Parasite-induced changes in the diet of a freshwater amphipod: field and laboratory evidence

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SUMMARY

Trophically transmitted parasites are likely to strongly influence food web-structure. The extent to which they change the trophic ecology of their host remains nevertheless poorly investigated and field evidence is lacking. This is particularly true for acanthocephalan parasites whose invertebrate hosts can prey on other invertebrates and contribute to leaf-litter breakdown. We used a multiple approach combining feeding experiments, neutral lipids and stable isotopes to investigate the trophic ecology of the freshwater amphipod *Gammarus roeseli* parasitized by the bird acanthocephalan *Polymorphus minutus*. Infected compared to uninfected amphipods consumed as many dead isopods, but fewer live isopods and less leaf material. Infection had no influence on the total concentration of neutral lipids. Contrary to what we expected based on laboratory findings, the nitrogen isotope signature, which allows for the estimation of consumer's trophic position, was not influenced by infection status. Conversely, the carbon isotope signature, which is used to identify food sources, changed with infection and suggested that the diet of infected *G. roeseli* includes less perilithon (i.e. fixed algae on rocks, stones) but more terrestrial inputs (e.g. leaf material) than that of uninfected conspecifics. This study shows evidence of changes in the trophic ecology of *P. minutus*-infected *G. roeseli* and we stress the need to complement feeding experiments with field data when investigating top-down effects of infection in an opportunistic feeder which adapts its diet to the available food sources.

Key words: *Gammarus roeseli*, manipulative parasite, neutral lipid contents, *Polymorphus minutus*, stable isotopes, trophic ecology.

INTRODUCTION

Since it is recognized that probably all living organisms are concerned with parasitism, either as hosts or as parasites (May, 1992; Thompson, 1994; Windsor, 1998; Poulin and Morand, 2000), researchers attempt to integrate parasitology and ecology to provide a better understanding of ecosystem functioning. Recent findings have shed light on how parasites modulate food-web length and community structure (for study cases see: Thompson *et al*. 2005; Hernandez and Sukhdeo, 2008*a*; Amundsen *et al*. 2009; for reviews see: Lafferty *et al*. 2006, 2008), and stress that these effects increase with the complexity of their life-cycle (simply because the more complex the cycle, the greater the number of organisms concerned with infection). The ecological significance of parasites is even more obvious for those whose transmission relies on the predation of an intermediate host by a definitive host (trophically transmitted parasites, Marcogliese, 2004). The reason for this is that most of the infected intermediate hosts display physiological, morphological, and/or behavioural alterations, and the manipulation hypothesis predicts that some of these parasite-induced changes are parasite adaptations to increase predation risk by definitive hosts (Holmes and Bethel, 1972; see Combes, 1991 and Moore, 2002 for reviews).

In their recent review on the ecological significance of manipulative parasites, Lefèvre *et al*. (2008) described how they affect apparent competition phenomena (e.g. competitor A better exploits the resource than competitor B but the reverse is true when they share a parasite), energy flow along food chains, and habitat creation. In most of the studies devoted to 'manipulated' food webs, the authors have investigated the relationship between infected intermediate hosts and upper-trophic-level species to test the predictions of the manipulation hypothesis that infection should make the intermediate host more susceptible to predation, hence strengthening the trophic links involved in transmission (Moore, 2002; Perrot-Minnot and Cézilly, 2009). As a consequence, infected intermediate hosts have been considered as potential predators, or at least, consumers, only in a few cases. The 'top-down influences' of infection (i.e. consequences downstream in the food chain) may

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nevertheless be of ecological importance when intermediate hosts are also key species in ecosystem functioning. For instance, in headwater streams, 20 to 73% of riparian leaf-litter inputs are estimated to be processed by benthic macroinvertebrates (Wallace and Webster, 1996; Covich *et al*. 1999). Infection with *Acanthocephalus tahlequahensis* (Acanthocephala: Echinorhynchidae) has been shown to significantly decrease the detritus consumption of the isopod *Caecidotea communis*, which thereby affects both the recycling of dead organic material and the dynamic of invertebrates using the material processed by isopods as food or habitat (Hernandez and Sukhdeo, 2008*a*,*b*).

Amphipods, which are also common hosts of various manipulative parasites, are considered as key species in this functional process (Dangles and Malmqvist, 2004; Piscart *et al*. 2009*b*). For instance, Piscart *et al*. (2009*b*) have shown that their leaf-litter breakdown activity represents about 75% of the overall leaf-litter recycling in the streams of Western France. However, the functional role of amphipods is not limited to their shredding activity, as several studies pointed out the predatory behaviour of *Gammarus* species (Dick, 1996; MacNeil *et al*. 1997; Kelly *et al*. 2002*a*,*b*, 2003, 2006; Piscart *et al*. 2009*a*). They can thus influence the size, location, growth and reproduction of prey populations, and such effects on lower trophic level organisms may be even greater since amphipods have been found to predominate the macroinvertebrate fauna in terms of biomass in many riverine communities (MacNeil *et al*. 1997; Dangles and Malmqvist, 2004; Piscart *et al*. 2009*b*).

