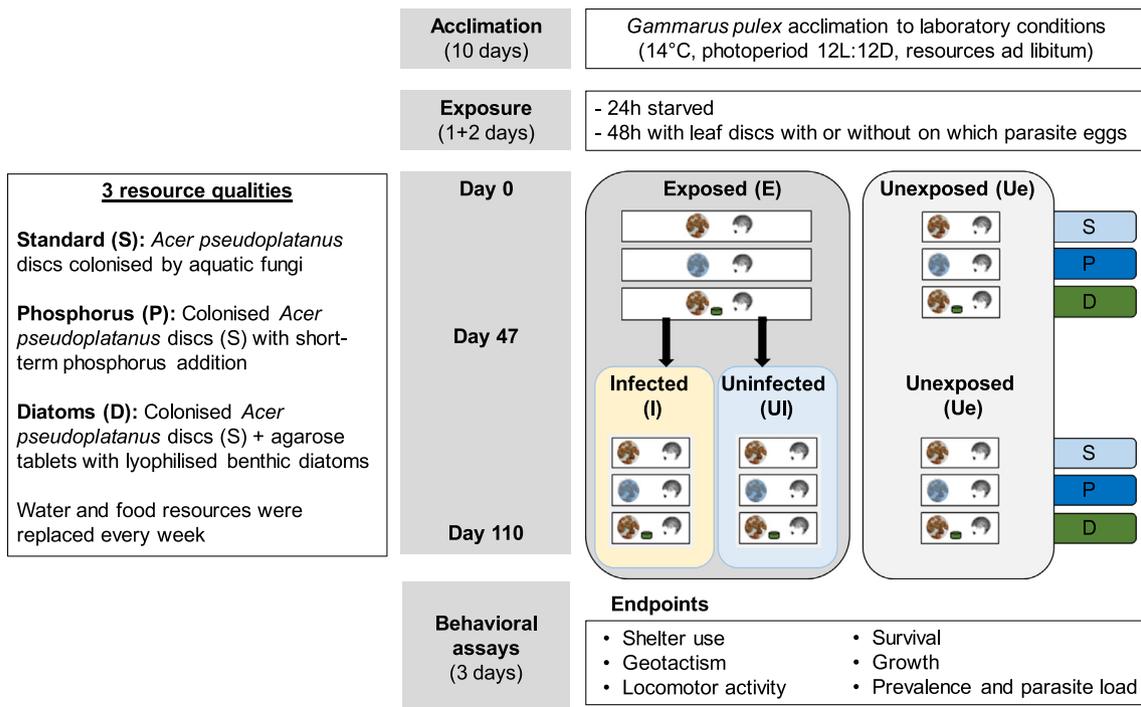

| | Acclimation (10 days) | *Gammarus pulex* acclimation to laboratory conditions (14°C, photoperiod 12L:12D, resources ad libitum) |

| | Exposure (1+2 days) | - 24h starved
- 48h with leaf discs with or without on which parasite eggs |

3 resource qualities

Standard (S): *Acer pseudoplatanus* discs colonised by aquatic fungi

Phosphorus (P): Colonised *Acer pseudoplatanus* discs (S) with short-term phosphorus addition

Diatoms (D): Colonised *Acer pseudoplatanus* discs (S) + agarose tablets with lyophilised benthic diatoms

Water and food resources were replaced every week

Day 0 — Exposed (E) — Unexposed (Ue) — S / P / D

Day 47 — Infected (I) — Uninfected (UI) — Unexposed (Ue)

Day 110 — S / P / D

Behavioral assays (3 days)

Endpoints
- Shelter use
- Geotactism
- Locomotor activity
- Survival
- Growth
- Prevalence and parasite load

Fig. 1. Description of the experimental design. Three resources qualities were tested (*Acer pseudoplatanus* leaves; S: without phosphorus addition; P: with phosphorus addition; and D: S + diatom supplementation using agarose pellets) cross-wise with the three parasite conditions (unexposed, uninfected and infected).

2.3. Resource treatments

Shed leaves of *A. pseudoplatanus* were collected from the riparian zone of a second-order forested stream located upstream of any direct anthropogenic pollution (La Maix stream, Vosges Mountains, north-eastern France; 7°03′14·2″E; 48°29′24 9″N). This litter has been used in previous experiments (e.g. Danger et al., 2013; Arce-Funck et al., 2016; Rollin et al., 2018) because it is uncontaminated and its physicochemical characteristics are well described. *Acer pseudoplatanus* litter was dried in the laboratory and stored at room temperature until use. Food resources were prepared in large quantities following the procedure described by Rollin et al. (2018).

2.3.1. Leaf phosphorus manipulation

The carbon/phosphorus ratio is an indicator of resource quality, whereby a low ratio indicates high food quality for herbivorous and detritivorous species because carbon is rarely limiting (Crenier et al., 2017; Arce-Funck et al., 2018; Rollin et al., 2018). To obtain marked differences in stoichiometric leave qualities, we selected *A. pseudoplatanus* leaves because they have an initially low phosphorus content and low carbon quality (Hladyz et al., 2009). Dried *A. pseudoplatanus* leaves were soaked for a few minutes in deionized water, and 21,000 discs (20 mm in diameter) were then cut using a disc borer while avoiding the main veins. Discs were placed in fine mesh bags (mesh: 200 μm; 15 × 25 cm; 200 discs per bag) to prevent macroinvertebrate access. Bags were transferred to the La Maix stream for 7 days for conditioning by microorganisms, such as hyphomycetes and bacteria.

In the laboratory, discs were then rinsed using La Maix water and randomly introduced into 105 Erlenmeyer flasks (500 mL, 200 discs per flask). Then, 200 mL of a sterile solution (autoclaved) for fungi culture adapted from Gessner and Chauvet (1993) but without phosphorus (KNO_3: 10 mg L^{-1}; $CaCl_2$: 5 mg L^{-1}; $MgSO_4$: 5 mg L^{-1}; $FeCl_3$, $6H_2O$: 62.5 μg L^{-1}; $FeSO_4$, $7H_2O$: 62.5 μg L^{-1}; H_3BO_3: 6 μg L^{-1}; $Mn(NO_3)_2$, $4H_2O$: 6 μg L^{-1}, $ZnSO_4$, $7H_2O$: 6 μg L^{-1}; $CoCl_2$, $6H_2O$: 6 μg L^{-1}; $(NH_4)_6Mo_7O_{24}$, $4H_2O$: 6 μg L^{-1}; $CuSO_4$, $5H_2O$: 3 μg L^{-1}) were added to each flask. This treatment served as the standard food treatment (low quality resource, S). In 35 randomly selected flasks, we introduced 4.64 mL of a 38.4 g L^{-1} KH_2PO_4 solution (corresponding to an input of 40.55 mg of phosphorus per flask), which served as the phosphorus supplemented food treatment (high quality resource 1, P; protocol fully described in Danger et al., 2013; Arce-Funck et al., 2016, 2018; Rollin et al., 2018). Discs were incubated in the flasks for 4 days in the dark at 14 °C on an orbital shaker, and then sorted, rinsed with deionized water, and stored at −20 °C until use.

2.3.2. Supplementation of diatoms in agarose pellets

An axenic strain of the diatom, *Nitzschia palea* (from the Canadian Phycological Culture Center, reference CPCC-160, Waterloo, Canada; high amount of EPA), was grown for 2.5 weeks in four 5 L Erlenmeyer flasks filled with 4 L of Combo medium (Kilham et al., 1998). This culture was maintained at 20 °C in a temperature-controlled chamber (50 μE, 16:8 h light:dark) under continuous shaking (150 rpm). Cells were harvested by centrifugation (4000*g*, 10 min) at the end of the exponential growth phase (2.5 weeks) and were freeze-dried and stored at −20 °C until use.

Food source pellets were prepared using low gelling temperature agarose (Sigma-Aldrich A9414) as a nutrient-free matrix, as fully described by Crenier et al. (2017). Briefly, agarose was dissolved in glass bottles containing demineralized water (2%), heated in a microwave, and placed in an agitated thermostatic water bath at 38 °C. The risk of fatty acid deterioration due to excessive temperature was limited as a result of the low gelling point of agarose and the low temperature applied. A mixture of 25 mg of diatoms and 1.6 mL of agarose solution was introduced in a 2 mL

Eppendorf® tube (to obtain a 50% carbon supply), homogenized using a vortex, and placed in a thermostatic bath until use to avoid agarose gelling. The pellets were formed by injecting the homogenate into the holes of a Plexiglas® mold comprising 40 holes (5 mm diameter × 2 mm height: 39.27 mm³). The pellets were unmolded after a few minutes and were kept at −20 °C in Petri dishes until use. Pellets were provided along with unmanipulated leaves (S) to constitute a second high quality treatment (D), which was the higher resource quality treatment. These pellets supplied EPA, phosphorus, and other elements potentially of use in the development of the organisms.

2.3.3. Resource content analysis

Four batches of 10 frozen leaf discs per treatment (S and P, see Section 2.3.1), and four agarose pellets were freeze-dried, ground, and weighed to the nearest 0.001 mg on a microbalance (Perkin Elmer AD6 Autobalance, Perkin Elmer Corp., USA). A CHN elemental analyzer (Carlo Erba NA2100, Thermo Quest CE International, Milan, Italy) was used to quantify the carbon and nitrogen contents of each resource. Leaves and pellets were burned at 550 °C for 12 h, solubilized in 10 μL of suprapure HNO_3, and 5 mL of nanopure water were then added. The phosphorus content was quantified by spectrophotometry.

