Variations in infection levels and parasite-induced mortality among sympatric cryptic lineages of native amphipods and a congeneric invasive species: Are native hosts always losing?

Matthias Galipaud a, Loic Bollache b, Clement Lagrue c,*

a Department of Evolutionary Biology, Bielefeld University, Konsequenz 45, 33615 Bielefeld, Germany
b UMR 6249 Chrono-environment, Universite Bourgogne Franche-Comte, 6 Boulevard Gabriel, 21000 Dijon, France
c Department of Zoology, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand

A R T I C L E   I N F O

Article history:
Received 9 February 2017
Accepted 24 April 2017

Keywords:
Biological invasion
Cryptic diversity
Gammarus fossarum/Gammarus pulex
species complex
Gammarus roeseli
Acanthocephalan parasites
Infection levels
Parasite-induced mortality

A B S T R A C T

Shared parasites can strongly influence the outcome of competition between congeneric, sympatric hosts, and thus host population dynamics. Parasite-mediated competition is commonly hypothesized as an important factor in biological invasion success; invasive species often experience lower infection levels and/or parasite-induced mortality than native congeneric hosts. However, variation in infection levels among sympatric hosts can be due to contrasting abilities to avoid infection or different parasite-induced mortality rates following infection. Low parasite infection levels in a specific host can be due to either factor but have drastically different implications in interaction outcomes between sympatric hosts.

We assessed acanthocephalan infection levels (prevalence and abundance) among cryptic molecular taxonomic units (MOTU) of the native G. pulex/G. fossarum species complex from multiple populations where they occur in sympatry. We concomitantly estimated the same parameters in the invasive G. roeseli commonly found in sympathy with G. pulex/G. fossarum MOTUs. We then tested for potential differences in parasite-induced mortality among these alternative hosts. As expected, the invasive G. roeseli showed relatively low infection level and was not subject to parasite-induced mortality. We also found that both acanthocephalan infection levels and parasite-induced mortality varied greatly among cryptic MOTUs of the native amphipods. Contrary to expectations, some native MOTUs displayed levels of resistance to their local parasites similar to those observed in the invasive G. roeseli. Overall, cryptic diversity in native amphipods coupled with high levels of variability in infection levels and parasite-induced mortality documented here may strongly influence inter-MOTU interactions and native population dynamics as well as invasion success and population dynamics of the congeneric invasive G. roeseli.