Despite the previous arguments, the feeding ecology of infected amphipods has received little interest. Crompton (1970) reported an increased food intake in *Gammarus pulex* when infected with the bird acanthocephalan *Polymorphus minutus*. On the other hand, *G. pulex* infected with the fish acanthocephalan *Pomphorhynchus laevis* takes longer to consume leaf material (McCahon *et al*. 1988), or brine shrimp eggs (Pascoe *et al*. 1995), than uninfected conspecifics. Similarly, infection of *G. pulex* with *Echinorhynchus truttae*, another fish acanthocephalan, has been shown to decrease the tendency to kill live *G. duebeni celticus* (MacNeil *et al*. 2003), and either to decrease (Fielding *et al*. 2003, using a single prey density) or increase (Dick *et al*. 2010, using the functional response approach) the tendency to kill live *Asellus aquaticus*. Such results suggest that infection may affect the regulation of prey populations but it is premature to draw conclusions about the top-down influences of parasitism at the community level when our knowledge of the predatory behaviour of infected hosts is based on laboratory findings only.

Here, we combined laboratory and field data to assess how infection with the acanthocephalan parasite *P. minutus* affects the trophic ecology of *Gammarus roeseli* (Gervais, 1835), a freshwater amphipod of Balkan-European origin which is widespread throughout Western Europe (Karaman and Pinkster, 1977; Barnard and Barnard, 1983). *P. minutus* is a trophically transmitted parasite which exploits amphipods as intermediate hosts and waterbirds as definitive hosts (Kennedy, 2006). Infection with *P. minutus* is known to influence *G. roeseli*'s distribution (Médoc and Beisel, 2009), reproduction (Dezfuli *et al*. 2008), physiology (Piscart *et al*. 2007; Sures and Radszuweit, 2007), and behaviour (Marriott *et al*. 1989; Bauer *et al*. 2005; Médoc *et al*. 2006, 2009; Médoc and Beisel, 2008), but nothing has been reported regarding its trophic ecology once infected.

We performed microcosm experiments under laboratory conditions to measure the consumption rates of uninfected and *P. minutus*-infected amphipods when feeding either leaf material, live or dead isopods (*Asellus aquaticus*). In the case where amphipods need to compensate for the cost of infection by increasing their food intake, we should observe a higher overall consumption activity in infected than in uninfected amphipods. However, the reverse could be observed if the parasite shifts the host's energy balance from costly feeding behaviours such as killing live prey to behaviours that are more conducive to transmission (e.g. swimming towards the water surface, Médoc *et al*. 2006). To get field information on the trophic ecology *P. minutus*-infected *G. roeseli*, we quantified the different neutral lipid classes of amphipods, namely triglycerides and free fatty acids, based on the assumption that feeding activities should be positively correlated to stocks of neutral lipids. We also determined the carbon (C) and nitrogen (N) isotopic signatures of amphipods and all the available food sources since they are known to indicate, respectively, the food sources and the trophic level of a consumer (DeNiro and Epstein, 1978; Peterson and Fry, 1987). We expected the $^{15}N/^{14}N$ ratio to be consistent with our laboratory findings on the predatory behaviour of amphipods, with lower values for infected compared to uninfected amphipods if infection reduces predation, or vice versa. We also expected the $^{13}C/^{12}C$ ratio to be influenced by the habitat shift induced by infection with *P. minutus* (see Médoc and Beisel, 2009), infected amphipods being mostly found in floating organic material.

MATERIALS AND METHODS

Feeding experiments

Amphipod sampling. Amphipods were collected with a 500 μm mesh pond net in the river Nied (Laquenexy, north-eastern France, 49°05′ N and 6°19′ E), where the prevalence of *P. minutus* exceeds 10% (Médoc *et al*. 2006). Concerning infected

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G. roeseli, we focused our sampling effort on floating materials since they are described as their main habitat in the study site (Médoc and Beisel, 2009). On the contrary, uninfected amphipods are widespread throughout the natural habitats of the study site (Médoc and Beisel, 2009), so we sampled all these habitats and caught the amphipods we needed from this subsample to avoid any habitat effect. All the amphipods used in this study (i.e. feeding experiments, neutral lipid and stable isotope analyses) were sampled in the same way. The yellow-orange cystacanth (the infective stage of *P. minutus* inside its intermediate host), visible through the invertebrate's translucent cuticle, distinguished infected *G. roeseli* from their uninfected counterparts. To avoid size or parasitic-load effects, only medium-sized amphipods (9 ± 1 mm in total length, from the tip of the rostrum to the base of the telson) harbouring 1 cystacanth were selected. We did not make distinction between the sexes.

In the laboratory, infected and uninfected individuals for feeding experiments were maintained separately in plastic tanks (33 cm long × 25 cm wide × 13 cm high) at a density of 20 ind./L. Each tank was filled with filtered site-water and equipped with glass pebbles and artificial plants to reduce cannibalism. The oxygen demand was supplied by a filter pump that also generated a flow inside the tanks. Alder-leaf discs (Ø: 20 mm) were provided to satiation as the sole food source. Amphipods were deprived of food for 24 h before experiments to standardize consumption rates. Housing took place in a thermo-regulated room to ensure a stable water temperature (14 ± 1 °C) and the alternating periods of light and dark were each 12 h.