Pooled resource material (leaves, diatoms; *n* = 5) were used to perform fatty acid analyses. According to the method proposed by Folch et al. (1957), a chloroform/methanol solution was used twice to extract lipids. The fatty acids were then converted into fatty acid methyl-esters (FAMEs) by acid catalyzed transesterification and analyzed on an Agilent Technologies™ 6850 gas chromatograph. FAMEs were identified by comparing retention times with those obtained from Supelco® and laboratory standards, and quantified against internal standards (13:0).

2.4. Infection procedure for G. pulex

Experimental infections were performed following the procedure described by Franceschi et al. (2008) and Labaude et al. (2015b). First, gammarids were starved for 24 h in individual 60 mL glass dishes (15 °C ± 1; 12:12 h light:dark cycle) to avoid cannibalism and maximize feeding during the infection procedure. Second, to enhance infection success, two gammarids were added to a glass dish (6 cm diameter) containing 50 mL of La Mance water at 15 ± 1 °C. Then, a conditioned *A. pseudoplatanus* leaf disk (S, see section 2.3.1.), on which 100 *P. laevis* eggs had been previously deposited, was added to each dish. Gammarids were allowed to feed on these disks for 48 h, thus initiating the infection procedure (i.e. *G. pulex* exposure (Exposed, E)). Under the same experimental conditions a control group, comprising unexposed organisms (S leaf disc but without parasite eggs, Ue), was used to control for potential confounding effects of the infection procedure on gammarid endpoints. Overall, 1014 gammarids were exposed to parasite eggs and 330 were unexposed under the same conditions.

After this infection step, live gammarids were rinsed and placed individually on new dishes under the same experimental conditions. Then, the three resource quality treatments were randomly allocated to the exposed and unexposed gammarids (338 and 110 gammarids per food quality treatment, respectively; Table 1). Dead organisms (*n* = 62) and gammarids naturally infected by other parasite species (*n* = 11) during the infection procedure were removed from the analyses (differences between the two first columns in Table 1).

2.5. Experimental setup

After the infection period, male gammarids were photographed for initial size measurements at the start of the experiment (see

Table 1
Number of *Gammarus pulex* alive in the different treatments groups at different times of the experiment. Infected and uninfected status of gammarids exposed to *Pomphorhynchus laevis* eggs were assessed after dissection and observation under a stereomicroscope at the time of death of each gammarid or at the end of the experiment.

			Number of living *Gammarus pulex* at different time of the experiment (day)					
			Day 2	Day 0	Day 47		Day 110	
			Infection period	Without dead gammarids and other parasites	First parasite observed		End of the experiment	
S: conditioned *Acer pseudoplatanus* leaves without phosphorus addition (low-quality treatment)	Unexposed (Ue)		110	104	94		16	
	Exposed (E)	Uninfected (Ui)	338	322	271	261	66	65
		Infected (I)				10		1
P: conditioned *Acer pseudoplatanus* leaves with phosphorus addition (high-quality treatment)	Unexposed (Ue)		110	107	91		30	
	Exposed (E)	Uninfected (Ui)	338	323	264	226	90	83
		Infected (I)				38		7
D: conditioned *Acer pseudoplatanus* leaves without phosphorus addition but with diatom supplementation using agarose pellets (higher quality treatment)	Unexposed (Ue)		110	102	98		30	
	Exposed (E)	Uninfected (Ui)	338	313	283	220	106	92
		Infected (I)				63		14

Section 2.6.1; ANOVA; no significant size differences between treatments were observed; mean size: 10.39 ± 0.11 mm; $P > 0.05$). The experiment was conducted for 110 days in natural unpolluted water at $15 \pm 1\,°C$ under a 12:12 h light:dark cycle, which represents conditions close to the optimum for gammarid growth and survival. Each replicate comprised a 60 mL glass dish containing 50 mL of La Mance water, a male *G. pulex* (unexposed or exposed to parasite eggs, and then successfully infected or not infected), and the corresponding food resource (one disc of *A. pseudoplatanus* leaf with or without phosphorus addition and with or without an agar pellet). To limit chemical variations and to ensure that *G. pulex* were fed ad libitum, water and food resources were renewed weekly.

Six weeks after the first infected *G. pulex* was observed (Day 42), all dead gammarids were dissected under a stereomicroscope to verify their infection status. Gammarids in which parasites failed to develop were referred to uninfected (Ui). After 110 days, gammarids were photographed before the behavioral assays. This 110 day period appeared to be a good compromise between measuring survival for a long enough time and obtaining enough animals to perform behavioral tests. After behavioral assays, gammarids were sacrificed and dissected under a stereomicroscope to determine their infection status (infected or uninfected) and their parasite load.

2.6. Endpoints

2.6.1. Gammarid survival

The number of dead gammarids was recorded daily. The date of the death of each individual was noted and dead gammarids were immediately photographed for size assessment (see Section 2.6.2) and dissected to assess the presence and the number of parasites (see Section 2.6.4).

2.6.2. Gammarid size and growth rate

Gammarids were photographed under a stereomicroscope either on the day that they were found dead or on Day 110 (end of the experiment). The height of their fourth coxal plate was measured using SigmaScan Image Analysis Version 5.000 (SPSS Inc, Chicago, IL, USA). This metric is a reliable proxy for gammarid size (Bollache et al., 2000) and is widely used. To determine gammarid body length (mm), we used the allometric relation established by Labaude (Labaude, 2016. Effect of the environment on the interaction between gammarids (Crustacea: Amphipoda) and their manipulative acanthocephalan parasites. PhD thesis, University of Burgundy, France) for male *G. pulex* from the same sampling site

(body length (mm) = 4.33 × fourth coxal plate length (mm); $R^2 = 0.61$, $P < 0.0001$).

Individual growth rates (day^{-1}) were calculated by dividing the difference between the final size and the initial size by the initial size multiplied by the number of days between the two measures.

2.6.3. Behavioral assays

The day after the end of the experiment, we assessed shelter use by each live gammarid. On the second day, we measured the geotaxis of the same gammarid, followed on the third day by its locomotor activity. For all the behavioral assays, blind recordings were ensured by labeling the testing device with a number that provided no indication of the treatment group to which the gammarid belonged.

The sheltering behavior of individual gammarids was recorded by quantifying refuge use according to Dianne et al. (2014). Sheltering behavior assays were performed on 75 unexposed *G. pulex* (Ue; 15, 31 and 29 for S, P, and D resource treatments, respectively) and 20 infected *G. pulex* (I; 1, 6, and 13 for S, P and D, respectively). Gammarids were individually placed in boxes (10.5×16 cm) filled with 250 mL of La Mance water. At one end, each box contained an opaque refuge, comprising half of a terracotta saucer (8.5 cm diameter) with a $1\,cm^2$ hole in the convex part. After 5 min of acclimatization, the gammarid position was recorded every 3 min for 1 h and scored as 1 when outside or 0 when inside the refuge. Therefore, the cumulated score of shelter use ranged from 0 (always inside the refuge) to 20 (always outside) for each individual.

Geotaxis was estimated as the average vertical position of individuals in the water column, according to Labaude et al. (2017b). In total, geotaxis assays were performed on 75 unexposed *G. pulex* (Ue; 15, 31 and 29 for S, P and D resource treatments, respectively) and 20 infected *G. pulex* (I; 1, 6 and 13 for S, P and D, respectively). After 110 days of exposure, gammarids were individually placed in 500 mL graduated cylinders (diameter: 6 cm; height: 35 cm) vertically divided into five zones of equal height and filled with 250 mL of La Mance water. To allow gammarids to choose their position without having to swim, the inner walls of the cylinders were covered with nylon mesh, to which the animals could cling. This was important because the parasite is able to alter both the swimming and the clinging behaviors of gammarids (Bauer et al., 2005). Each cylinder was placed at 15 °C in a temperature-controlled chamber in which the light came in only horizontally to avoid any confounding phototactic reaction. After 3 min acclimatization, the position of each gammarid was recorded every 15 s for 5 min and scored from 1 (bottom) to 5 (top compartment). Therefore, the cumulated

geotaxis score ranged from 20 (always in the bottom zone) to 100 (always in the top zone) for each individual.

The locomotor activity was assessed following the method of Sornom et al. (2010). This method usually measures the proportion of time spent moving. In total, locomotor activity assays were performed on 57 unexposed *G. pulex* (Ue; 14, 22 and 21 for S, P and D resource treatments, respectively) and 13 infected *G. pulex* (I; 1, 6 and 6 for S, P and D, respectively). We placed the gammarids individually in 8 cm diameter Petri dishes open to the air and filled with La Mance water. Petri dishes were then placed in a hermetic box linked to a digital video camera. After 5 min acclimatization, the behavior of each individual was video-recorded under red light at 15 frames per second for 5 min. Locomotor activity was scored as the proportion of time swimming. Below 15 mm s^{-1}, moving gammarids were considered to be crawling rather than swimming.

2.6.4. Prevalence and parasite load

All gammarids were dissected under stereomicroscope ($\times$6 to $\times$40 magnification) either on the day that they were found dead or at the end of the experiment. This step enabled us to remove gammarids infected by parasites other than *P. laevis*, define the status of exposed *G. pulex* (infected versus uninfected), the parasite load (number of parasites in one *G. pulex*) and the parasite prevalence (% of infected *G. pulex*) in each experimental condition.