© 2017 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Parasitism is an important biotic determinant of animal population dynamics and community structure (Hudson and Greenman, 1998). Parasites can influence host numbers within a population by increasing mortality rate (Goater and Ward, 1982; Rousset et al., 1996). For parasites with complex life cycles, intermediate host mortality attributable to parasites can occur through reduction in host survival due to the pathological consequences of parasitic infection, or through host manipulation increasing predation on infected hosts (Thomas et al., 1995; Latham and Poulin, 2002; Parker et al., 2003; Hansen and Poulin, 2005; Benesh and Valtonen, 2007; Violante-González et al., 2016). However, host species often differ in their susceptibility to infection and/or parasite-induced mortality (Sánchez et al., 2012). For example, invasive species generally suffer less from parasitism than native species (Dunn and Dick, 1998; Torchin et al., 2003; Genner et al., 2008). Native parasites are often less effective at infecting invasive hosts due to the lack of co-evolutionary history between the novel hosts and local parasites (Ebert, 1994; Kaltz and Shykoff, 1998; Embleg Fromme and Dybdahl, 2006; Genner et al., 2008). Host-parasite interactions proceed in a co-evolutionary context; both species must continually adapt to each other (May and
Anderson, 1983; van Baalen, 1998; Dieckmann et al., 2002; Vale and Little, 2009). Parasites are generally considered to stay ahead of host defenses during co-evolution and are often expected to be adapted to their local hosts (Kaltz and Shykoff, 1998; Morgan et al., 2005). Even if invasive hosts are congeneric species taxonomically close to native hosts, parasites may be so closely adapted to their local host species that they are unable to infect or induce pathogenic effects in the invader (Cornet et al., 2010; Westram et al., 2011a). Local parasites that are able to infect novel, invasive hosts may still reach lower infection levels and pathogenic effects in native than invasive host species (Lagruè et al., 2016). In turn, local, native hosts are also continuously mounting counter-adaptations specifically targeting their local parasites and may be better at dealing with native parasites than a naïve, invasive host (Lagruè et al., 2016). Overall, native and invasive hosts can vary wildly in their vulnerability to infection and local parasites in their host specificity and pathogenic effects (Redon et al., 2015). Generally, two closely related and sympatric host species infected by the same parasite can exhibit strongly contrasted vulnerability to infection and/or parasite-induced mortality (Thomas et al., 1995; Rouset et al., 1996). Although evidence for parasite-induced host mortality from field data remains difficult to obtain (Anderson and Gedon, 1982), multiple studies have inferred parasite-induced host mortality from distribution of parasite abundances within host populations (Gordon and Rau, 1982; Thomas et al., 1995; Rouset et al., 1996; Médoc et al., 2006). But the direct links between parasite prevalence or abundance and host mortality can be difficult to determine (Benesh, 2011). Differences in parasite prevalence and/or abundance among sympatric host species can be due to one host being more efficient at avoiding the parasite and/or resisting infection following encounter; i.e. lower infection levels result from higher resistance to the parasite. Alternatively or concomitantly, contrasting parasite-induced mortality between co-occurring host species can in turn induce widely different parasite prevalence and/or abundance between hosts. Hosts in which survival is not affected by the infection will tend to accumulate parasites, thus displaying higher prevalence and/or abundance levels than hosts with high parasite-induced mortality, even if their encounter rates and vulnerability to infection are similar. Assessing which of these potential factors affects apparent infection levels among alternative host species in wild host populations remains extremely difficult in the field however. In France, the native Gammarus fossarum and G. pulex are often found co-existing with the invasive Gammarus roeseli, an established Balkan species (Bauer et al., 2000). These species are also hosts to a variety of acanthocephalan parasites (Bauer et al., 2000, 2005; Westram et al., 2011a). Previous studies have shown that acanthocephalan parasites can induce various behavioural, phenotypic, and physiologic changes in G. fossarum and G. pulex. These include altered reaction to light (Bauer et al., 2000, 2005; Cézilly et al., 2000; Perrot-Minnot, 2004; Tain et al., 2006), impaired antipredator behaviour (Kaldonski et al., 2007; Dianne et al., 2014), partial castration (Bollache et al., 2002), and lower immune activity (Rigaud and Moret, 2003). No such effects were apparent, or were significantly lower, in the invasive G. roeseli (Bauer et al., 2000, 2005; Rigaud and Moret, 2003). Contrasting host manipulation in native compared to invasive amphipod hosts reflected a difference in the ability of the acanthocephalan Pnomophorhynchus laevis to alter brain serotonergic activity of the two host species (Tain et al., 2007). As a result, predation rates on acanthocephalan-infected G. fossarum/G. pulex were significantly higher than on infected individuals of the invasive G. roeseli (Lagruè et al., 2007; Tain et al., 2007). Consequently, mean parasite abundance relative to host size displayed the characteristic bell-shaped curve supporting a higher mortality in acanthocephalan-infected G. fossarum/G. pulex individuals compared to uninfected amphipods (Lagruè et al., 2007). Here, parasite-induced host mortality likely resulted from increased predation on infected amphipods induced by host manipulation (Lafferty, 1999; Outreman et al., 2007; Violante-González et al., 2016). Again, no such pattern was observed in G. roeseli; mean parasite abundance simply increased with amphipod host size, indicating an accumulation of acanthocephalan parasites over time by invasive hosts with no apparent induced mortality (Lagruè et al., 2007). Furthermore, although acanthocephalan prevalence is higher in the invasive G. roeseli than in the native G. fossarum/G. pulex in the field, laboratory controlled infections showed that native amphipods were more susceptible to infection by local acanthocephalan (Lagruè et al., 2007; Bauer and Rigaud, 2015). Overall, differences in vulnerability to infection, acanthocephalan prevalence and size distributions of infected individuals suggest a differential parasite-induced mortality between G. fossarum/G. pulex and the invasive G. roeseli. Such pattern of enemy release is commonly observed in a biological invasion context and provides a competitive advantage to the invader (Clay, 2003; Cornet et al., 2010; Arundell et al., 2015). Differential susceptibility to infection by local parasites and parasite-induced mortality are often seen as key factors, among others, for the ability of G. roeseli to invade, establish and invade populations of the native G. fossarum/G. pulex (Lagruè et al., 2011). Gammarus pulex and Gammarus fossarum are widely distributed “morphological” species and are often considered to be single, independent functional taxonomic units (Karaman and Pinkster, 1977; Müller, 1998). However, extensive use of DNA barcoding have recently allowed the detection of high degrees of cryptic diversity (i.e. genetically diverging lineages that differ little or not at all in their morphology) across a variety of taxa (Hebert et al., 2003; Pfenninger and Schwenk, 2007; Fontaneto et al., 2009; Trontelj and Fiser, 2009). Amphipods are no exception with a number of cases of morphologically cryptic lineages reported (Hogg et al., 1998, 2006; Witt and Hebert, 2000; Witt et al., 2006; Lefèbvre et al., 2007; Sutherland et al., 2010; Grabowski et al., 2012). Several recent studies showed extensive genetic divergence within G. fossarum and G. pulex (Müller, 1998, 2000; Westram et al., 2011b; Lagruè et al., 2014; Weiss et al., 2014; Weiss and Leese, 2016). Indeed, Lagruè et al. (2014) showed that, in Eastern France, G. fossarum and G. pulex form a complex of cryptic lineages, or molecular operational taxonomic units (i.e. MOTU; Blaxter et al., 2005), that are morphologically undistinguishable and often co-occur in sympatric populations. Whether these cryptic MOTUs display differential vulnerability to shared parasites remains unclear. For instance, the respective vulnerability to parasites of each G. fossarum/G. pulex lineage compared to that of the sympatric invasive G. roeseli is completely unknown. Many of the studies cited above were conducted on amphipod populations that have proved, in hindsight, to be made of several sympatric MOTUs of G. fossarum/G. pulex (Bauer et al., 2000, 2005; Lagruè et al., 2007, 2014), thus preventing reliable comparisons of parasitism between native and invasive species (Bauer and Rigaud, 2015). Morphologically cryptic lineages of amphipod hosts may be physiologically, behaviourally and/or ecologically different and thus not be cryptic to parasites (Cothran et al., 2013a, b). In our study system, whether acanthocephalan parasites are generalists and able to infect any available native amphipod host or are highly specific to particular host MOTUs is yet to be assessed. However, the occurrence of sympatric but genetically divergent native host lineages, coupled with the presence of the invasive G. roeseli, has the potential to affect acanthocephalan infection levels, host-parasite interactions and ultimately host-host competition among lineages and/or species (Gandon, 2004; Rigaud et al., 2010; Westram et al., 2011a). In such cases, contrasting parasite-induced mortality
among sympatric host species or MOTUs may result in differences in their relative competitiveness (Kennedy et al., 1978; Raque et al., 2003; Lively, 2010).