Isopod consumption. The isopod *Asellus aquaticus* was collected by hand sorting in the river Moselle (Metz, North-eastern France, 49°07′ N and 6°10′ E). We deliberately chose a prey that is absent from the location where *G. roeseli* was collected in order to use naïve prey during predation tests (i.e. no common history between the predator and its prey). Furthermore, isopods are common prey of amphipods of the *Gammarus* genus (MacNeil *et al.* 1997). Medium-sized isopods were selected regardless of the sex (6 ± 1 mm in total length), but gravid females were excluded to avoid the confounding influence of eggs on prey consumption. In the laboratory, they were maintained for 15 days under the regime described above for amphipods to acclimatize them to the water of the river Nied.

The experimental design consisted of 10 aquaria (33 cm long × 25 cm wide × 13 cm high), each with an air pump, enclosed between 2 hermetically fixed, porous partitions. Each aquarium was thus equally divided into 2 Experimental Units (EU). This design was expected to protect individuals from air-pump perturbations while allowing for proper oxygenation and water circulation. Each EU (13 cm long × 14·5 cm wide × 25 cm high) contained 2·3 L of filtered water drawn from the river Nied and 20 translucent glass pebbles (Ø: ~15 mm) were placed on the bottom, convex size down, to provide a standard substrate. The aquaria were placed inside a wide vat (140 cm long × 110 cm wide × 40 cm high) filled with tap water and equipped with a cooler (Huber TC40E) and a pump to ensure water circulation. This device acted as a 'water bath' system allowing a high degree of thermal stability among the various EUs (14 ± 1 °C), water temperature being checked regularly. The experimental design was illuminated by 4 light tubes (Philips, 36 W) mounted 60 cm above the water's surface, with alternating periods of light and dark each being 12 h.

Twelve isopods were placed inside each of the 16 EUs. After a 30-min acclimatization period, 8 uninfected amphipods were added to half of the EUs while the other half received 8 *P. minutus* infected amphipods (i.e. $N = 8$ per infection status). One control group of 4 EUs with isopods alone allowed to monitor for deaths due to experimental conditions. To test whether *P. minutus* infection affects *G. roeseli*'s tendency to kill live prey or its affinity for this prey type, the same feeding experiment was repeated by replacing live isopods with dead individuals previously sacrificed by thermal shock (immersion in water at 45 °C for 30 s) ($N_{\text{replicates}} = 12$ per infection status). The EUs were checked regularly in search of dead amphipods and the number of totally consumed isopods was recorded after a 48-h exposure to *G. roeseli*. At the end of the experiments, amphipods were dissected to confirm parasitic load and infection status.

Leaf consumption. Freshly fallen alder leaves (*Alnus glutinosa*) were collected from the natural habitat and 220 disks were cut using a cork borer (Ø: 18 mm). All the disks were dried in an oven at 60 °C for 48 h and pooled in groups of 5, corresponding to the number of disks amphipods were allowed to feed on during the experiment. Each group was weighed to the nearest mg to determine the amount of leaf material provided to amphipods at the beginning of the experiment, and placed in stream water using 250 μm-mesh net bags for 2 weeks prior to the experiment to establish fungal and bacterial biofilms on the disks. A net of 250 μm-mesh prevented invertebrate colonization. When conditioned, the disks were distributed in 44 top-opened cylindrical EUs (Ø: 11 cm, 1 group of 5 disks per EU) each equipped with 4 glass pebbles serving as a refuge for amphipods. The EUs were placed into a large vat (the same as for predation tests) filled with aerated stream water. The bottom of each EU was made of a 1 mm-mesh net and all the EUs were placed on a rigid grid 15 cm above the bottom of the vat to allow the supply of the aerated stream water to each EU (≈0·76 L of water per EU).

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This design was expected to minimize the sources of disturbance while allowing standard conditions between EUs.

The experiment started with the addition of medium-sized amphipods: 3 uninfected in 20 randomly selected EUs and 3 infected in 20 others. Four supplementary EUs were free of *G. roeseli* to control for disk weight loss resulting from leaching. Four EUs per infection status (i.e. $N_{\text{replicates}}=4$) were removed at 5 time-intervals: 4, 8, 12, 24 and 48 h. Amphipods and disks were washed and dried (48 h at 60 °C) to determine individual feeding rates (i.e. the dry weight of leaf consumed divided by the dry weight of amphipods).

Neutral lipid analysis

The concentration of the different neutral lipid classes and especially triglycerides was used as a measure of nutritional condition (Fraser *et al*. 1987; Napolitano and Ackman, 1989; Falk-Petersen *et al*. 2001). The use of neutral lipids was hence more powerful than the total lipid content which is an indicator of the physical condition of individuals (Plaistow *et al*. 2001). For each infection status, 30 medium-sized amphipods were sampled without distinction between the sexes from the river Nied within the same day. Since a single invertebrate provided insufficient lipid material, neutral lipids were analysed from the whole body of 3 pooled individuals ($N_{\text{replicates}}=10$ per infection status). Before the extraction, cystacanths were removed from infected specimens. Lipids were extracted according to the method described by Folch *et al*. (1957). Briefly, samples were homogenized in 20 vols of mixture of chloroform–methanol (2:1, v/v) and aliquots removed for protein determination (Bradford, 1976) (after evaporation of the solvent). Samples were filtered and washed twice with water containing 0·25% KCl (w/v). The chloroform lower phase was evaporated and total lipid contents were determined by weight. Neutral lipid classes, corresponding to triglycerides, free fatty acids, and cholesterol were resolved from each other on thin layer chromatography (TLC) plates using hexane-diethyl ether–acetic acid (80:20:1, v:v:v) as the developing solvent. The TLC plate was subsequently dried and neutral lipid contents were revealed after spraying with a solution of 10% cupric sulfate (w/v) in 8% phosphoric acid (v/v) and charred at 180 °C for 15 min. Neutral-lipid spots were identified using authentic standards.