2.7. Statistical analyses

The first infected gammarid was observed on Day 47. Thus, two datasets were used for survival and parasite load statistical analysis to integrate or not the delay of infection into the analysis (from Day 0 to Day 110 versus from Day 47 to Day 110). Prior to Day 47, it was not always possible to identify whether gammarids were infected, due to the minimal parasite development. For other endpoints, only the results obtained at the end of the experiment were considered.

Statistical analyses were conducted using R software (R Core Team, 2016) to the 5% level of significance. Survival data were analyzed by Kaplan–Meier survival curves and log-rank tests were performed to compare curves and determine the effects of infection status and resource quality on *G. pulex* survival throughout the experiment: from Day 0 to Day 110 (difference between exposed and unexposed) and from Day 47 to Day 110 (difference between infected and uninfected; in the last case, mortality before Day 47 (first infection detected) was not considered). Given that the number of infected gammarids was very low, log-rank tests were also performed (Day 47 to Day 110) by pooling infected and uninfected gammarid data or data of gammarids fed on S, P and D resources to better assess the global effect of food resource or of infection status, respectively. Bonferroni adjustments were performed on log-rank tests to determine the effects of resource quality and infection status on survival.

Given the extremely low survival in infected-S treatment ($n = 1$), only main effects were testable for gammarid growth rate and behavioral responses. The influence of food quality and infection status on *G. pulex* growth was tested using Kruskal–Wallis tests, and post-hoc tests were performed on main effects (Mann–Whitney *U*-test and Bonferroni adjustment).

A test for proportion comparison enabling the calculation of 95% confidence intervals (CIs) was used to assess the effect of resource quality on the proportion of infected gammarids. Significance between differences was assessed using CIs. A multiple Poisson regression model was used to investigate the effect of resource quality on individual parasite load. In the analyses, the S resource treatment was considered as a reference level.

Kruskal–Wallis tests were used to assess the effect of resource quality on each behavior score (geotaxis, shelter use and locomotor activity). Cliff's deltas (Cliff, 1996) were calculated to evaluate the behavior score differences of infected (I) and unexposed (Ue) organisms fed with different resource qualities (S, P and D). Then, effect sizes for each score between unexposed (Ue) and infected (I) organisms were also compared for P and D resource qualities (not for S because the number of surviving organisms was too low). Cliff's delta, robust to non-normally distributed data, is a unitless parameter ranging from -1 to 1. It is used to represent the size of the effect and the direction of this effect size difference. Significance between differences was assessed using CIs. The R-package "orddom" (version 3.1; Rogmann, J.J. (2013). orddom: Ordinal Dominance Statistics. R package version 3.1. https://CRAN.R-project.org/package=orddom) was used to calculate Cliff's deltas medians and 95% CIs.

3. Results

3.1. P and D resource treatments showed higher phosphorus content, with the resource D also containing PUFAs

Low quality leaves (S) contained 0.36 ± 0.08 mg g^{-1} phosphorus and manipulated leaves (P) contained 1.24 ± 0.16 mg g^{-1} phosphorus. The dominant fatty acids in conditioned leaves (S or P) were palmitic acid (16:0), oleic acid (18:1ω9), linoleic acid (18:2ω6), and α-linolenic acid (18:3ω3), whereas long-chain PUFAs (number of Cs $\geq$ 20) were completely lacking from these resources.

Agarose pellets contained 1.95 ± 0.58 mg g^{-1} phosphorus. In diatoms the major fatty acids were palmitic (16:0), palmitoleic acid (16:1ω7), and EPA (20:5ω3). As mentioned by Rollin et al. (2018), these compounds accounted for more than 65% of the total fatty acids (16:0 = 28.2%, 16:1ω7 = 21%, and 20:5ω3 = 16.9%). Docosahexaenoic acid (22:6ω3) represented 2.2% of diatom fatty acids.

3.2. Low quality resource and P. laevis infection reduce G. pulex survival

After 47 days of exposure (day of the first observed parasite), the survival of gammarids was high, ranging from 81.7% in exposed (E; Ui+I)-P treatment to 96% in Ue-D treatment groups (Table 1). At Day 110, survival was low, between 15.4% in the Ue-S treatment and 33.9% in E-D treatment groups. The highest *G. pulex* survival was observed in animals fed the highest resource qualities (D > P > S), regardless of the infection status (Table 1).

The log-rank global comparison of *G. pulex* exposed or not to *P. laevis* eggs (Fig. 2A, Day 0 to 110) and fed one of the three resource qualities revealed highly significant differences in gammarid survival (Chi^2 = 30.9, degrees of freedom (d.f.) = 5, $P = 9.68 \times 10^{-6}$). Pairwise log-rank tests with Bonferroni adjustment showed that survival in the E-D and Ue-D treatment groups (highest quality resources) was significantly higher than that in the E-S and Ue-S treatment groups (lower quality resources) (E-D versus E-S: Chi^2 = 21.6, d.f. = 1, $P = 3.82 \times 10^{-6}$; E-D versus Ue-S: Chi^2 = 9, d.f. = 1, $P = 0.0027$; Ue-D versus Ue-S: Chi^2 = 11, d.f. = 1, $P = 9 \times 10^{-4}$; Ue-D versus E-S: Chi^2 = 17.3, d.f. = 1, $P = 3.21 \times 10^{-5}$). P resources led to intermediate but not significantly different survival.

Among exposed gammarids, between Day 47 and Day 110, survival ranged from 10% in the I-S treatment to 41.8% in the Ui-D treatment group. Infected gammarids exhibited significantly lower survival compared with unexposed and uninfected animals (S: 10% versus 17 and 24.9%; S: 18.4% versus 33 and 36.7%; S: 22.2% versus 30.6 and 41.8%; Table 1).

The log-rank global comparison performed on *G. pulex* infected or not by *P. laevis* (Fig. 2B, Day 47 to Day 110) fed one of the three resource qualities also showed significant differences in gammarid
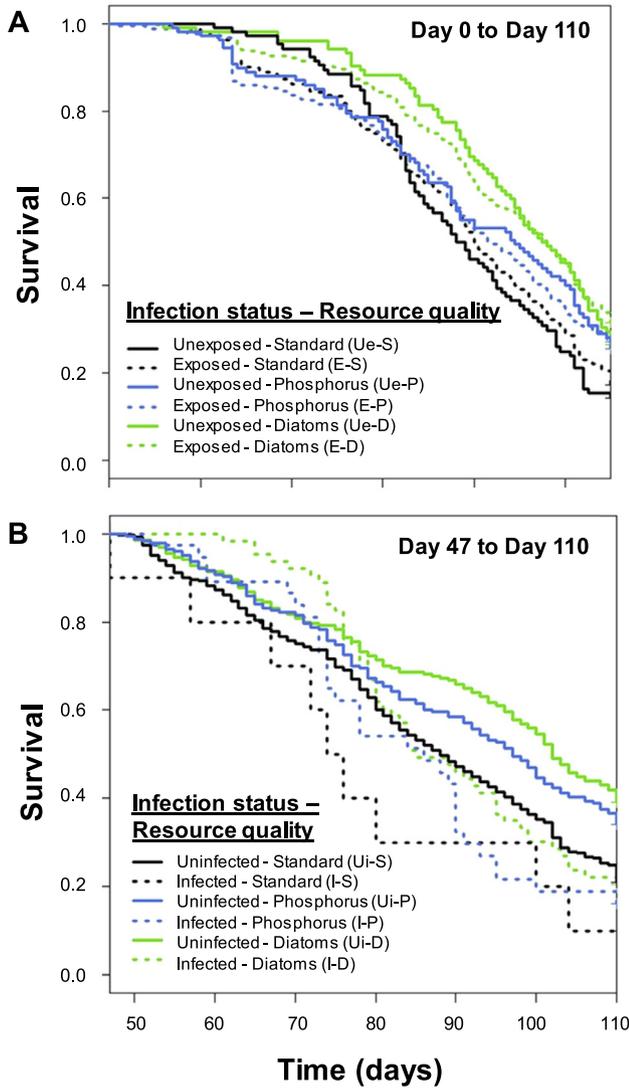
Fig. 2. Kaplan–Meier curves representing the survival of *Gammarus pulex* fed one of the three resource qualities and exposed/infected or not to/by parasites, (A) from Day 0 to Day 110 (difference between exposed (E) and unexposed (Ue)) and (B) from Day 47 to Day 110 (difference between infected (I) and uninfected (Ui)).

survival (Chi2 = 32.08, d.f. = 5, $P = 4.09 \times 10^{-6}$). Pairwise log-rank tests with Bonferroni adjustment showed that survival in the Ui-S treatment groups was significantly lower than that in the Ui-P and Ui-D treatment groups (Ui-S versus Ui-P: $Chi^2 = 9.4$, d.f. = 1, $P = 0.0022$; Ui-S versus Ui-D: $Chi^2 = 20.1$, d.f. = 1, $P = 7.33 \times 10^{-6}$) and that the Ui-D treatment group had significantly higher survival than the I-P and I-D treatment groups (Ui-D versus I-P: $Chi^2 = 12.2$, d.f. = 1, $P = 4.8 \times 10^{-4}$; Ui-D versus I-D: $Chi^2 = 8$, d.f. = 1, $P = 4.64 \times 10^{-3}$). No other significant differences were found.