Here, we investigated potential differences in acanthocephalan infection levels (prevalence and abundance; see Bush et al., 1997 for terminology) among the invasive G. roeseli and cryptic MOTUs of the native G. pulex/G. fossarum species complex from multiple populations/sampling sites where they occur in sympathy. In populations containing two or more G. pulex/G. fossarum MOTUs, we assessed the effects of host cryptic diversity on apparent infection patterns and potential differences in infection susceptibility and parasite-induced mortality among sympatric MOTUs. In amphipod populations where it was found, we also tested whether the invading G. roeseli was less vulnerable to infection by local acanthocephalan parasites and, when infected, less subject to parasite-induced mortality. We hypothesized that the invader should be less vulnerable than native amphipod MOTUs, both in terms of infection levels (prevalence and abundance) and pathological effects (parasite-induced mortality). We also predicted variations among MOTUs in parasitism and predicted that cryptic diversity would influence apparent acanthocephalan infection levels and pathological effects documented in the field. Finally, we discussed how parasites may influence inter-MOTU competition as much as interspecific competition among native and invasive amphipod host species.

2. Materials and methods

A previous study revealed high cryptic diversity among and within populations of the native G. fossarum/G. pulex species complex in Burgundy, France (Lagrue et al., 2014). Multiple divergent molecular operational taxonomic units (i.e. MOTU; Blaxter et al., 2005) were found and populations/sampling sites often contained several MOTUs occurring in sympathy. For the present study, we collected amphipods from 15 sampling sites/rivers containing two or more divergent MOTUs of G. fossarum/G. pulex or at least one G. fossarum/G. pulex MOTU and G. roeseli in sympathy (Table S1), and where acanthocephalan parasites also occurred.

2.1. Field sampling

Amphipods from the Gammarus fossarum/Gammarus pulex (hereafter referred to as GF and Gp) species complexes were collected using kick nets from 15 different rivers (Table S1) in Autumn 2010 (From October to December). Amphipods were collected on only one occasion in each river to avoid potential temporal variation effects on our data. When present in the river, invasive Gammarus roeseli (Gr) amphipods were also collected. All potential habitats present at any given site were sampled. Amphipods collected were pooled in a large container from which between 250 and 1000 were haphazardly sampled and preserved individually in 1.5 ml Eppendorf tubes filled with 100% ethanol. Remaining amphipods were then released. Back in the laboratory, ethanol was renewed in each tube and samples kept at −20 °C until needed for measurements, genetic identification and dissections.

2.2. Laboratory processing

All amphipods were sexed and measured using height of the fourth coxal plate as a proxy for body size (Bollache et al., 2000). Amphipods were then dissected under a dissecting microscope using fine forceps and all acanthocephalan parasites found were identified and counted. Amphipods from sampling sites containing Gammarus roeseli and MOTU(s) of G. fossarum/G. pulex were first sorted morphologically as Gammarus roeseli is easily identified by its dorsal spines (Bollache et al., 2006). Furthermore, as a relatively recent invader, G. roeseli differs little genetically among populations and all individuals collected during our study can be considered as a single Gr MOTU (Moret et al., 2007). Individuals of G. fossarum/G. pulex were genetically identified using a PCR-RFLP method (Levy et al., 2002; Pfeiffer et al., 2004) and assigned to their respective Molecular Operational Taxonomic Unit (MOTU): GF-I, GF-II, GF-III, GF-VI, GF-VII or Gp-D as determined in Lagrue et al., 2014. These MOTUs show COI genetic divergences ranging from about 3% up to 26% (Fig. 1; Lagrue et al., 2014).

