Manipulative parasites may not alter intermediate host distribution but still enhance their transmission: field evidence for increased vulnerability to definitive hosts and non-host predator avoidance

C. LAGRUE[1,2]*, A. GÜVENATAM[2] *and* L. BOLLACHE[2]

[1] *University of Otago, Department of Zoology, Dunedin, New Zealand*
[2] *Université de Bourgogne, UMR CNRS 6282 Biogéosciences, Dijon, France*

(*Received 17 June 2012; revised 6 August 2012; accepted 21 August 2012; first published online 15 October 2012*)

SUMMARY

Behavioural alterations induced by parasites in their intermediate hosts can spatially structure host populations, possibly resulting in enhanced trophic transmission to definitive hosts. However, such alterations may also increase intermediate host vulnerability to non-host predators. Parasite-induced behavioural alterations may thus vary between parasite species and depend on each parasite definitive host species. We studied the influence of infection with 2 acanthocephalan parasites (*Echinorhynchus truttae* and *Polymorphus minutus*) on the distribution of the amphipod *Gammarus pulex* in the field. Predator presence or absence and predator species, whether suitable definitive host or dead-end predator, had no effect on the micro-distribution of infected or uninfected *G. pulex* amphipods. Although neither parasite species seem to influence intermediate host distribution, *E. truttae* infected *G. pulex* were still significantly more vulnerable to predation by fish (*Cottus gobio*), the parasite's definitive hosts. In contrast, *G. pulex* infected with *P. minutus*, a bird acanthocephalan, did not suffer from increased predation by *C. gobio*, a predator unsuitable as host for *P. minutus*. These results suggest that effects of behavioural changes associated with parasite infections might not be detectable until intermediate hosts actually come in contact with predators. However, parasite-induced changes in host spatial distribution may still be adaptive if they drive hosts into areas of high transmission probabilities.

Key words: intermediate host manipulation, *Echinorhynchus truttae*, *Polymorphus minutus*, host distribution, trophic transmission.

INTRODUCTION

Multiple species of parasites rely on trophic transmission (consumption of infected intermediate hosts by an appropriate definitive-host predator) to complete their life cycle (Parker *et al*. 2003). Such strategy creates strong selective pressures on these parasites to increase the probability of intermediate hosts predation by final hosts (Lafferty, 1999; Moore, 2002). Some trophically transmitted parasites have evolved the ability to alter intermediate host phenotypes in ways that should increase transmission probabilities to definitive hosts (Moore, 2002; Thomas *et al*. 2005; Kaldonski *et al*. 2007). Acanthocephalan parasites are known to manipulate several behavioural traits of crustacean hosts, increasing their vulnerability to predation by definitive host predators (Poulin, 1995; Lafferty, 1999; Cézilly *et al*. 2000; Baldauf *et al*. 2007; Médoc and Beisel, 2011). Some species have been documented to alter habitat selection by intermediate hosts, infected individuals showing spatially divergent distributions compared to uninfected ones, resulting in patches of high parasite prevalence (MacNeil *et al*. 2003; Wellnitz *et al*. 2003; Médoc *et al*. 2009). For example, *Gammarus pulex* amphipods infected with *Echinorhynchus truttae* aggregated in fast-flowing, shallower stretches of river, areas below which drift-feeding fish, the parasite definitive hosts, usually congregate (MacNeil *et al*. 2003). Changes in the micro-distribution of intermediate hosts are often interpreted as a consequence of host behavioural manipulation, an adaptive strategy of the parasite to increase transmission probabilities. Infected hosts move in or closer to definitive hosts' habitats and/or feeding areas, thus increasing their vulnerability to predation and parasite transmission probabilities (MacNeil *et al*. 2003; Lagrue *et al*. 2007; Médoc and Beisel, 2009). However, the direct link between infected intermediate host altered spatial distribution and parasite transmission to definitive hosts is almost never tested.

Alterations of intermediate host distributions could also expose infected hosts to predation by other predators, non-definitive hosts (i.e. dead-end predators that parasites cannot infect; resulting in parasite death after intermediate host consumption).

* Corresponding author: Department of Zoology, University of Otago, 340 Great King Street, PO Box 56, Dunedin 9054, New Zealand. Tel: +64 3479 7986. Fax: +64 3479 7584. E-mail: clement.lagrue@gmail.com

Parasitology (2013), **140**, 258–265.
doi:10.1017/S0031182012001552

This may strongly reduce the advantage of host behavioural manipulation (Marriott *et al*. 1989; Mouritsen and Poulin, 2003), although not necessarily eliminating all benefits of such strategy (Seppälä and Jokela, 2008). Infected host distribution may be different in response to the presence of suitable hosts compared to other predators, preferentially exposing infected hosts to predation by the appropriate definitive host and/or decreasing its vulnerability to predation by non-host predators (Levri, 1998; Médoc *et al*. 2006, 2009). *Polymorphus minutus*-infected amphipods aggregate onto floating material while their uninfected conspecifics preferentially shelter in river-bottom substrate. Such parasite-induced alterations in host micro-distribution patterns are thought to move infected individuals closer to *P. minutus* definitive bird hosts and away from dead-end predators like fish and invertebrates, thus potentially increasing the parasite's transmission probabilities while avoiding non-host predator species (Médoc and Beisel, 2009; Médoc *et al*. 2009). However, counter examples exist where *P. minutus* made their crustacean hosts significantly more vulnerable to consumption by fish (Marriott *et al*. 1989). *Polymorphus minutus*-infected *G. pulex* vulnerability to dead-end predators also varies depending on habitat complexity (i.e. refuge availability) and predator type (i.e. sit-and-wait or active hunter), potentially eroding host manipulation benefits for the parasite (Kaldonski *et al*. 2008; Médoc and Beisel, 2008). Also, most studies looking at infected host vulnerability to definitive host and/or non-host predators are performed under laboratory conditions rather than *in situ*, and usually focused on a single parasite-predator species association (Médoc and Beisel, 2009).