The effect of food resource was significant, but due to a low number of infected gammarids, the effect of infection was not observed using the 2×2 log-rank tests. When log-rank tests were performed on pooled infection status data (infected and uninfected; Day 47 to Day 110), a significant global effect of food treatment was observed ($Chi^2 = 16.9$, d.f. = 2, $P = 2.19 \times 10^{-4}$) with gammarids fed with S resource showing significant lower survival compared with those fed with P ($Chi^2 = 6.9$, d.f. = 1, $P = 0.00865$) and D resources ($Chi^2 = 16.4$, d.f. = 1, $P = 5.25 \times 10^{-5}$). In the same way, when log-rank tests were performed on pooled food resource data (S, P, and D; Day 47 to Day 110), a significant global effect of

infection status was reported ($Chi^2 = 10.9$, d.f. = 2, $P = 0.00433$), with uninfected gammarids showing significantly higher survival compared with those infected by *P. laevis* ($Chi^2 = 9.4$, d.f. = 1, $P = 2.155 \times 10^{-3}$). Thus, survival was influenced by both resource quality and infection status, but no interactive effect was observed. No global survival differences were reported between unexposed and uninfected gammarids ($Chi^2 = 3.4$, d.f. = 1, $P = 0.0638$), showing the absence of impacts of the infestation procedure.

3.3. Low quality resource and P. laevis infection reduce G. pulex growth

Resource quality had a significant effect on *G. pulex* growth (Kruskal–Wallis; $Chi^2 = 140.69$, d.f. = 2, $P = 2.2 \times 10^{-16}$; Fig. 3). Pairwise Mann–Whitney *U*-tests with Bonferroni adjustment revealed that growth of *G. pulex* fed the D resource was significantly higher than that of gammarids fed either P or S resources (D versus P: $Chi^2 = 2343.5$, d.f. = 1, $P = 2.2 \times 10^{-16}$; D versus S: $Chi^2 = 1398$, d.f. = 1, $P = 2.2 \times 10^{-16}$) and that the growth of *G. pulex* fed with the P resource was significantly higher than that of gammarids fed with the S resource ($Chi^2 = 2680$, d.f. = 1, $P = 4.4 \times 10^{-8}$). On average, compared with the S treatment, gammarid growth increased by 17% and 56.4% in individuals fed with P and D resources, respectively.

Infection status also had a significant effect on *G. pulex* growth (Kruskal–Wallis, resource quality: $Chi^2 = 9.66$, d.f. = 2, $P = 0.008$). The growth of infected gammarids was similar regardless of the resource quality. Pairwise Mann–Whitney *U*-tests with Bonferroni adjustment revealed that the growth of *G. pulex* infected by *P. laevis* was significantly lower than that of uninfected gammarids (I versus Ui: $Chi^2 = 3703$, d.f. = 1, $P = 0.002$). On average, compared with uninfected gammarids, the growth of infected gammarids was reduced by 20.3%.
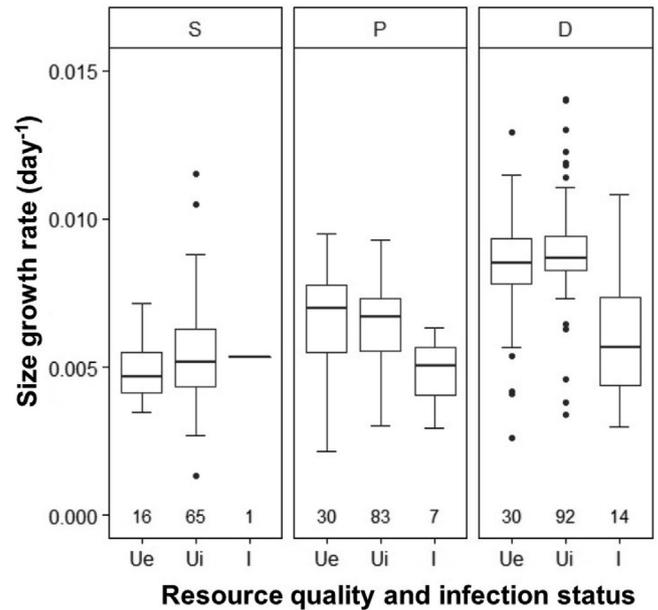

Fig. 3. Box plot (median, quartiles) representing the size growth in male *Gammarus pulex* exposed for 110 days to the nine treatments (three infection statuses × three resource qualities). Infection statuses were: *G. pulex* unexposed to *Pomphorhyncus laevis* (Ue); *G. pulex* exposed but uninfected by *P. laevis* (Ui); and *G. pulex* infected by *P. laevis* (I). Resource quality treatments were: conditioned *Acer pseudoplatanus* leaves without phosphorus addition (S) or with phosphorus addition (P) or conditioned *A. pseudoplatanus* leaves without phosphorus addition but with diatom supplementation using agarose pellets (D). Numbers of *G. pulex* per treatment are indicated above the X-axis.

3.4. Low quality resource reduces G. pulex infection success by P. laevis (prevalence and load)

Gammarus pulex infection success by *P. laevis* was significantly affected by resource quality, both when considering all gammarids (dead and alive) after Day 47 (Chi^2 = 47.29, d.f. = 2, $P = 5.38 \times 10^{-11}$, Fig. 4A) and those still alive at Day 110 (Chi^2 = 6.21, d.f. = 2, $P = 0.045$, Fig. 4B); the number of infected *G. pulex* also increased with increasing resource quality.

Among the gammarids exposed to *P. laevis* eggs, 2.7% (1.2–5.2), 11.4% (8.2–14.4) and 20.1% (15.8–25.0) of *G. pulex* (mean % (95% CI)) were infected and alive after 47 days of feeding on S, P and D resources, respectively (n = 10, 38 and 63; Table 1). Based on the 95% CIs, it can be deduced that infection success significantly differed between the three resource qualities. At Day 110, only 1.7% (0.04–8.5), 7.2% (2.6–15), and 20.1% (7.1–21.4) of gammarids, representing 1, 7 and 14 individuals, were alive and infected in the S, P, and D treatment groups, respectively (Table 1).

Among infected *G. pulex*, amphipods fed with the D resource had a significantly higher *P. laevis* loads when considering all individuals, dead or not (Day 47 to Day 110; S as reference level; $P = 2.73 \times 10^{-13}$, Fig. 4C; Table 2), or only the surviving gammarids (Day 110; S as reference level; $P = 0.012$, Fig. 4D). The P resource also significantly increased *P. laevis* load in individual gammarids but only when all individuals, dead or not, were considered (Day 47 to Day 110; S as reference level; $P = 4.13 \times 10^{-6}$, Fig. 4C; Table 2). Considering all individuals, the relative proportion of infected gammarids with only one *P. laevis* was reduced by a factor of 1.4 between S and P resource groups and by a factor of 1.9 between S and D resource groups (by 2 and by 2.6 at Day 110, but only one organism was present in the infected-S treatment group).

3.5. High quality resource increases G. pulex geotaxis, and P. laevis infection increases G. pulex geotaxis and shelter use

Resource quality had a marginally significant effect on the *G. pulex* geotaxis score (Kruskal–Wallis; Chi^2 = 6.15, d.f. = 2,
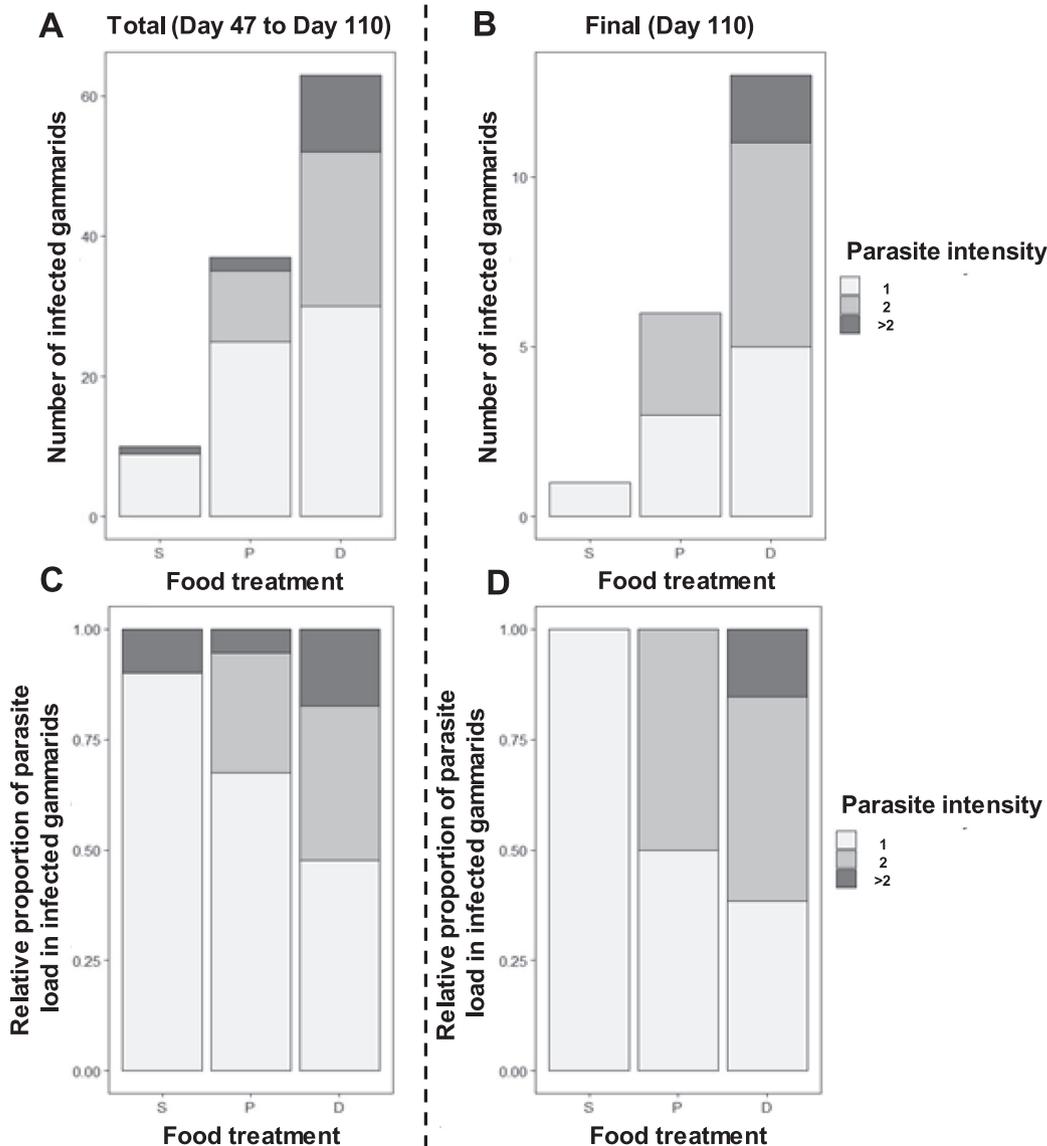