Stable isotope study

Because the stable isotope ratios of C (13C/12C, expressed as δ13C) and N (15N/14N, δ15N) of a consumer depends on its diet (DeNiro and Epstein, 1978; Peterson and Fry, 1987), we determined the isotopic signatures of both infected and uninfected amphipods, and all the available food sources, to get field information on their trophic ecology. An enrichment in 15N is indeed observed between a consumer and its prey (Minigawa and Wada, 1984), thus allowing estimation of the consumer's trophic position (Post, 2002; Vanderklift and Ponsard, 2003). Conversely, δ13C varies between the different sources of C, for instance benthic *vs* pelagic, freshwater *vs* marine, or C-3 *vs* CAM/C-4 plants (Post, 2002; Bearhop *et al*. 2004), with little or no 13C enrichment along the food chain.

For each infection status, 15 medium-sized amphipods were sampled without distinction between the sexes from the river Nied within the same day ($N=15$). We also picked up 3 samples ($N_{\text{replicates}}=3$) of all the food sources available so as to have at least 0·5 and 1 mg of matter per sample (in dry weight) for animal and vegetal sources, respectively. This included 2 other macroinvertebrate consumers: *Baetis rhodani* (Ephemeroptera) and *Calopteryx* sp. (Odonata) larvae, 2 macrophytes: *Myriophyllum spicatum* and *Potamogeton pectinatus*, 2 terrestrial inputs: leaf litter and *Salix babylonica* roots, and the bacterial-fungal biofilm covering mineral substrates (perilithon, hereafter). Samples were frozen as soon as possible and stored at −20 °C until processing. All animals were defrosted and digestive tracts were removed and discarded. Cystacanths were removed from infected amphipods. Samples were cleaned with distilled water and acidified by adding of 0·5 ml of 1 M hydrochloric acid to remove inorganic carbon from the cuticle. The resulting material was oven-dried (60 °C for 48 h) and ground using a ball mill grinder (Mixer Mill MM 200, Retsch, Haan, Germany) to produce homogenous powder. Invertebrates were small (<10 mm) and/or lightweight (<7 mg dry weight) so whole bodies were analysed. Approximately 0·5 mg of each animal sample and 1 mg of each vegetation sample were weighed into tin cups. The stable-isotope ratios of C and N were measured using a continuous-flow stable isotope ratio mass spectrometer (CF-IRMS, Isoprime Ltd, Manchester, UK) interfaced with a Eurovector elemental analyser (EuroEA3028-HT) at the stable isotope geochemistry facility of the "PaléoEnvironnements et Paléobio-Sphère" laboratory (Lyon, France). Isotope ratios were reported as delta (δ) in part per thousand (‰) relative to international standard V-PDB (C) and atmospheric nitrogen (N). Repeated analyses of international standards (IAEA N1, USGS 25, IAEA CH6 and C3), along with 2 laboratory standards (tyrosine and triphenylamine), showed that the maximum standard deviations for δ^{15}N and δ^{13}C values were, respectively, 0·03‰ and 0·08‰. Standard deviations of samples analysed in duplicate ($n=4$) averaged 0·39‰ and 0·51‰ for C and N, respectively, and single measurements were carried out on all remaining samples.

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To quantify the food source contributions to consumer's diet, linear mixing models based on isotopic mass balance are commonly used (e.g. Phillips, 2001). For instance, using 2 isotopic signatures (δ^{15}N and δ^{13}C), the contribution (f) of 3 food sources (A, B, C) to the diet of a consumer (M) is calculated with the following 3 end-member mixing model:

$$\begin{aligned} \delta^{13}C_M &= f_A\delta^{13}C_A + f_B\delta^{13}C_B + f_C\delta^{13}C_C \\ \delta^{15}N_M &= f_A\delta^{15}N_A + f_B\delta^{15}N_B + f_C\delta^{15}N_C \\ f_A + f_B + f_C &= 1 \end{aligned} \quad (1)$$

For a dual isotope system, this model is underdetermined and no unique solution exists for more than 3 food sources.

Here, the IsoSource mixing model (Phillips and Gregg, 2003) was used to calculate the frequency distributions of feasible source contributions since the number of potential food sources exceeded 3 (leaf litter, *S. babylonica* roots, *Potamogeton pectinatus*, and perilithon). With this method, all possible combinations of each food source contribution (0–100%) are examined in small increments (1%) and those that sum to the observed mixture isotopic signatures within a small tolerance (0·1‰) are considered to be feasible solutions. Food sources were then combined using the aggregation method provided by Phillips *et al*. (2005) to reduce the number of sources. We pooled leaf litter and *S. babylonica* roots into a single 'terrestrial inputs' source since the frequency distributions generated by IsoSource were similar. The contribution of terrestrial inputs, *Potamogeton pectinatus*, and perilithon to *G. roeseli*'s diet was then calculated using the previous equations (1) (Phillips, 2001). Uncertainties about source proportions that derive from variability in the stable-isotope composition of consumers and sources were determined using IsoError 1.04 developed by Phillips and Gregg (2001). C and N isotope ratios had to be corrected for trophic fractionation before the use of the model. A trophic enrichment of 3·4‰ was chosen for δ^{15}N based on commonly reported values (e.g. Post, 2002), and we assumed no trophic fractionation for δ^{13}C between food sources and primary consumers (France, 1996).