Overall, field data on links between possible parasite-induced changes in infected intermediate host distribution, host and non-host predator presence, and predation rates on infected hosts are generally lacking. In particular, little is known about the specificity of parasite-induced habitat shifts in intermediate hosts (Médoc and Beisel, 2009). The present study investigated the micro-distribution of acanthocephalan-infected intermediate hosts in response to the absence or presence of predators, either suitable definitive hosts or non-host predators. First, we examined the prevalence of 2 acanthocephalan parasites, *Echinorhynchus truttae* and *Polymorphus minutus*, in their common intermediate host (*Gammarus pulex*), in the absence or immediate vicinity of both suitable definitive hosts and non-host predators in the field. Second, we assessed whether infected intermediate host micro-distributions resulted in increased parasite transmission to the definitive host and/or avoidance of non-host predators; these two aspects of parasite transmission being often the main characteristics required to qualify host behavioural manipulation as adaptive (Poulin, 1995; Cézilly and Perrot-Minnot, 2005; Seppälä and Jokela, 2008; Médoc and Beisel, 2011).

MATERIALS AND METHODS

Study site and animal material

Field experiments were carried out in spring 2011 (25 March to 25 April) in a second-order permanent karst spring, tributary of the Suzon River (47°24′14.45″N, 4°53′1.46″E), about 20 km north-west of Dijon, Eastern France. It is characterized by very stable water levels and temperatures, due to subterranean karstic origins, and a succession of small riffle-pool structures. The stream contains high densities of *Gammarus pulex* amphipod crustacean. Low densities of bullhead (*Cottus gobio*) can be found all year, while juvenile brown trout (*Salmo trutta*) are observed in spring and adults in late autumn during spawning season (Klemetsen *et al*. 2003); the stream being used as a spawning ground by adult *S. trutta* from the larger, adjacent Suzon River. Adult *S. trutta*, migrating in the stream to spawn, are likely to be the main source of *E. truttae* infection to *G. pulex* as the highest prevalence of mature cystacanths are observed in spring, 3–4 months after trout spawning, the time necessary for *E. truttae* to reach the infective cystacanth stage (Awachie, 1966). Field experiments were thus run at pick prevalences of mature *E. truttae* cystacanths and at a time when host behavioural manipulation should be maximal (Benesh *et al*. 2009).

Gammarus pulex amphipods present in the stream are intermediate hosts to 2 species of acanthocephalan parasites, *Echinorhynchus truttae* and *Polymorphus minutus*. *Echinorhynchus truttae* uses both *C. gobio* and *S. trutta* as definitive hosts (Awachie, 1973; Okada and Koura, 2000). Adult *P. minutus* are bird parasites (Holmes and Bethel, 1972), mainly infecting mallard duck (*Anas platyrhynchos*) and white-throated dipper (*Cinclus cinclus*) in our study stream. These acanthocephalan parasites are characterized by a complex, 2-host life-cycle. They mature and sexually reproduce in vertebrate definitive hosts. Eggs are then released in the water with host feces and must be consumed by an amphipod crustacean intermediate host. The life cycle is completed when the infected crustacean is eaten by the appropriate definitive host (Crompton and Nickol, 1985).

Predators used in our study were bullheads (*C. gobio*), being suitable definitive hosts to *E. truttae*, and the noble crayfish (*Astacus astacus*) as the non-host predator to both *E. truttae* and *P. minutus*. Both species are present in the Suzon river catchment and are known to feed on invertebrates, including amphipods (Lagrue *et al*. 2007; Kaldonski *et al*. 2008; Haddaway *et al*. 2012). Bullheads were captured by electro-fishing in an upstream stretch of the Suzon River where the parasites are absent.

https://doi.org/10.1017/S0031182012001552 Published online by Cambridge University Press

Crayfish were bought from a fish farm (Loro's Farm, Loromontzey, North-Eastern France; 48°26′38.42″N, 6°22′1.95″E) where *A. astacus* is raised in large ponds, feeding on naturally occurring prey, including amphipods.

Host density and parasite prevalence and distribution

Field experiments consisted of 5 blocks of 3 cages (15 in total), each cage haphazardly assigned to one of 3 treatments: control, crayfish or bullhead. Blocks 1 to 5 consisted in structurally similar riffles evenly spaced (20 m) along a 100 m reach of the stream. Cages were cylindrical (Ø = 30 cm and height = 50 cm), made of 10 mm steel mesh and designed such that predator (bullhead and crayfish) foraging behaviour and access to prey would not be restricted. Mesh aperture and cage height allowed all prey movements across cage boundaries and over the entire water column (15–30 cm water depth) while preventing other naturally occurring fish predators from accessing amphipod traps. Cages were imbedded 5 cm deep into the streambed and anchored to the river bottom using iron rods. Gravel was then added so that substratum surfaces inside and outside the cages were levelled. Control treatment cages contained no predator, each crayfish cage contained 1 adult individual of the noble crayfish (*Astacus astacus*; total length >10 cm) and each fish cage contained 3 adult bullheads (*C. gobio*; mean total length ± S.E. = 8·1 ± 0·3 cm). Cages within each block were at a distance of 2 m to avoid possible chemical or visual interactions between predators. Predator numbers were such that there was an equivalent predator biomass between crayfish and bullhead treatments and reflected natural predator densities. Crayfish were selected as natural predators of amphipods and unsuitable hosts for *E. truttae* and *P. minutus; Cottus gobio* being a definitive host for *E. truttae* but not *P. minutus*.