Fig. 4. Number (A, B) and proportion (C, D) of infected gammarids according to their infection status and the three resource quality conditions from baseline on Day 0 to Day 110 (B, D) or from Day 47 to Day 110 (A, C). Treatment names are described in the legend to Fig. 3.

Table 2
Regression coefficients estimated from a Poisson regression model fitted to individual parasite load relative to the proportion of the parasite (*Pomphorhyncus laevis*) load in infected *Gammarus pulex* considering: (A) all data from Day 47 to Day 110; or (B) only data for Day 110. Bold *P* values show statistically significant differences between the different resource treatments compared with S resource, which was considered as the reference level.

	Estimate	*Z* value	Standard Error (coefficient)	*P* value
A- Day 47 to Day 100				
P resource	1.5308	4.605	0.3324	**4.13×10^{-6}**
D resource	2.3126	7.307	0.3165	**2.73×10^{-13}**
B- Day 110				
P resource	1.804	1.711	1.054	0.087
D resource	2.566	2.512	1.022	**0.012**

P = 0.046; Fig. 5A), but not on shelter use (Chi^2 = 1.53, d.f. = 2, P = 0.47; Fig. 5B) or locomotor activity (Chi^2 = 2.69, d.f. = 2, P = 0.26; Fig. 5C). Pairwise Mann–Whitney *U*-tests with Bonferroni adjustment showed that mean geotaxis scores among resource treatments were similar (mean values of 28.9, 36.8 and 38.6 for S, P, and D, respectively), whereas Cliff's deltas revealed a significantly higher geotaxis score in the Ue-D and Ue-P groups compared with the Ue-S group (Fig. 6A). Unexposed gammarids fed a higher quality resource occupied a significantly higher position in the water column.

Compared with unexposed *G. pulex*, those infected with *P. laevis* showed a significantly higher geotaxis score (Mann–Whitney; U = 338, d.f. = 1, P = 1.652×10^{-4}; mean 32.7 versus 53.6; Fig. 5A), a significantly higher shelter use score (Mann–Whitney; U = 505.5, d.f. = 1, P = 0.0244; mean 6.7 versus 10.3; Fig. 5B), but no differences in locomotor activity (Mann–Whitney; U = 340, d. f. = 1, P = 0.6504; mean 0.364 versus 0.378; Fig. 5B). Thus, infected gammarids tended to be higher in the water column and were more often outside of the refuge. However, when considering Cliff's delta approach, no significant differences were reported between treatments.

4. Discussion

To date, only a few studies have evaluated the effect of stoichiometric quality of resource, excluding confounding factors (e.g. changes in microbial biomass or alteration of detrital carbon quality), on the survival and life-history traits of detritivorous species (Danger et al., 2013; Arce-Funck et al., 2016; Rollin et al., 2018). Similarly, studies on the effect of the stoichiometric quality of resources on parasitic infection are scarce and have not included detritivorous hosts (Frost et al., 2008a,b; Schlotz et al., 2013; Stephens et al., 2017).

In the present study, comparison of S (microbial-conditioned *A. pseudoplatanus* leaves) and P (phosphorus-manipulated *A. pseudoplatanus* leaves) resource treatments enabled us to investigate stoichiometric effects resulting from the higher phosphorus content in a food resource (Danger et al., 2013). In addition, a supplementation of leaf litter with diatom pellets (D) was hypothetized to greatly increase resource quality for consumers. Indeed, diatoms are easier to assimilate than detritus, show high elemental quality (a higher phosphorus content compared with leaf litter), and have a high EPA concentration, which enhances gammarid survival, growth and reproduction (Crenier et al., 2017).

Resource quality was the main driver of *G. pulex* survival, infected or not by *P. laevis*. Higher survival was related to the higher quality of resources consumed by *G. pulex* (S < P < D; confirming hypothesis 1, that high quality resources would reduce *G. pulex* mortality, regardless of the infection status). Higher growth rates were also reported for *G. pulex* fed higher quality resources (confirming hypothesis 2, that high quality resources

would increase *G. pulex* growth, regardless of the infection status). These results are in accordance with studies reporting a beneficial effect on survival and growth rate of aquatic organisms of resources containing high phosphorus or EPA levels (*Daphnia*: Makino et al., 2002; Masclaux et al., 2009; Schlotz et al., 2012; Prater et al., 2016; *Oncorhynchus mykiss*: Lellis et al., 2004; *Gammarus*: Kraufvelin et al., 2006; Danger et al., 2013; Gergs et al., 2014; Crenier et al., 2017; Rollin et al., 2018). According to Rollin et al. (2018), the higher *G. pulex* growth rate seen when fed higher quality resources was linked to a higher molt frequency. This is in accordance with the growth rate hypothesis stating that growth requires high amounts of phosphorus, phosphorus being involved in the synthesis of phosphorus-rich RNA necessary for ensuring organismal growth (Elser et al., 2003). Beneficial effects of PUFAs on the growth of gammarids might result from their inability to maintain their PUFA content and/or to synthesize these essential compounds (Crenier et al., 2017).

In the present study, *G. pulex* infected by *P. laevis* showed lower survival and lower growth rates than those gammarids that were unexposed and uninfected. These results are in accordance with previous studies (Lafferty et al., 2008; Aalto and Pulkkinen, 2013; Labaude et al., 2015b; Labaude, 2016, PhD Thesis, cited earlier). Growth reduction could be related to the energetic costs of harboring a parasite. Crustaceans infected by acanthocephalan parasites showed significant modifications in their energetic reserves (Plaistow et al., 2001; Gismondi et al., 2012; Caddigan et al., 2014), and isopods infected by acanthocephalans were reported to allocate approximately 21% of their energy production to parasite growth, to the detriment of their own reproduction (Lettini and Sukhdeo, 2010).

Leaving enough resources for their hosts might be a strategy selected by parasites, allowing them to manipulate host behaviors (Maure et al., 2013). According to this hypothesis, when the host condition is reduced, lower parasite exploitation is expected. In the present study, both a low quality resource (S) and infection with *P. laevis* significantly reduced *G. pulex* survival, but no clear interactive effects were identified. Survival was systematically lowered for infected *G. pulex* compared with uninfected organisms, regardless of the resource quality. In addition, the growth of infected gammarids was similar regardless of the resource quality. Therefore, our results did not confirm the hypothesis of Maure et al. (2013), and were in accordance with those of Labaude et al. (2015b). Similarly, Frost et al. (2008b) showed that low phosphorus containing resources increased the deleterious effects of bacterial infection on *Daphnia* reproduction.