Statistics

All data were examined for normality and homogeneity of variance using Shapiro-Wilk and Bartlett's tests, respectively. Because of the heterogeneity of variance, data concerning feeding experiments were tested non-parametrically. We used the Mann-Whitney *U*-test to assess differences in isopod or leaf consumption between infected and uninfected *G. roeseli*. Bonferroni's correction was used to account for the multiple comparisons. A Wilcoxon's signed rank test was used to compare the dry weight of control disks before and after the experiment. Data on lipids and isotopes met the assumptions underlying parametric tests. The total neutral-lipid concentration of uninfected amphipods was thus compared to that of infected ones with a Student's *t*-test. Differences among neutral-lipid classes were assessed using a multivariate analysis of variance (MANOVA), with infection status as independent variable and lipid classes as dependent variables. Concerning stable isotopes, we performed a one-way analysis of variance (ANOVA) with Tukey's HSD tests for multiple comparisons to compare the C and N ratios of both sources and consumers. Differences in the proportional contributions of organic matter sources to the diet of *G. roeseli* were assessed using Fisher's exact test. Statistical analyses were performed with Statistica software (v. 6.0, Statsoft Inc.). All tests were two-tailed and used a 5% type I error risk.

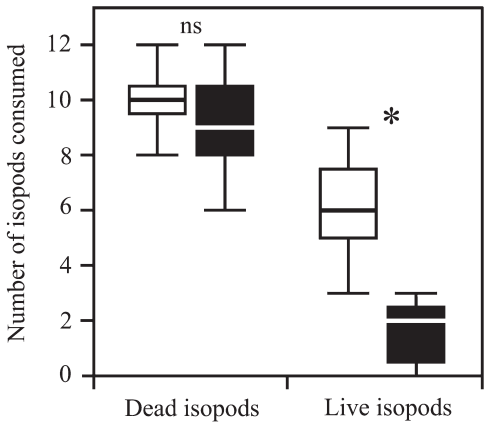

Fig. 1. Isopod consumption by *Gammarus roeseli* infected with *Polymorphus minutus*. The number of dead or live isopods consumed (median and interquartile range) was obtained for uninfected (white bars) and infected (black bars) amphipods at the end of a 48-h experiment. Significant differences in isopod consumption between the two infection statuses are indicated with an asterisk above bars (Mann-Whitney *U* tests, * $P \leqslant 0{\cdot}05$, ns: non significant).

RESULTS

Isopod consumption

There were no deaths during the experiment, neither for isopods in controls, nor for amphipods feeding on *A. aquaticus*. This means that mortality in isopods was caused by predation only, while amphipods did not exhibit cannibalism. Regardless of the infection status of *G. roeseli*, there was a higher number of isopods consumed when dead than when alive (Mann-Whitney's *U* tests, uninfected amphipods: $Z = 3{\cdot}43$, $P < 0{\cdot}001$, infected: $Z = 3{\cdot}70$, $P < 0{\cdot}001$, Fig. 1). This is simply because, in a given time-interval, it takes longer to kill and consume a live prey than to consume a dead animal. There was no significant difference in the number of dead isopods consumed between the two infection statuses ($Z = -1{\cdot}38$, $P = 0{\cdot}18$) whereas uninfected amphipods

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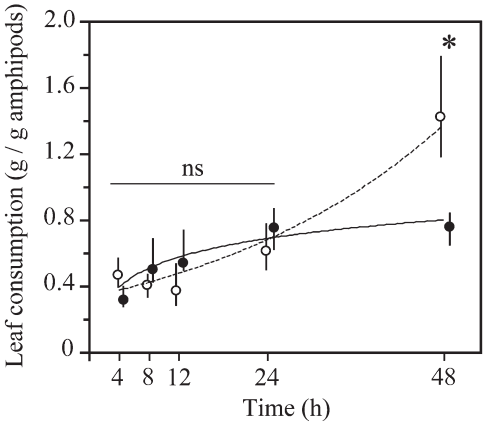

Fig. 2. Leaf consumption by *Gammarus roeseli* infected with *Polymorphus minutus*. The dry weight of leaf consumed per dry weight of amphipods (median and interquartile range) was obtained for uninfected (white dots) and infected (black dots) amphipods at different time-intervals. Adjustment curves (dotted for uninfected amphipods, solid for infected amphipods) were calculated with raw data. Significant differences in leaf consumption between the two infection statuses are indicated with an asterisk above dots (Mann-Whitney *U* tests without Bonferroni's correction, * $P \leqslant 0{\cdot}05$, ns: non significant).

consumed a higher number of live isopods than did infected ones ($Z = -3{\cdot}26$, $P < 0{\cdot}001$, Fig. 1).