Autumn-shed leaves of field elm [*Ulmus minor* (Mill.)] were used as food/habitat in the amphipod traps set out in treatment cages. We used air-dried leaves to make standardized leaf packs to capture *G. pulex* amphipods. After wetting, leaves were enclosed in 10 mm steel mesh cages (15 × 10 × 1 cm) to constitute a trap and assigned to one of the 3 treatments (5 control, 5 crayfish and 5 bullhead, respectively); the large mesh size and low trap height (1 cm) allowed both bullheads and crayfish to access and predate on amphipods present within the traps or migrating towards and from these. One trap was then added in each treatment cage, maintained flat against the substrate using iron rods and left in the cage for 10 days before retrieval. Elm leaf mass used in each trap allowed amphipods to feed without noticeably impacting the resource; food never ran out between sampling dates. Traps were designed to allow immigration and emigration of amphipods colonizing elm leaves so that samples reflected an amphipod distribution equilibrium, and not amphipod accumulation, in each trap at each sampling date. Amphipods were sampled 3 times during the experiment (4, 14 and 25 April, respectively), allowing 10 days for amphipod colonization before each sampling date. Traps were swiftly recovered from treatment cages and put into a plastic tray to capture all amphipods contained within. Amphipods were sorted from elm leaves, collected in a 0·5 mm mesh sieve and preserved in 70% ethanol before laboratory analyses. Leaf packs were replaced by fresh ones within each trap after each sampling date. In the laboratory, all amphipods were counted and *G. pulex* densities expressed as captures per unit effort (i.e. CPUE), corresponding to the number of *G. pulex* individuals per amphipod trap. Individuals were measured (total body length to the closest 0·5 mm) and, when possible, sexed from the shape and size of segment 6 (propodus) of gnathopods 1 and 2 (Bollache and Cézilly, 2004). Amphipods were then dissected under a dissecting microscope to record and identify possible acanthocephalan parasites. Only mature cystacanths, developmental stage infective to definitive hosts, were recorded and considered in our analyses (Awachie, 1966); host manipulation and thus infected amphipod aggregation around predator hosts being expected only for mature, infective acanthocephalan cystacanths (Dianne *et al*. 2011). Prevalence of each parasite in each treatment could thus be determined and compared.

Potential differences in amphipod densities between treatments, sampling dates and blocks were tested using main-effects ANOVAs and trap/cage as the sampling unit. Amphipod numbers (CPUE) were log-transformed before analyses to normalize the data. Generalized Linear Models (GLMs) were used to analyse the effects of the different factors on the presence or absence of acanthocephalan parasites in amphipods, hereafter referred to as parasite prevalence (proportion of infected hosts). We examined the effects of treatment (control, crayfish or bullhead), amphipod host sex (male or female), sampling date (4, 14 or 25 of April) and block (1 to 5) as fixed factors and amphipod host size as a covariate, on acanthocephalan parasite prevalence (dependent variable), using individual amphipods as the sampling unit. Two GLMs were run, one for each parasite species, *E. truttae* and *P. minutus*. Finally, linear regressions between amphipod CPUE and parasite prevalence in each block were used to test for a potential relationship between *G. pulex* density and *E. truttae* or *P. minutus* prevalences and thus possible differences in microdistribution of infected and uninfected hosts.

Fish diet and parasite transmission

Fish diet was analysed *in situ* by collecting stomach contents of *C. gobio* maintained in the 5 bullhead

https://doi.org/10.1017/S0031182012001552 Published online by Cambridge University Press

Table 1. Generalized Linear Models testing for the effects of treatment (control, crayfish and fish), sampling date (4, 14 and 25 April 2011), sampling block (1–5), and amphipod host sex and size (total body length) on the prevalence of (A) *Echinorhynchus truttae* and (B) *Polymorphus minutus* in *Gammarus pulex* captured in amphipod traps

(Non-significant main effects are shown in the tables whereas non-significant interactions were removed. Degree of freedom (df), Log Likelihood (Log (*L*)), chi-square (χ^2) and *P* values are given for each factor. Significant *P* values are indicated by *.)

	df	Log (*L*)	χ^2	*P*
(A)				
Treatment	2	−573·94	0·192	0·909
Host size	1	−574·04	8·127	0·004*
Host sex	1	−557·93	9·303	0·002*
Date	2	−571·19	5·509	0·064
Block	4	−562·58	17·21	0·002*
(B)				
Treatment	2	−293·95	3·225	0·199
Host size	1	−295·56	6·813	0·009*
Host sex	1	−291·82	0·935	0·334
Date	2	−293·08	1·728	0·422
Block	4	−292·28	1·599	0·809

treatment cages described above, allowing comparisons between parasite prevalences recorded in the bullhead treatment (i.e. amphipod traps) and actual bullhead diet. Because *C. gobio* do not have pharyngeal teeth, their diet can be analysed by collecting bolus, using stomach-flushing methods (Lagrue and Bollache, 2006; Lagrue *et al*. 2007). Fish stomach contents were collected every 5 days for a total of 9 sampling dates using a simplified stomach flushing technique from Gaudin *et al*. (1981; see also Lagrue and Bollache, 2006). Amphipods recovered from fish stomachs were preserved in 70% ethanol before analyses. In the laboratory, intact amphipods, i.e. defined as the carcass possessing a head still connected to the pereon (MacNeil *et al*. 2001; Lagrue and Bollache, 2006), were sexed, measured and dissected as described above. Other amphipod remains were disregarded since they could not be measured and/or positively categorized as infected or not. As for amphipods collected in traps, prevalences of both acanthocephalan parasites were determined.