One of the important results of our study concerns the impact of food quality on *G. pulex* infection by *P. laevis*: high quality resources were associated with higher parasite prevalence. Several studies have shown that high nutrient availabilities in ecosystems tend to increase the prevalence of parasites (Johnson et al., 2010; Altman and Byers, 2014). However, most of these studies mainly
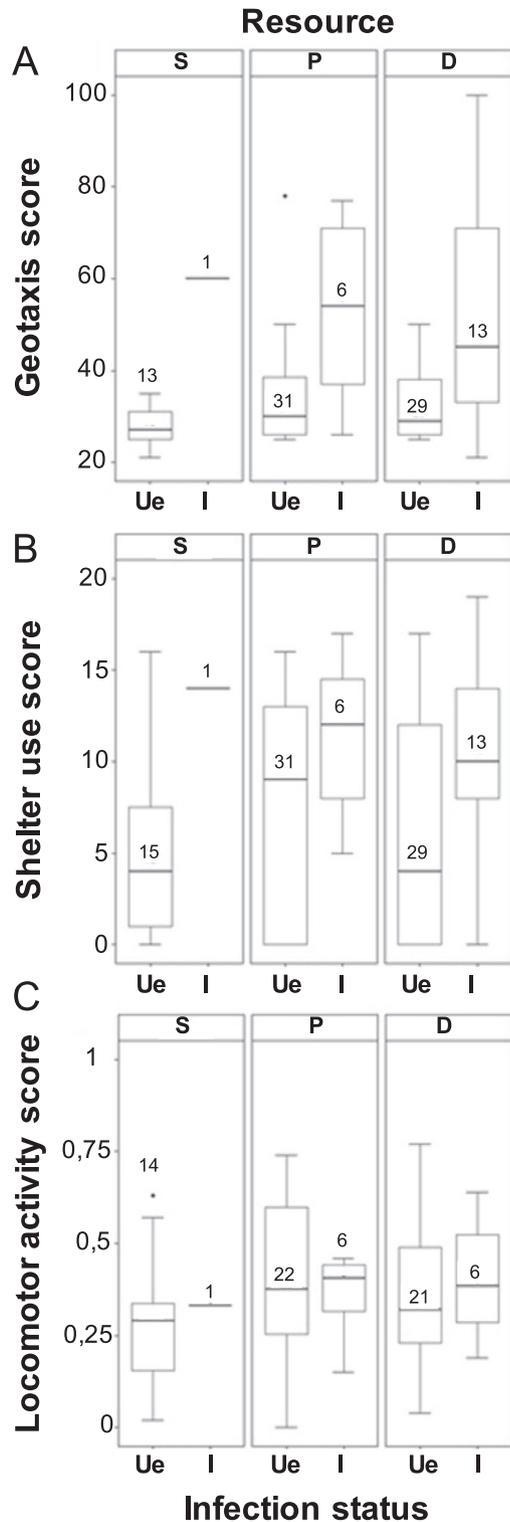
Fig. 5. Box plot (median, quartiles) representing the behavioral responses ((A) geotaxis, (B) shelter use and (C) locomotor activity) of *Gammarus pulex* after 110 days of exposure to the nine treatments (three infection statuses × three resource qualities). The cumulated geotaxis scores range from 20 (always in the bottom zone) to 100 (always in the top zone). The cumulated scores of shelter use range from 0 (always inside the refuge) to 20 (always outside). Treatment names are described in the legend to Fig. 3. Sample sizes are indicated on the median or above box plot.

considered resource quantity and rarely resource quality. Finally, we showed that diatom supplementation led to significantly higher infection rates compared with phosphorus-enriched resources alone, suggesting that in ecosystems, consumption of such a high quality resource, bringing both nutrients and PUFAs, might strongly impact parasite development, at least more than ingestion of leaf litter conditioned in streams with high nutrient loads. This is particularly important in the global context of the degradation of riparian vegetation and canopy opening in the vicinity of streams (Hladyz et al., 2011). Thus our results provide support for the positive effect of the quality of resources on parasitic infections. This is in line with the results of Frost et al. (2008b), showing that the proportion of *Daphnia* infected, as well as the number of parasites per *Daphnia*, increased when organisms were fed low carbon/phosphorus resources, or those of Stephens et al. (2017), which showed that higher leaf litter quality did not decrease the susceptibility of amphibians to parasitic infection. Therefore, in these cases, more energy in the host might permit improved parasite development, rather than allowing the host to increase its energy, which would result in improved defenses (Arce-Funck et al., 2016; Stephens et al., 2017).

Another interesting result was the increase in the parasite load (number of *P. laevis* per gammarid) for organisms fed the highest quality resources (P and D). This suggested that the higher energy content of gammarids fed high quality resources, previously observed by Arce-Funck et al. (2016), favored the coexistence of several parasites within a single host by reducing competition between parasites (thus confirming hypothesis 3: high quality resources increased the parasite load). Interestingly, Schlotz et al. (2013) also observed that daphnids infected with the parasite *Pasteuria ramosa* showed the highest spore load per animal when fed high quality food. Even though the number of infected organisms at the end of the experiment precluded strong statistical testing, the number of parasites per gammarid appeared to be unrelated to higher mortality and the proportion of multi-infected gammarids remained unchanged throughout the experiment. This result is in accordance with the results of Franceschi et al. (2008) who did not observe any effect of infection intensity on host survival.

Despite the important effects of resource quality on host survival and growth, as well as on parasite prevalence and infection intensity, our study did not reveal clear impacts of resource quality on the modulation of parasite-induced behavioral changes in their hosts (effect on geotaxis behavior only; partially verifying hypothesis 4, that high quality resources would modify the behavior of infected gammarids). As expected, at Day 110, *P. laevis* infection led to increases in host geotaxis and shelter use. Behavioral changes have long been described in this host–parasite combination (Bakker et al., 1997; Bauer et al., 2005; Lagrue et al., 2007). These changes, according to the manipulation hypothesis (Holmes and Bethel, 1972), predispose manipulated intermediate hosts to be preyed upon by definitive hosts (i.e. a fish). Such behavioral changes are mainly related to parasite-induced changes in host neurotransmitter production (Tain et al., 2006; Perrot-Minnot et al., 2014). Maure et al. (2013) proposed the "host energetic resource constraint hypothesis" (HERC) stating that a largely overlooked proportion of infected host energy might be used during the behavioral manipulation of the host. Following this hypothesis, higher resource quality should have enabled the parasite to amplify the behavioral manipulation of its host. The absence of such an effect suggests that HERC does not apply to gammarids infected with *P. laevis* (rejection of hypothesis 5: high quality resources do not increase parasite impacts on host behavioral
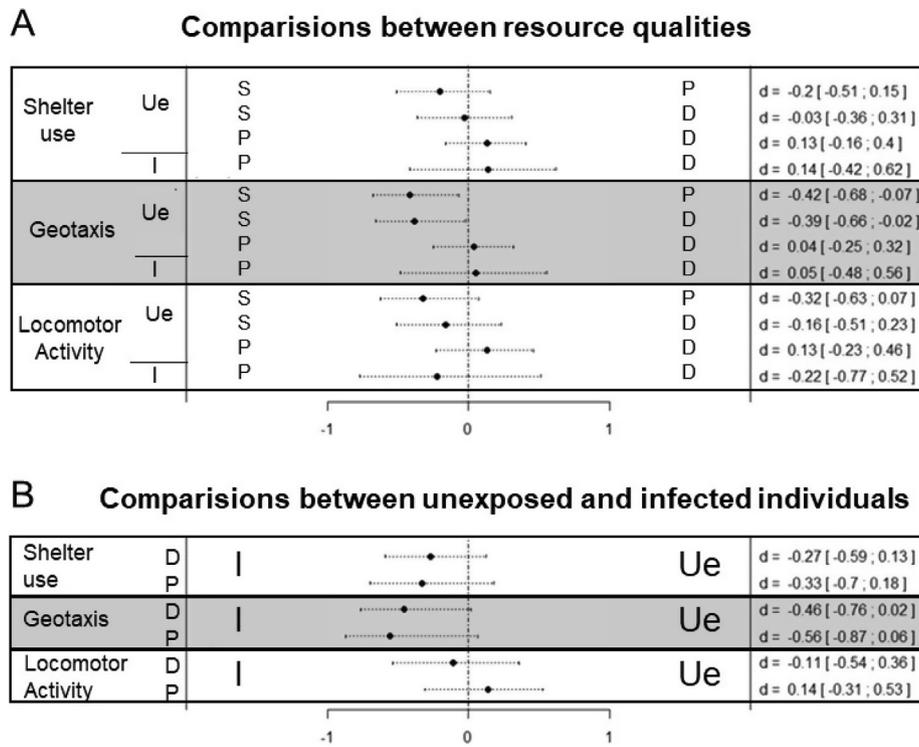
Fig. 6. Effect sizes (Cliff's delta, d) of the behavioral scores (shelter use, geotaxis, locomotor activity) of *Gammarus pulex* (A) between resource qualities and for the different infection statuses (infected (I) and unexposed (Ue)), and (B) between I and Ue individuals, for each test (geotaxis, shelter use and locomotor activity) for each resource quality (P, D). Treatment names are described in the legend of Fig. 3. Values of Cliff's delta effect sizes are given with their 95% confidence intervals. Values inferior to 0 (dotted line) show that the behavioral score was higher for the group specified on the left, whereas values above zero show higher scores for the group mentioned on the right. The difference is significant when the bar does not overlap zero.

changes). However, this result must be interpreted with caution due to the low statistical power generated by the low survival of infected gammarids fed with standard food resources. Results of Franceschi et al. (2010) suggested a trade-off between *P. laevis* growth rate and behavioral manipulation. However, working on the same host and parasite species, Labaude et al. (2015b) did not report any effect of resource quality on parasite growth. Finally, although resource quality had no amplification effect on parasite-mediated host manipulation, resource quality had a small but significant effect on the geotaxis of uninfected gammarids. As proposed by Arce-Funck et al. (2016), who observed a change in (another) behavior following increased food quality, the observed effect on geotaxis probably relies on the higher energetic content of well-fed organisms.