Leaf consumption

The dry weight of control disks did not significantly vary during the experiment (Wilcoxon's signed rank test, $Z = 1{\cdot}46$, $P = 0{\cdot}14$), and there were no deaths of amphipods suggesting that they did not exhibit cannibalism. Overall, leaf consumption increased with time following an exponential relationship for uninfected amphipods ($R^2 = 0{\cdot}78$, $P < 0{\cdot}001$, $N = 20$), and a logarithmic relationship for infected ones ($R^2 = 0{\cdot}50$, $P < 0{\cdot}001$, $N = 20$, Fig. 2). There was no significant difference in leaf consumption between the two infection statuses within the first 24 h (Mann-Whitney's *U* tests at 4, 8, 12 and 24 h: all $P > 0{\cdot}05$) but, after a 48-h exposure, leaf consumption was 2-fold higher for uninfected amphipods (≈1·4 g/g amphipod) than for infected ones (≈0·75 g/g amphipod, $Z = 2{\cdot}31$, $P = 0{\cdot}028$, Fig. 2). Because of the limited number of replicates ($N = 4$ per infection status), this difference was not significant after Bonferroni's correction (0·05/5 = 0·01).

Lipid contents

The total neutral-lipid concentration of infected amphipods (20·62 ± 6·0 µg per 100 mg proteins) was not significantly different from that of uninfected amphipods (18·65 ± 5·0 µg per 100 mg proteins, Student's *t*-test, $t_{18} = 1{\cdot}4$, $P = 0{\cdot}621$). However, when comparing the mean concentrations of

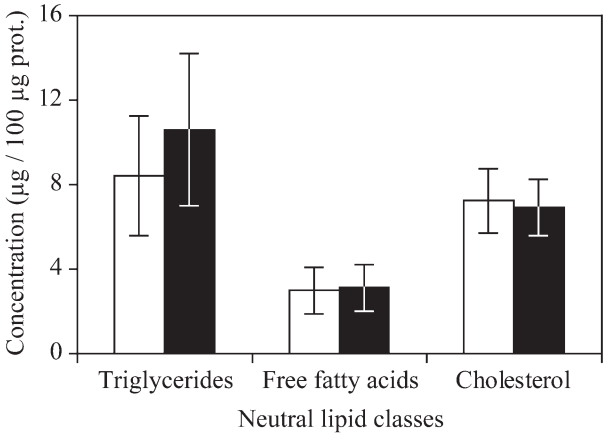

Fig. 3. Concentrations of the neutral lipid classes (mean ± S.D.) in uninfected (white bars) and *Polymorphus minutus* – infected *Gammarus roeseli* (black bars).

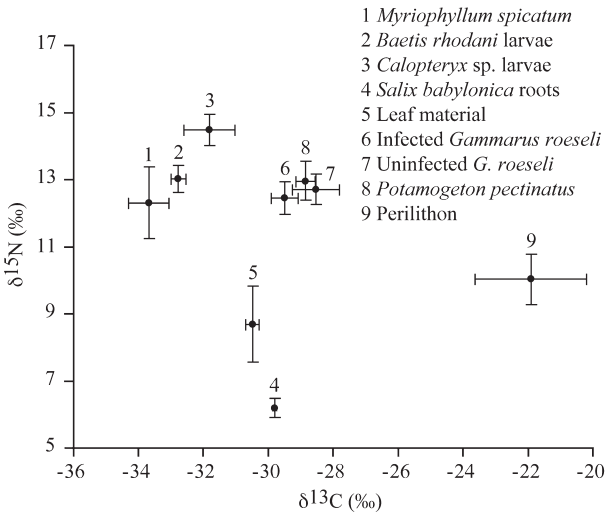

Fig. 4. Nitrogen and carbon stable isotope bi-plot (mean ± S.D.) for uninfected and *Polymorphus minutus*-infected *Gammarus roeseli*, two other invertebrate consumers (*Baetis rhodani* and *Calopteryx* sp. larvae), and five potential food sources (leaf material; *Myriophyllum spicatum; Potamogeton pectinatus*; perilithon; *Salix babylonica* roots).

triglycerides, free fatty acids, and cholesterol, the analysis of variance indicated a small but significant effect of infection status on the amphipod's lipid metabolism (MANOVA, $F_3 = 5{\cdot}16$, $P = 0{\cdot}011$). This may be due to the triglyceride concentration that tends to be higher in infected compared to uninfected *G. roeseli* even if the difference was not significant (F-test, $F_{18} = 2{\cdot}20$, $P = 0{\cdot}155$, Fig. 3).

Stable isotope study

When depicted in a $\delta^{13}C$-$\delta^{15}N$ bi-plot space, the stable-isotope ratios of both consumers and food sources suggested the existence of 2 distinct food webs (Fig. 4). The first (on the left of Fig. 4) showed

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Table 1. Contribution of organic matter sources (terrestrial inputs, *Potamogeton pectinatus*, perilithon) to the diet assimilated by uninfected and *Polymorphus minutus*-infected *Gammarus roeseli*

Mean proportions are given ± S.E. and were calculated from dual isotopic signatures (δ^{13}C and δ^{15}N, see text.)