Echinorhynchus truttae and *P. minutus* prevalences (proportions of infected amphipods) in fish diet were compared with parasite prevalences in amphipod traps from the bullhead treatment using Fisher's exact tests. Possible differences in amphipod size between treatments were tested using a non-parametric test (Mann-Whitney U test). We performed all tests with a 5% type I error risk, using STATISTICA Software 6.0 (StatSoft Inc., France).

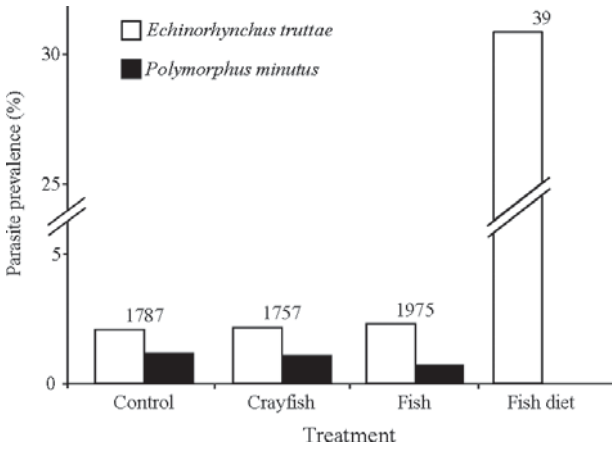

Fig. 1. Prevalences of *Echinorhynchus truttae* (□) and *Polymorphus minutus* (■) in *Gammarus pulex* individuals captured in amphipod traps in the different treatments (control, crayfish and fish, respectively) and recovered in *Cottus gobio* stomach samples (fish diet). Sample size (i.e. number of *G. pulex* amphipods) for each treatment is indicated above bars.

RESULTS

Parasite prevalence and distribution

In total, 8919 amphipods were captured in leaf traps, 2430 males, 3089 females and 3400 juveniles. Amphipods qualified as 'juveniles' were immature individuals that could not be sexed and measured less than 5 mm in body length. Juvenile amphipods were never infected by acanthocephalan parasites or predated upon by *C. gobio* and were therefore discarded from the following analyses. Of the 5519 adult amphipods collected, 1787 were captured in the control treatment, 1757 in the crayfish and 1975 in the fish treatment. At the trap/cage level, treatment had no effect on amphipod densities (119·1 ± 16·5, 117·5 ± 19·0 and 131·7 ± 17·2 mean CPUE ± S.E. for control, crayfish and fish treatments, respectively; ANOVA, $F_{2,42} = 0{\cdot}452$, $P = 0{\cdot}640$). However, *G. pulex* CPUE (i.e. mean number of amphipods per trap) was significantly different between sampling blocks (156·6 ± 25·3, 114·8 ± 27·2, 87·4 ± 17·8, 107·4 ± 14·0 and 149·9 ± 21·1 for blocks 1 to 5, respectively; ANOVA, $F_{4,40} = 2{\cdot}921$, $P = 0{\cdot}034$). CPUE also decreased between sampling dates (178·2 ± 17·5, 106·1 ± 13·5 and 78·3 ± 8·3 for 4, 14 and 25 of April, respectively; ANOVA, $F_{2,42} = 11{\cdot}04$, $P = 0{\cdot}0002$).

Generally, parasite prevalences did not vary between sampling dates (Table 1). More importantly, treatment had no overall effect on either *E. truttae* or *P. minutus* (Table 1 and Fig. 1). *Echinorhynchus truttae* prevalence was very similar between control, crayfish and bullhead treatments (2·07, 2·16 and 2·28%, respectively; Fig. 1) while *P. minutus* prevalence was slightly lower in the bullhead treatment (0·66%) compared to the two others (1·18 and 1·08% in control and crayfish treatments, respectively;

https://doi.org/10.1017/S0031182012001552 Published online by Cambridge University Press