Thus, the results of the current study revealed that higher quality resources, regardless of infection status, reduced *G. pulex* mortality (hypothesis 1), whereas only uninfected gammarids exhibited higher growth rates when fed high quality resources (partial validation of hypothesis 2). In addition, higher quality resources increased the infection rates of parasites (verifying hypothesis 3). Although slightly modifying the geotaxis behavior of uninfected organisms (partially verifying hypothesis 4), resource quality did not modulate the impacts of parasites on host behavior (rejecting hypothesis 5). Finally, for most of the parameters investigated, consumption of algal resources had a greater impact than consumption of phosphorus-enriched leaf litter. Therefore, our study confirmed that, in addition to changes in resource quantity, changes in resource quality might have a major role in parasite development. This stresses the need for future research to investigate in natura the relationships between resource availability, resource quality and parasite prevalence. In particular, it might be important to verify whether rivers with higher algal production (e.g. those with open canopies; Feio et al., 2010) exhibit higher

parasite prevalence. Moreover, given that all algal groups do not have the same biochemical qualities (e.g. chlorophytes and cyanobacteria lack EPA, Bec et al., 2010) and that diatoms are progressively replaced by chlorophytes or cyanobacteria along nutrient load gradients (Leflaive et al., 2015), stream eutrophication might lead first to increased parasite prevalence until a decline related to shifts in algal communities occurs. Finally, it would be interesting to evaluate whether parasites could modify the feeding behavior of their hosts (food selection) toward food with high phosphorus and/or algal contents, to allow them better development. In this context, using the conceptual frameworks of trophic ecology, such as ecological stoichiometry (Bernot and Poulain, 2018), might enable us to understand in more depth the host–parasite interaction in ecosystems, and the impact that the anthropogenically driven changes might have on these interactions. Such research might be especially important when considering the important role of parasites in ecological processes, and the resulting ecosystem services (Lafferty and Kuris, 2012).

References

Aalto, S.L., Pulkkinen, K., 2013. Food stoichiometry affects the outcome of *Daphnia*–parasite interaction. Ecol. Evol. 3 (5), 1266–1275.

Aalto, S.L., Decaesestecker, E., Pulkkinen, K., 2015. A three-way perspective of stoichiometric changes on host-parasite interactions. Trends Parasitol. 31, 333–340.

Altman, I., Byers, J.E., 2014. Large-scale spatial variation in parasite communities influenced by anthropogenic factors. Ecology 95, 1876–1887.

Arce-Funck, J., Crenier, C., Danger, M., Cossu-Leguille, C., Guérold, F., Felten, V., 2016. Stoichiometric constraints modulate impacts of silver contamination on stream detritivores: an experimental test with *Gammarus fossarum*. Freshwater Biol. 61, 2075–2089.

Arce-Funck, J., Crenier, C., Danger, M., Billoir, E., Usseglio-Polatera, P., Felten, V., 2018. High stoichiometric food quality increases moulting organism vulnerability to pollutant impacts: an experimental test with *Gammarus fossarum* (Crustacea: Amphipoda). Sci. Total Environ. 645, 1484–1495.

Arts, M.T., Brett, M.T., Kainz, M. (Eds.), 2009. Lipids in Aquatic Ecosystems. Springer Science & Business Media, New York.

Bakker, T., Mazzi, D., Zala, S., 1997. Parasite-induced changes in behavior and color make *Gammarus pulex* more prone to fish predation. Ecology 78 (4), 1098–1104.

Bauer, A., Haine, E.R., 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*. J. Zool. 267 (1), 39–43.

Bec, A., Martin-Creuzburg, D., von Elert, E., 2006. Trophic upgrading of autotrophic picoplankton by the heterotrophic nanoflagellate *Paraphysomonas sp.* Limnol. Oceanogr. 51 (4), 1699–1707.

Bec, A., Perga, M.-E., Desvilettes, C., Bourdier, G., 2010. How well can the fatty acid content of lake seston be predicted from its taxonomic composition? Freshwater Biol. 55 (9), 1958–1972.

Bernot, R.J., Poulain, R., 2018. Ecological stoichiometry for parasitologists. Trends Parasitol. 34 (11), 928–933.

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

Brown, A.F., Pascoe, D., 1989. Parasitism and host sensitivity to cadmium: an acanthocephalan infection of the freshwater amphipod *Gammarus pulex*. J. Appl. Ecol. 26, 473–487.

Caddigan, S.C., Barkauskas, R.T., Sparkes, T.C., 2014. Intra-population variation in behavior modification by the acanthocephalan *Acanthocephalus dirus*: are differences mediated by host condition? Parasitol. Res. 113, 4307–4311.

Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., Smith, V.H., 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol. Appl. 8 (3), 559–568.

Civitello, D.J., Penczykowski, R.M., Smith, A.N., Shocket, M.S., Duffy, M.A., Hall, S.R., 2015. Resources, key traits, and the size of fungal epidemics in *Daphnia* populations. J. Anim. Ecol. 84 (4), 1010–1017.

Cliff, N., 1996. Ordinal Methods for Behavioral Data Analysis. Lawrence Erlbaum Associates Inc, New Jersey.

Crenier, C., Arce-Funck, J., Bec, A., Perrière, F., Billoir, E., Leflaive, J., Guérold, F., Felten, V., Danger, M., 2017. Minor food sources can play a major role in secondary production in detritus-based ecosystems. Freshwater Biol. 62, 1155–1167.

Crompton, D.W.T., Nickol, B.B. (Eds.), 1985. Biology of the Acanthocephala. Cambridge University Press, Cambridge.

Cross, W.F., Benstead, J.P., Rosemond, A.D., Wallace, J.B., 2003. Consumer-resource stoichiometry in detritus-based streams. Ecol. Lett. 6 (8), 721–732.

Danger, M., Arce-Funck, J., Devin, S., Heberle, J., Felten, V., 2013. Phosphorus content in detritus controls life-history traits of a detritivore. Funct. Ecol. 27, 807–815.

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 acanthocephalan *Pomphorhynchus laevis*. Int. J. Parasitol. 44, 211–216.

Dobson, A., Lafferty, K.D., Kuris, A.M., Hechinger, R.F., Jetz, W., 2008. Homage to Linnaeus: how many parasites? How many hosts? P.N.A.S. 105 (1), 11482–11489.

Elser, J.J., Hayakawa, K., Urabe, J., 2001. Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. Ecology 82, 898–903.

Elser, J.J., Dobberfuhl, D., MacKay, N.A., Schampel, J.H., 1996. Organism size, life history, and N: P stoichiometry: towards a unified view of cellular and ecosystem processes. BioScience 46, 674–684.

Elser, J.J., Acharya, K., Kyle, M., Cotner, J., Makino, W., Markow, T., Watts, T., Hobbie, S., Fagan, W., Schade, J., Hood, J., Sterner, R.W., 2003. Growth rate-stoichiometry couplings in diverse biota. Ecol. Lett. 6, 936–943.

Feio, M.J., Alves, T., Boavida, M., Medeiros, A., Graça, M.A.S., 2010. Functional indicators of stream health: a river-basin approach. Freshwater Biol. 55 (5), 1050–1065.

Folch, J., Lees, M., Stanley, H.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497–509.

Franceschi, N., Bauer, A., Bollache, L., Rigaud, T., 2008. The effects of parasite age and intensity on variability in acanthocephalan-induced behavioural manipulation. Int. J. Parasitol. 38, 1161–1170.

Franceschi, N., Bollache, L., Corne, S., Bauer, A., Motreuil, S., Rigaud, T., 2010. Co-variation between the intensity of behavioural manipulation and parasite development time in an acanthocephalan–amphipod system. J. Evol. Biol. 23 (10), 2143–2150.

Frost, P.C., Elser, J.J., 2002. Growth responses of littoral mayflies to the phosphorus content of their food. Ecol. Lett. 5, 232–240.

Frost, P.C., Ebert, D., Smith, V.H., 2008a. Bacterial infection changes the elemental composition of *Daphnia magna*. J. Anim. Ecol. 77 (6), 1265–1272.

Frost, P.C., Ebert, D., Smith, V.H., 2008b. Responses of a bacterial pathogen to phosphorus limitation of its aquatic invertebrate host. Ecology 89 (2), 313–318.

Fuller, C.L., Evans-White, M.A., Entrekin, S.A., 2015. Growth and stoichiometry of a common aquatic detritivore respond to changes in resource stoichiometry. Oecologia 177 (3), 837–848.

Gergs, R., Steinberger, N., Basen, T., Martin-Creuzburg, D., 2014. Dietary supply with essential lipids affects growth and survival of the amphipod *Gammarus roeselii*. Limnologica 46, 109–115.

Gessner, M.O., Chauvet, E., 1993. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. Appl. Environ. Microbiol. 59, 502–507.

Gismondi, E., Cossu-Leguille, C., Beisel, J.-N., 2012. Does the acanthocephalan parasite *Polymorphus minutus* modify the energy reserves and antitoxic defences of its intermediate host *Gammarus roeseli*? Parasitology 139 (8), 1054–1061.

Grabner, D.S., 2017. Hidden diversity: parasites of stream arthropods. Freshwater Biol. 62 (1), 52–64.

González, A.L., Romero, G.Q., Srivastava, D.S., 2014. Detrital nutrient content determines growth rate and elemental composition of bromeliad-dwelling insects. Freshwater Biol. 59 (4), 737–747.

Hladyz, S., Gessner, M.O., Giller, P.S., Pozo, J., Woodward, G., 2009. Resource quality and stoichiometric constraints on stream ecosystem functioning. Freshwater Biol. 54, 957–970.