	Terrestrial inputs	*P. pectinatus*	Perilithon
Uninfected *G. roeseli*	57·9 (6·9)	26·8 (8·0)	15·3 (4·0)
95% confidence interval	42·3–73·5	8·7–45·0	6·8–23·7
Infected *G. roeseli*	69·8 (7·9)	27·2 (9·3)	3·0 (2·8)
95% confidence interval	51·2–88·4	4·4–49·9	0–8·8

the trophic links occurring between *Myriophyllum spicatum, Baetis rhodani* and *Calopteryx* sp. larvae. The stable-isotope ratios of this food web varied significantly between sources (ANOVA, ^{15}N content: $F_{2,8}=19{\cdot}8$, $P<0{\cdot}001$; ^{13}C content: $F_{2,8}=90{\cdot}2$, $P<0{\cdot}001$, Fig. 4). The increase in δ ^{15}N (+0·7‰) and δ ^{13}C (+0·9‰) between *M. spicatum* and *B. rhodani* indicated that *B. rhodani* mainly fed on *M. spicatum*. However, the ^{15}N enrichment was lower than expected (+3·4‰ ± 1 between two trophic levels; Post, 2002) and suggested that terrestrial inputs (leaf litter and/or *S. balylonica* roots) could be a significant part of *B. rhodani*'s diet. The increase in δ ^{15}N (+1·5‰) and δ ^{13}C (+1‰) between *B. rhodani* and *Calopteryx* sp. were congruent with the hypothesis of a predator–prey interaction, *B. rhodani* representing a major food source for *Calopteryx* sp. larvae.

On the other hand, the δ^{13}C and δ^{15}N values of infected and uninfected *G. roeseli* indicated that their diet was mainly made of terrestrial inputs, with a potential contribution from *P. pectinatus* and perilithon, thus constituting a separate food web (on the right of Fig. 4). The stable isotope ratios along this food web varied significantly between sources (^{15}N content: $F_{4,34}=128{\cdot}3$, $P<0{\cdot}001$; ^{13}C content: $F_{4,34}=12{\cdot}1$, $P<0{\cdot}001$, Fig. 4). The δ^{15}N values of infected and uninfected *G. roeseli* were similar (Tukey's HSD post-hoc test, $P=0{\cdot}677$), indicating the same trophic level, whereas δ^{13}C values were significantly different (Tukey's HSD post-hoc test, $P<0{\cdot}001$), strongly suggesting differences in food sources.

The contribution of each food source to the diet of infected and uninfected *G. roeseli* was calculated using the equations previously described (1) (Phillips and Gregg, 2001). Based on a ^{15}N enrichment of 3·4‰ (Post, 2002) and assuming no trophic fractionation for ^{13}C, terrestrial inputs represented a major proportion of the diet for infected (70 ± 8%) as well as for non-infected amphipods (58 ± 7%). The contribution of *P. pectinatus* was about 27 ± 9% regardless of infection status. Although perilithon was a consistent part of the assimilated diet of uninfected amphipods (15 ± 4%), its contribution to that of infected ones was negligible (3 ± 3%, Table 1).

DISCUSSION

The present study combined laboratory and field data with the aim of revealing changes in the trophic ecology of acanthocephalan-infected amphipods relative to their uninfected conspecifics. Stable isotopes and neutral lipids were successfully used together, to complement the results of feeding experiments whose ecological relevance, when alone, may be questionable.

Contrary to Crompton's assumption (Crompton, 1970) that infected animals need to feed more to compensate for the cost associated with infection, we found no difference in the number of dead isopods consumed between infected and uninfected *G. roeseli*. Similarly, infection with the fish acanthocephalan *Echinorhynchus truttae* has no influence on the consumption of dead chironomids by *G. pulex* (Fielding *et al.* 2003). Although our experiment was not designed to quantify the energy diverted to *P. minutus*, this result means that under laboratory conditions and in the presence of an 'easy-to-eat' food source (i.e. dead prey), *G. roeseli* did not feed more to compensate for the cost associated with infection. This is consistent with the fact that we did not find a marked difference in the total amount of neutral lipid contents between infected and uninfected amphipods. Thus, even under natural conditions, infected amphipods, which have a more important content of triglycerides than uninfected ones, seem to eat as much as uninfected ones and did not show apparent neutral lipid depletion resulting from a decrease in the feeding activity.

The results of our feeding experiments differed greatly when isopods were alive, the number of isopods consumed being significantly lower for infected than for uninfected amphipods. Similar differences were found in *G. pulex* infected with *E. truttae* and feeding on live isopods (Fielding *et al.* 2003), or live amphipods (MacNeil *et al.* 2003). Conversely, Dick *et al.* (2010) showed that *G. pulex* preying on isopods displays a Type-II functional response rising more steeply and with a higher asymptote when infected with *E. truttae*, which suggests that infection increases predation. How

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infection modifies the diet of infected hosts may thus depend on the host-parasite system while laboratory results may vary with the experimental approach used. Concerning our results, one may argue that the parasite larva encysted in the abdomen of *G. roeseli* was a physical constraint to its motion, hence decreasing its efficiency in capturing live prey. This is, however, improbable because a previous study investigating the same host/parasite system demonstrated that infected amphipods swim faster than their uninfected counterparts (Médoc and Beisel, 2008). Because infected amphipods ate as many dead isopods as uninfected ones, the assumption that infection reduced the affinity of the host for this particular prey types can be rejected. The low number of live isopods consumed by infected amphipods thus argues for a reduced tendency to kill living prey. In other words, *P. minutus* infection significantly reduced the predatory behaviour of *G. roeseli*.