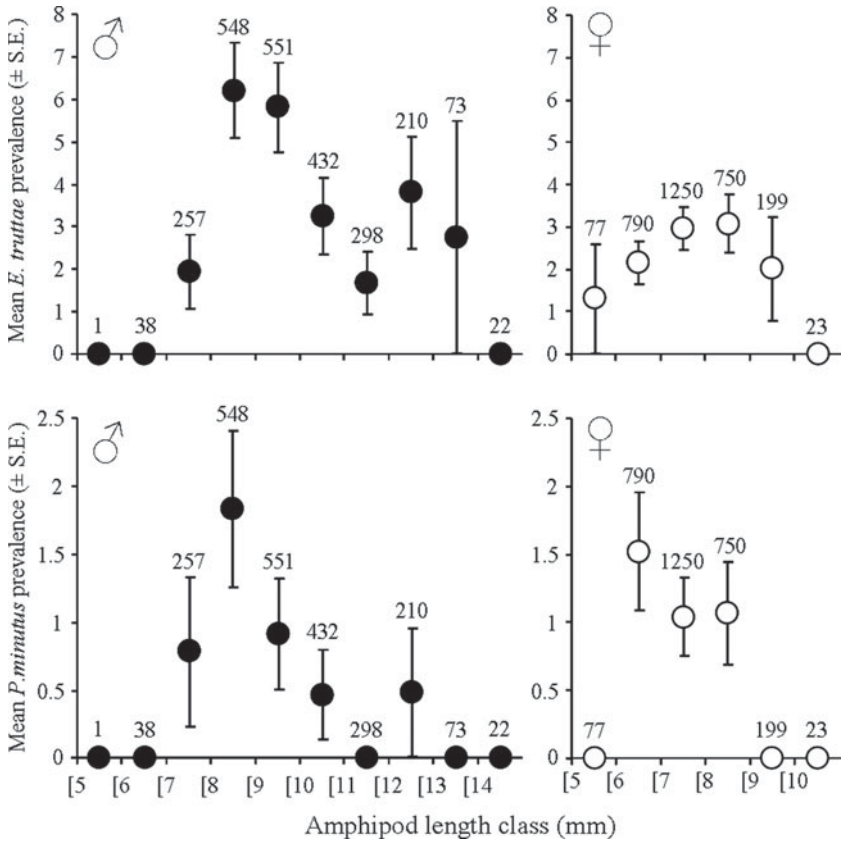

Fig. 2. Mean prevalences (± S.E.) of *Echinorhynchus truttae* (top graphs) and *Polymorphus minutus* (bottom) in relation to host size (amphipod length class) in male (left graphs) and female (right) *Gammarus pulex* captured in amphipod traps. Sample size (i.e. number of *G. pulex* amphipods) for each host size class is indicated above each data-point.

Fig. 1). Sampling blocks did not significantly influence *P. minutus* infection (Table 1B); prevalence varying between 0·64 and 1·14% among blocks. Contrastingly, *E. truttae* infection was variable across sampling blocks. Observed prevalences were highly dependent upon blocks (Table 1A). This pattern was mainly due to variations in overall amphipod densities since *E. truttae* infected *G. pulex* numbers did not vary between sampling blocks (2·22 ± 0·76, 2·56 ± 0·65, 3·56 ± 0·94, 2·33 ± 0·60 and 2·67 ± 0·73 CPUE ± S.E. for blocks 1–5, respectively; Kruskal-Wallis ANOVA, $H_{4,45} = 1{\cdot}51$, $P = 0{\cdot}826$).

Individual amphipod size significantly influenced the probability of infection by both parasite species (Table 1 and Fig. 2). *Echinorhynchus truttae*-infected individuals were larger (mean body length ± S.E. = 8·75 ± 0·14 mm) than uninfected amphipods (8·29 ± 0·02 mm, Fig. 2). The trend was opposite for *P. minutus* with infected individuals being smaller (7·72 ± 0·16 mm) than their uninfected conspecifics (8·30 ± 0·02 mm, Fig. 2). However, in both species, the highest mean parasite prevalence occurred in amphipod hosts of intermediate size (Fig. 2), a pattern classically used as an estimate of parasite-induced mortality in intermediate hosts (Thomas *et al*. 1995; Rousset *et al*. 1996). Host sex also significantly influenced *E. truttae* infection but had no effect on *P. minutus* (Table 1 and Fig. 2). Male amphipods were 2·2 times more likely to be infected by *E. truttae* than females (3·13 and 1·42% prevalence, respectively) and seemed to carry heavier parasite loads (Fig. 2). No significant interaction was detected between the different factors included in the models.

Finally, we found a significant relationship between *G. pulex* densities (CPUE) and *E. truttae* prevalence ($r = 0{\cdot}933$, $n = 5$, $P = 0{\cdot}021$); prevalence being higher in sampling blocks with lower densities of amphipod hosts (Fig. 3A). Contrastingly, *P. minutus* prevalence seemed to increase with host densities although the trend was not significant ($r = 0{\cdot}589$, $n = 5$, $P = 0{\cdot}296$; Fig. 3B).

Fish diet and parasite transmission

A total of 39 intact amphipods were recovered in *C. gobio* stomach samples (6, 8, 6, 8 and 11 from blocks 1–5 respectively); 12 were infected by *E. truttae* (2, 2, 2, 2 and 4 from blocks 1–5 respectively) and zero contained *P. minutus*. Because of the small sample size, Amphipods from all individual fish and all sampling blocks were grouped before analyses.

Host size was not significantly different between amphipods recovered in fish stomachs (mean body length ± S.E. = 7·91 ± 0·30 mm) and individuals

https://doi.org/10.1017/S0031182012001552 Published online by Cambridge University Press

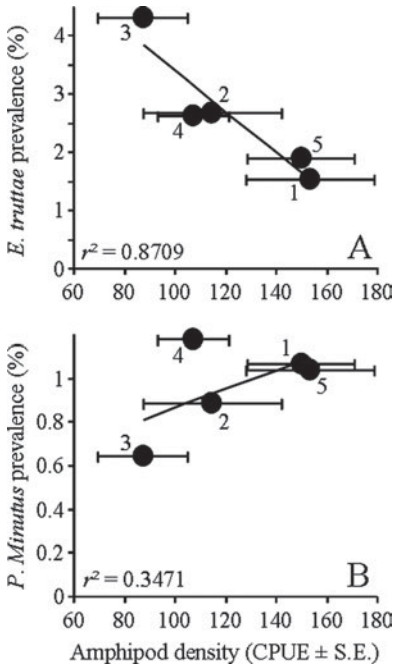

Fig. 3. Relationship between *G. pulex* amphipod density (CPUE ± S.E.) and parasite prevalence for (A) *Echinorhynchus truttae* and (B) *Polymorphus minutus* across sampling blocks (1–5). Lines of best fit and coefficients of determination are shown on each figure. Block number is indicated next to each data-point.