Hladyz, S., Åbjörnsson, K., Chauvet, E., Dobson, M., Elosegi, A., Ferreira, V., Fleituch, T., Gessner, M.O., Giller, P.S., Gulis, V., Hutton, S.A., Lacoursière, J.O., Lamothe, S., Lecerf, A., Malmqvist, B., McKie, B.G., Nistorescu, M., Preda, E., Riipinen, M.P., Rîşnoveanu, G., Schindler, M., Tiegs, S.D., Vought, L.B.M., Woodward, G., 2011. Stream ecosystem functioning in an agricultural landscape: the importance of terrestrial-aquatic linkages. Adv. Ecol. Res. 44 (3), 211–276.

Holmes, J.C., Bethel, W.M., 1972. Modification of intermediate host behaviour by parasites. In: Canning, E.U., Wright, C.A. (Eds.), Behavioural Aspects of Parasite Transmission. Academic Press, London, pp. 123–149.

Johnson, P.T., Townsend, A.R., Cleveland, C.C., Glibert, P.M., Howarth, R.W., McKenzie, V.J., Rejmankova, E., Ward, M.H., 2010. Linking environmental nutrient enrichment and disease emergence in humans and wildlife. Ecol. Appl. 20 (1), 16–29.

Kennedy, C.R., 2006. Ecology of the Acanthocephala. Cambridge University Press, Cambridge.

Kilham, S.S., Kreeger, D.A., Lynn, S.G., Goulden, C.E., Herrera, L., 1998. COMBO: A defined freshwater culture medium for algae and zooplankton. Hydrobiologia 377, 147–159.

Kraufvelin, P., Salovius, S., Christie, H., Moy, F.E., Karez, R., Pedersen, M.F., 2006. Eutrophication-induced changes in benthic algae affect the behaviour and fitness of the marine amphipod *Gammarus locusta*. Aquat. Bot. 84, 199–209.

Labaude, S., Rigaud, T., Cézilly, F., 2015a. Host manipulation in the face of environmental changes: ecological consequences. Int. J. Parasitol. 4 (3), 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*. Parasit. Vectors 8 (1), 403.

Labaude, S., Rigaud, T., Cézilly, F., 2017a. Additive effects of temperature and infection with an acanthocephalan parasite on the shredding activity of *Gammarus fossarum* (Crustacea: Amphipoda): the importance of aggregative behavior. Glob. Chang. Biol. 23 (4), 1415–1424.

Labaude, S., Cézilly, F., Tercier, X., Rigaud, T., 2017b. Temperature-related intraspecific variability in the behavioral manipulation of acanthocephalan parasites on their gammarid hosts. Biol. Bull. 232 (2), 82–90.

Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., De Leo, G., Dobson, A.P., Dunne, J.A., Johnson, P., Kuris, A., Marcogliese, D.J., Martinez, N., Memmott, J., Marquet, P.A., McLaughlin, J., Mordecai, E.A., Pascual, M., Poulin, R., Thieltges, D., 2008. Parasites in food webs: the ultimate missing links. Ecol. Lett. 11, 533–546.

Labaude, S., 2016. Effect of the environment on the interaction between gammarids (Crustacea: Amphipoda) and their manipulative acanthocephalan parasites. Thesis of the University of Burgundy, 235.

Lafferty, K.D., Kuris, A.M., 2012. Ecological consequences of manipulative parasites. In: Hughes, D.P., Brodeur, J., Thomas, F. (Eds.), Host manipulation by parasites. Oxford University Press; Oxford, pp. 158–171.

Lagrue, C., Kaldonski, N., Perrot-Minnot, M.-J., Motreuil, S., Bollache, L., 2007. Modification of the hosts' behavior by a parasite: field evidence for adaptive manipulation. Ecology 88 (11), 2839–2847.

Leflaive, J., Felten, V., Ferriol, J., Lamy, A., Ten-Hage, L., Bec, A., Danger, M., 2015. Community structure and nutrient level control the tolerance of autotrophic biofilm to silver contamination. Environ. Sci. Poll. R. 18, 13739–13752.

Lellis, W.A., Barrows, F.T., Hardy, R.W., 2004. Effects of phase-feeding dietary phosphorus on survival, growth, and processing characteristics of rainbow trout *Oncorhynchus mykiss*. Aquaculture 242, 607–616.

Lettini, S.E., Sukhdeo, M.V.K., 2010. The energetic cost of parasitism in isopods. Ecoscience 17, 1–8.

Makino, W., Urabe, J., Elser, J.J., Yoshimizu, C., 2002. Evidence of phosphorus-limited individual and population growth of *Daphnia* in a Canadian Shield lake. Oikos 96, 197–205.

Maltby, L., Clayton, S.A., Wood, R.M., McLoughlin, N., 2002. Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: robustness, responsiveness, and relevance. Environ. Toxicol. Chem. 21 (2), 361–368.

Marcarelli, A.M., Baxter, C.V., Mineau, M.M., Hall, R.O., 2011. Quantity and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. Ecology 92 (6), 1215–1225.

Marcogliese, D.J., Pietrock, M., 2011. Combined effects of parasites and contaminants on animal health: parasites do matter. Trends Parasitol. 27 (3), 123–130.

Masclaux, H., Bec, A., Kainz, M., Desvilettes, C., Jouve, L., Bourdier, G., 2009. Combined effects of food quality and temperature on somatic growth and reproduction of two freshwater cladocerans. Limnol. Oceanogr. 54, 1323–1332.

Maure, F., Brodeur, J., Hughes, D.P., Thomas, F., 2013. How much energy should manipulative parasites leave to their hosts to ensure altered behaviours? J. Exp. Biol. 216, 43–46.

Moore, J., 2002. Parasites and host behaviour. Trends Ecol. Evol. 17, 585–586.

Moore, A.A., Palmer, M.A., 2005. Invertebrate biodiversity in agricultural and urban headwater streams: implications for conservation and management. Ecol. Appl. 15 (4), 1169–1177.

Muller-Navarra, D.C., Brett, M.T., Park, S., Chandra, S., 2004. Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. Nature 427 (6969), 69.

Naiman, R.J., Decamps, H., McClain, M.E., 2010. Riparia: Ecology, Conservation, and Management of Streamside Communities. Elsevier Academic Press, London.

Perrot-Minnot, M.-J., Sanchez-Thirion, K., Cézilly, F., 2014. Multidimensionality in host manipulation mimicked by serotonin injection. Proc. R. Soc. Lond. B Biol. Sci. 281, 20141915.

Peterson, B.J., Wollheim, W.M., Mulholland, P.J., Webster, J.R., Meyer, J.L., Tank, J.L., Marti, E., Bowden, W.B., Valett, H.M., Hershey, A.E., McDowell, W.H., Dodds, W. K., Hamilton, S.K., Gregory, S., Morrall, D.D., 2001. Control of nitrogen export from watersheds by headwater streams. Science 292 (5514), 86–90.

Plaistow, S.J., 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*. Int. J. Parasitol. 31, 346–351.

Power, M.E., 1992. Top-down and bottom-up forces in food webs: do plants have primacy. Ecology 73 (3), 733–746.

Prater, C., Wagner, N.D., Frost, P., 2016. Effects of calcium and phosphorus limitation on the nutritional ecophysiology of *Daphnia*. Limnol. Oceanogr. 61, 268–278.

Rollin, M., Coulaud, R., Danger, M., Sohm, B., Flayac, J., Bec, A., Chaumot, A., Geffard, O., Felten, V., 2018. Additive effect of calcium depletion and low resource quality on *Gammarus fossarum* (Crustacea, Amphipoda) life history traits. Environ. Sci. Poll. R. 25, 11264–11280.

Schade, J., Kyle, M., Hobbie, S., Fagan, W., Elser, J.J., 2003. Stoichiometric tracking of soil nutrients by a desert insect herbivore. Ecol. Lett. 6, 91–101.

Schlotz, N., Sørensen, J.G., Martin-Creuzburg, D., 2012. The potential of dietary polyunsaturated fatty acids to modulate eicosanoid synthesis and reproduction in *Daphnia magna*: a gene expression approach. Comp. Biochem. Physiol. A 162, 449–454.

Schlotz, N., Ebert, D., Martin-Creuzburg, D., 2013. Dietary supply with polyunsaturated fatty acids and resulting maternal effects influence host-parasite interactions. BMC Ecol. 13, 41.

Sims, J.T., Simard, R.R., Joern, B.C., 1998. Phosphorus loss in agricultural drainage: Historical perspective and current research. J. Environ. Qual. 27 (2), 277–293.

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. Environ. Poll. 158, 1288–1295.

Stanley-Samuelson, D.W., 2006. Prostaglandins and other eicosanoids in insects: biological significance. Annu. Rev. Entomol. 51 (1), 25–44.

Sterner, R.W., Elser, J.J., 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press, Princeton, USA.

Stephens, J.P., Altman, K.A., Berven, K.A., Tiegs, S.D., Raffel, T.R., 2017. Bottom-up and trait-mediated effects of resource quality on amphibian parasitism. J. Anim. Ecol. 86 (2), 305–315.

Tain, L., Perrot-Minnot, M.-J., Cézilly, F., 2006. Altered host behaviour and brain serotonergic activity caused by acanthocephalans: evidence for specificity. Proc. R. Soc. Lond. B Biol. Sci. 273, 3039–3045.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., Gushing, E., 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37, 130–137.

Wright, A.N., Gompper, M.E., 2005. Altered parasite assemblages in raccoons in response to manipulated resource availability. Oecologia 144 (1), 148–156.