Data on leaf consumption are more surprising. Indeed, as shown in the first feeding experiments, infection had no apparent influence on the consumption of dead isopods, and thus we expected the same with leaf disks. This was not the case and after 48 h the amount of leaf material consumed by infected amphipods was half of that consumed by uninfected ones. Amphipods shred the leaves to subsequently feed on bacteria and fungi from their attached biofilms (Dangles and Malmqvist, 2004; Piscart *et al*. 2009*b*). We expect this shredding activity to be to some extent costly, thus the difference we observed does not necessarily mean that infected compared to uninfected amphipods ate less leaf material, but rather that they invested less energy in shredding. Similarly, *G. pulex* takes longer to consume leaf material when infected with the fish acanthocephalan *Pomphorhynchus laevis* (McCahon *et al*. 1988) but, conversely, it shows unchanged daily feeding rates when infected with *E. truttae* (Fielding *et al*. 2003). The way a host's biology is influenced by infection may thus differ among host/parasite systems.

Overall, our findings suggest that *P. minutus* changes the life-history budgeting of its intermediate host *G. roeseli*. Natural selection among parasites favours those having the ability to manipulate the phenotype of their host in a way that increases their own transmission. Host behavioural manipulation by *P. minutus* is well known and includes a reverse geotaxis (Bauer *et al*. 2005; Médoc *et al*. 2006), a higher refuge use (Médoc *et al*. 2009), and a higher swimming speed (Médoc and Beisel, 2008). Although their associated fitness gains in terms of increased trophic transmission are assumed and remain to be proven, we expect parasites that are able to shift the energy balance of their host in favour of manipulated traits to be favoured by natural selection. In turn, infected hosts should reduce their investment in behaviours that are less conducive to the parasite's transmission, for instance costly feeding activities (e.g. predation, shredding) or reproduction. This assumption is supported by the results of our feeding experiments and by the literature on the reproduction of *P. minutus*-infected *G. pulex*. Its pairing success is indeed significantly decreased while females are totally castrated (Bollache *et al*. 2001, 2002). *P. minutus* should hence shift the energy balance of *G. roeseli* in such a way that its overall energy output is reduced. This could explain why infected compared to uninfected amphipods had a higher concentration of triglycerides without necessarily feeding more.

At the community level, 2 distinct food webs were identified based on stable-isotope tracers: a first one relying on the predator/prey interaction between Odonata and Ephemeroptera larvae, the latter being herbivorous, and a second one with *G. roeseli* feeding mostly on terrestrial inputs. Both Ephemeroptera and amphipods (whether infected or not) shared the same N-isotope signature, that of primary consumers, whereas the N-isotope signature of Odonata larvae argued for a higher trophic position, that of a predator. This may first appear inconsistent with laboratory findings stressing the potential predatory behaviour of (uninfected) amphipods (Dick and Platvoet, 1996, 2000; Kelly *et al*. 2002*b*; and present study). Actually, although amphipods are omnivorous, they are first of all opportunistic, feeding on what they find where they are (MacNeil *et al*. 1997; Maazouzi *et al*. 2009). For instance, the feeding strategy of the amphipod *Dikerogammarus villosus* was recently found to be much more plastic than previously expected (Maazouzi *et al*. 2009). Nicknamed 'killer shrimp', *D. villosus* was depicted as a strong predator by almost all the experimental studies (Dick and Platvoet, 2000). Maazouzi *et al*. (2009) determined the fatty acid composition of the *D. villosus* population from an artificial reservoir. The fatty acid composition, which is an efficient trophic marker, significantly differed between seasons and habitats. *D. villosus* is thus rather unspecialized; it adapts its diet and switches from carnivorous to herbivorous, or detritivorous, depending on spatially and temporally available food sources.

As shown by the N-isotope signatures and because of the previous arguments, the reduced predation due to *P. minutus* infection observed in the laboratory was not found under natural conditions. So, based on the predation experiment only, it would be premature to conclude that infection may affect the regulation of prey populations. This result emphasizes the need to complement laboratory results with field data before drawing conclusions at the community level.

The small but significant difference in $\delta^{13}C$ values between infected and uninfected *G. roeseli* was probably due to a lower perilithon consumption by infected (< 9% of the diet) than by uninfected

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amphipods (7 to 24% of the diet). *P. minutus* is known to induce a reverse geotaxis in *G. roeseli* (Bauer *et al.* 2005; Médoc *et al.* 2006), which therefore is found inhabiting floating materials in its natural habitat (Médoc and Beisel, 2009). Infected amphipods are thus located far from benthic food sources and, because they are opportunistic, they feed on what is available at the surface, for instance decomposing leaves found among floating materials. This could explain the apparent shift from perilithon to terrestrial inputs in the diet of infected amphipods.

To conclude, our findings emphasize that the knowledge of parasite life-history strategies is essential to fully understand the feeding behaviour of infected hosts. From an ecological perspective, predicting the top-down influences of infection may be confusing when the host is an opportunistic feeder and, as such, this study shows the benefits of combining field and laboratory data through a multiple approach.

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