captured in the fish treatment traps (8·28 ± 0·04 mm; Mann-Whitney U test, $Z = -1{\cdot}367$, $P = 0{\cdot}172$). Again, *E. truttae*-infected amphipods tended to be larger than their uninfected conspecifics, both in *C. gobio* diet (8·38 ± 0·47 and 7·70 ± 0·39 mm, respectively; Mann-Whitney U test, $Z = -1{\cdot}297$, $P = 0{\cdot}195$) and fish treatment (8·27 ± 0·04 and 8·74 ± 0·27 mm, respectively; Mann-Whitney U test, $Z = -2{\cdot}179$, $P = 0{\cdot}029$); although the difference was not significant in *C. gobio* diet. Prevalence of *E. truttae* was significantly higher in *C. gobio* diet (30·77%) than in the fish treatment (2·28%; Fisher's exact test, $\chi^2 = 112{\cdot}88$, $P < 0{\cdot}0001$; Fig. 1). *Gammarus pulex* hosts recovered in fish stomach contents were 13·5 times more likely to be infected than individuals captured in amphipod traps from the fish treatment (Fig. 1). Finally, although *P. minutus*-infected amphipod were absent from *C. gobio* stomach contents, no significant difference in *P. minutus* prevalence was detected between amphipod hosts in fish diet and fish treatment traps (0 and 0·66%, respectively; Fisher's exact test, $\chi^2 = 0{\cdot}26$, $P = 0{\cdot}611$; Fig. 1).

DISCUSSION

Uninfected *G. pulex* densities were variable across sampling blocks, but unaffected by predator presence or species (fish or crayfish) within blocks. Additionally, *E. truttae*- and *P. minutus*-infected amphipods showed homogeneous distributions (i.e. similar densities) across sampling blocks. These contrasting patterns induce variations in *E. truttae* prevalences, apparently negatively correlated with amphipod densities. Furthermore, *E. truttae* infected *G. pulex* micro-distribution (i.e. within blocks) was not linked to predator presence and/or type (i.e. suitable host or dead-end predator). Overall, amphipod distribution differed between infected and uninfected individuals but was not affected by predators. Although *G. pulex* is known to be able to use chemical cues from predators to adjust habitat use and efficiently avoid predator proximity (Dahl *et al.* 1998), this capacity did not influence *G. pulex* distribution in our study. Heterogeneity in amphipod density was detected across sampling blocks but within a block *G. pulex* micro-distribution did not depend on predator presence, in contrast to suggestions from MacNeil *et al.* (2003). Overall, in our study, uninfected *G. pulex* did not seem to avoid habitat patches occupied by predators. Micro-distribution of *E. truttae* and *P. minutus* infected individuals was not influenced by suitable definitive or non-host predator presence although these parasites could still alter host spatial distribution in other ways that may be beneficial (MacNeil *et al.* 2003; Médoc and Beisel, 2009, 2011).

Nevertheless, evidence exists that crustacean amphipods can use chemical cues from predators and develop adaptive anti-predator responses (Mathis and Hoback, 1997; Wudkevich *et al.* 1997; Åbjörnsson *et al.* 2000). In particular, amphipods tend to reduce activity and increase refuge use upon chemical detection of predators (Kaldonski *et al.* 2007; Perrot-Minnot *et al.* 2007; Benesh *et al.* 2008*a*). While our results suggest that such anti-predator behaviours do not affect amphipod distribution, they still constitute ideal targets for manipulation by acanthocephalans to increase transmission to definitive hosts. For example, *G. pulex* infected with the fish acanthocephalans, *Pomphorhynchus laevis* or *P. tereticollis*, showed increased activity levels, decreased use of shelter and/or reversed reactions to fish chemical cues, being attracted to it while uninfected individuals were clearly repulsed (Baldauf *et al.* 2007; Perrot-Minnot *et al.* 2007). Similarly, *E. truttae*-infected *G. pulex* are more active and less photophobic than uninfected individuals (MacNeil *et al.* 2003). Movement being a major stimulus eliciting predator attack on prey, *E. truttae*-infected amphipods should be more likely to be seen and consumed by predators than their hidden and motionless uninfected conspecifics (Poulin, 1995; MacNeil *et al.* 1999, 2003). This is clearly suggested by our results; *E. truttae*-infected *G. pulex* were 13·5 times more likely to be consumed by *C. gobio*, the parasite's definitive host, than uninfected individuals, even though *E. truttae* did not influence amphipod distribution. Parasitized host vulnerability

https://doi.org/10.1017/S0031182012001552 Published online by Cambridge University Press

to fish predation could thus be increased regardless of infected host micro-distribution. Our results suggest that, even though the parasite does not seem to alter amphipod hosts micro-distribution into areas with fish definitive hosts, *E. truttae*-induced behavioural manipulation may still operate at very close range, in the immediate vicinity of definitive host predators. However, *E. truttae*-induced changes in *G. pulex* anti-predatory responses remain to be tested and characterized under controlled, laboratory conditions if we are to understand fully how the parasite is able to increase its transmission at such close range.

Upon detection of a specific predator, prey should adjust their behaviour so that they are less likely to encounter, be detected and captured by that particular predator (Lima, 1998). Parasites could thus increase transmission probabilities accordingly through manipulation of specific intermediate host anti-predator behaviours, thus combining predation enhancement by suitable hosts and predation suppression by non-host predators (Lagrue *et al*. 2007; Médoc *et al*. 2009; Médoc and Beisel, 2011). Such multidimensionality in host manipulation means that a single parasite may alter more than one behavioural trait in its intermediate host in order to target a unique definitive host species and avoid dead-end predators (Benesh *et al*. 2008*b*; Cézilly and Perrot-Minnot, 2010; Thomas *et al*. 2010). Recent empirical evidence suggests that acanthocephalans may be able to reduce intermediate host predation risk by non-host predators through parasite-induced increase in specific anti-predator responses (Médoc and Beisel, 2011), although several counter-examples exist (Holmes and Bethel, 1977; Marriott *et al*. 1989; Kaldonski *et al*. 2008; Seppälä *et al*. 2008). For example, the amphipod *Gammarus roeseli* reacts to predator chemical cues by increasing refuge use, regardless of infection status. However, amphipods infected with *P. minutus*, a bird parasite, showed increased shelter use and tend to exploit refuges close to the surface, away from non-host benthic predators (Médoc *et al*. 2006, 2009). Infected amphipods were consequently less preyed upon by fish predators (Médoc *et al*. 2009). Whether this is the case with the crayfish predator could not be determined from our study since crayfish stomach contents cannot be sampled on live animals. However, results show that, while *E. truttae* infected *G. pulex* were significantly over-predated by *C. gobio*, *P. minutus*-infected individuals were never found in fish diet. Results suggest that different acanthocephalans render common intermediate host species specifically vulnerable to particular predators, thus increasing parasite transmission to appropriate final hosts only. While *P. minutus* did not seem to suffer from increased predation by dead-end fish predators, we cannot conclude from our results whether infected amphipods are capable of actively avoiding predation by fish, thus increasing *P. minutus* survival when faced with non-host predators.

Overall, our study showed that, although parasites did not influence amphipod host micro-distribution, intermediate host manipulation by acanthocephalans may still increase parasite transmission to the definitive hosts in the field by operating at very close range. Results from fish stomach samples also point to potential parasite species-specific behavioural alterations in *G. pulex*. *Echinorhynchus truttae* induced host behaviour alterations increased parasite transmission probabilities through over-predation of infected hosts. Contrastingly, *P. minutus* is unlikely to suffer from increased predation by non-host fish predators. However, whether this manipulative parasite is capable of enhancing *G. pulex* anti-predator responses, when facing dead-end predators, would require further investigations.

ACKNOWLEDGEMENTS

We thank three anonymous reviewers for very constructive comments on a previous version of the manuscript.

REFERENCES

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

Awachie, J. B. E. (1966). The development and life history of *Echinorhynchus truttae* Schrank, 1788 (Acanthocephala). *Journal of Helminthology* **40**, 11–32.

Awachie, J. B. E. (1973). The status of the infection of the bullhead, *Cottus gobio* L., by *Echinorhynchus truttae* Schrank, 1788 (Acanthocephala) in Mon Terrig. *Acta Parasitologica Polonica* **21**, 481–484.

Baldauf, S. A., Thünken, T., Frommen, J. G., Bakker, T. C. M., Heuprl, O. and Kullmann, H. (2007). Infection with an acanthocephalan manipulates an amphipod's reaction to a fish predator's odours. *International Journal for Parasitology* **37**, 61–65.

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

Benesh, D. P., Kitchen, J., Pulkkinen, K., Hakala, I. and Valtonen, E. T. (2008*a*). The effects of *Echinorhynchus borealis* (Acanthocephala) infection on the anti-predator behaviour of a benthic amphipod. *Journal of Parasitology* **94**, 542–545.

Benesh, D. P., Valtonen, E. T. and Seppälä, O. (2008*b*). Multidimensionality and intra-individual variation in host manipulation by an acanthocephalan. *Parasitology* **135**, 617–626.

Bollache, L. and Cézilly, F. (2004). State-dependent pairing behaviour in male *Gammarus pulex* (L.) (Crustacea, Amphipoda): effects of time left to moult and prior pairing status. *Behavioural Processes* **66**, 131–137.

Cézilly, F., Gregoire, A. and Bertin, A. (2000). Conflict between co-occurring manipulative parasites? An experimental study of the joint influences of two acanthocephalan parasites on the behaviour of *Gammarus pulex*. *Parasitology* **120**, 625–630.

Cézilly, F. and Perrot-Minnot, M.-J. (2005). Studying adaptive changes in the behaviour of infected hosts: a long and winding road. *Behavioural Processes* **68**, 223–228.

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

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

Dahl, J., Anders Nilsson, P. and Pettersson, L. B. (1998). Against the flow: chemical detection of downstream predators in running waters. *Proceedings of the Royal Society of London, B* **265**, 1339–1344.

Dianne, L., Perrot-Minnot, M. J., Bauer, A., Gaillard, M., Leger, E. and Rigaud, T. (2011). Protection first then facilitation: a manipulative

parasite modulates the vulnerability to predation of its intermediate host according to its own developmental stage. *Evolution* **65**, 2692–2698.

Gaudin, P., Martin, E. and Caillere, L. (1981). Le tubage gastrique chez les poissons: mise an point d'un équipement et test chez le chabot *Cottus gobio* (L.). *Bulletin Français de la Pêche et de la Pisciculture* **282**, 8–15.

Haddaway, N. R., Wilcox, R. H., Heptonstall, R. E. A., Griffiths, H. M., Mortimer, R. J. G., Christmas, M. and Dunn, A. M. (2012). Predators functional response and prey choices identify predation differences between native/invasive and parasitized/unparasitised crayfish. *Plos One* **7**, e32229. doi:10.1371/journal.pone.0032229.

Holmes, J. C. and Bethel, W. M. (1972). Modification of intermediate host behaviour by parasites. *Zoological Journal of the Linnean Society* **51**, 123–149.

Holmes, J. C. and Bethel, W. M. (1977). Increased vulnerability of amphipods to predation owing to altered behaviour induced by larval acanthocephalans. *Canadian Journal of Zoology* **55**, 110–115.

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

Kaldonski, N., Perrot-Minnot, M.-J., Motreuil, S. and Cézilly, F. (2008). Infection with acanthocephalans increases the vulnerability of *Gammarus pulex* (Crustacea, Amphipoda) to non-host predators. *Parasitology* **135**, 627–632.

Klemetsen, A., Amundsen, P.-A., Dempson, J. B., Jonsson, B., Jonsson, N., O'Connell, M. F. and Mortensen, E. (2003). Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecology of Freshwater Fish* **12**, 1–59.

Lafferty, K. D. (1999). The evolution of trophic transmission. *Parasitology Today* **15**, 111–115.

Lagrue, C. and Bollache, L. (2006). Effects of temperature on persistence times of native and invasive gammarid species in the stomachs of bullhead, *Cottus gobio*. *Journal of Fish Biology* **68**, 318–322.

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

Levri, E. P. (1998). Perceived predation risk, parasitism, and the foraging behaviour of a freshwater snail (*Potamopyrgus antipodarum*). *Canadian Journal of Zoology* **76**, 1878–1884.

Lima, S. L. (1998). Nonlethal effects in the ecology of predator-prey interactions – What are the ecological effects of anti-predator decision-making? *Bioscience* **48**, 25–34.

MacNeil, C., Elwood, R. W. and Dick, J. T. A. (1999). Brown trout predation on native and introduced amphipods in N. *Ireland, a behavioural study*. Ecography **22**, 686–697.

MacNeil, C., Elwood, R. W. and Dick, J. T. A. (2001). Persistence times of four amphipod species in the stomachs of brown trout. *Journal of Fish Biology* **59**, 1401–1404.

MacNeil, C., Fielding, N. J., Hume, K. D., Dick, J. T. A., Elwood, R. W., Hatcher, M. J. and Dunn, A. M. (2003). Parasite altered micro-distribution of *Gammarus pulex* (Crustacea: Amphipoda). *International Journal for Parasitology* **33**, 57–64.

Marriott, D. R., Collins, M. L., Paris, R. M., Gudgin, D. R., Barnard, C. J., McGregor, F. S., Gilbert, F. S., Hartley, J. C. and Behnke, J. M. (1989). Behavioural modifications and increased predation risk of *Gammarus pulex* infected with *Polymorphus minutus*. *Journal of Biological Education* **23**, 135–141.

Mathis, A. and Hoback, W. W. (1997). The influence of chemical stimuli from predators on precopulatory pairing by the amphipod, *Gammarus pseudolimnaeus*. *Ethology* **103**, 33–40.

Médoc, V. and Beisel, J.-N. (2008). An acanthocephalan parasite boosts the escape performance of its intermediate host facing non-host predators. *Parasitology* **135**, 977–984.

Médoc, V. and Beisel, J.-N. (2009). Field evidence for non-host predator avoidance in a manipulated amphipod. *Naturwissenschaften* **96**, 513–523.

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

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

Médoc, V., Rigaud, T., Bollache, L. and Beisel, J.-N. (2009). A manipulative parasite increasing an antipredator response decreases its vulnerability to a nonhost predator. *Animal Behaviour* **77**, 1235–1241.

Moore, J. (2002). Parasites and the behaviour of animals. *Oxford Series in Ecology and Evolution*. Oxford University Press, NY, USA.

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

Okada, C. E. and Koura, E. A. (2000). The ecology of endohelminth parasites of fish in the Driffield trout stream, Yorkshire, England. *Journal of Aquatic Sciences* **15**, 41–45.

Parker, G. A., Chubb, J. C., Michaud, M. and Milinski, M. (2003). Optimal growth strategies of larval helminths in their intermediate hosts. *Journal of Evolutionnary Biology* **16**, 47–54.

Perrot-Minnot, M. J., Kaldonski, N. and Cézilly, F. (2007). Increased susceptibility to predation and altered anti-predator behaviour in an acanthocephalan-infected amphipod. *International Journal for Parasitology* **37**, 645–651.

Poulin, R. (1995). "Adaptive" changes in the behaviour of parasitized animals: A critical review. *International Journal for Parasitology* **25**, 1371–1383.

Rousset, F., Thomas, F., de Meeüs, T. and Renaud, F. (1996). Inference of parasite-induced mortality from distributions of parasite loads. *Ecology* **77**, 2203–2211.

Seppälä, O. and Jokela, J. (2008). Host manipulation as a parasite transmission strategy when manipulation is exploited by non-host predators. *Biology Letters* **4**, 663–666.

Seppälä, O., Valtonen, P. and Benesh, D. P. (2008). Host manipulation by parasites in the world of dead-end predators: adaptation to enhance transmission? *Proceedings of the Royal Society of London, B* **275**, 1611–1615.

Thomas, F., Renaud, F., Rousset, F., Cézilly, F. and de Meeüs, T. (1995). Differential mortality of two closely related host species induced by one parasite. *Proceedings of the Zoological Society of London* **260**, 349–352.

Thomas, F., Adamo, S. A. and Moore, J. (2005). Parasitic manipulation: where are we and where should we go? *Behavioural Processes* **68**, 185–199.

Thomas, F., Poulin, R. and Brodeur, J. (2010). Host manipulation by parasites: a multidimensional phenomenon. *Oikos* **119**, 1217–1223.

Wellnitz, T., Giari, L., Maynard, B. and Dezfuli, B. S. (2003). A parasite spatially structures its host population. *Oikos* **100**, 263–268.

Wudkevich, K., Wisenden, B. D., Chivers, D. P. and Smith, R. J. F. (1997). Reactions of *Gammarus lacustris* to chemical stimuli from natural predators and injured conspecifics. *Journal of Chemical Ecology* **23**, 1163–1173.