Amphipod DNA was extracted from one to three pereopods (i.e. ‘walking legs’ in amphipod crustaceans), depending on individual size, following methods described in Lagrue et al., 2014. A total of 7261 individuals were amplified for the 5’ part of the mtDNA cytochrome c oxidase subunit I (COI) using universal primers (LCO1490 and HCO2198; Folmer et al., 1994) and then assigned to their respective MOTU using RFLP. Restriction endonucleases specific to a single MOTU were identified from the 565bp long sequence of the COI previously obtained by sequencing using the Cleaver Software (Jarman, 2006; for details on DNA extraction, PCR and sequencing, see Lagrue et al., 2014). The PCR amplified DNA products were then digested overnight using the appropriate restriction endonuclease(s) (Table S2), following manufacturer’s instructions (New England Biolabs). Resulting fragments were separated by gel electrophoresis in a 2% agarose gel. Restriction enzyme profiles were visualized with ethidium bromide on a UV bench and used to assign each individual amphipod to its respective MOTU.

2.3. Infection level estimations

Parasite prevalence and abundance were calculated for each MOTU at each sampling site as the proportion of infected individuals (%) and the mean number of parasites per amphipod host, respectively (Bush et al., 1997). Dissections recovered acanthocephalan cystacanths belonging to three different species occurring at varying infection levels: Pomphorhynchus laevis, Pomphorhynchus tereticollis and Poly morphus minutus. Pomphorhynchus laevis and P. tereticollis use fish as definitive hosts while P. minutus is a bird parasite (Holmes and Bethel, 1972; Kennedy, 2006; Jacquin et al., 2014). Prevalence and abundance were thus estimated for each parasite species separately but overall prevalence and abundance were also calculated with all three parasite species grouped together.

2.4. Statistical analyses

We tested our hypotheses using four different statistical models. With the first two models we tested for potential differences in parasite prevalences and abundances among MOTUs using mixed
effect general linear models with a logit and a log link function, respectively. In these models, the sampling site (i.e. river) was considered as a random effect to account for potential differences in parasite prevalence or abundance among rivers. Given that amphipods were frequently infected by parasites of more than one species simultaneously, amphipod mortality induced by parasites could not be assessed independently for each parasite species. We therefore performed the subsequent models with no regards to parasite species, investigating overall parasitism effect on infection avoidance and parasite-induced mortality among MOTUs. Using a third model, we then analyzed parasite abundance as a function of amphipod body size, taken as a proxy for amphipod age. We modeled parasite abundance using a general mixed effect model with a Poisson distribution and a log link function. We considered amphipod MOTU, body size and the interaction between the two variables as fixed effects and the sampled river as a random effect. Under the hypothesis that parasites induce mortality in their host, parasite abundance is first expected to increase with amphipod's age/size before decreasing due to the death of heavily infected hosts. Accordingly, we considered the effect of amphipod body size on parasite abundance as second degree polynomial of the form abundance = β₂size² + β₁size + intercept, rather than linear. A significant effect of the interaction between amphipod MOTU and body size would suggest that parasite induced mortality differs significantly among MOTUs. This model was performed using the R package “lme4” (Bates et al., 2015) and subsequent post hoc analyses were performed using the R package “multcomp” (Hothorn et al., 2008). The data contained an excess of uninfected individuals, as indicated by the excess of zeros in the distribution of parasite abundance across MOTUs. In the context of our study, amphipods can be uninfected for two different reasons: (1) they have never encountered any parasite by chance or as a result of strategic avoidance, or (2) they have encountered parasites but resisted their infection. Statistically, the strategic avoidance, or (2) they have encountered parasites but have never encountered any parasite by chance or as a result of amphipods can be uninfected for two different reasons: (1) they could not be assessed independently for each parasite species. We therefore performed the subsequent models with no regards to parasite species, investigating overall parasitism effect on infection avoidance and parasite-induced mortality among MOTUs. Using a third model, we then analyzed parasite abundance as a function of amphipod body size, taken as a proxy for amphipod age. We modeled parasite abundance using a general mixed effect model with a Poisson distribution and a log link function. We considered amphipod MOTU, body size and the interaction between the two variables as fixed effects and the sampled river as a random effect. Under the hypothesis that parasites induce mortality in their host, parasite abundance is first expected to increase with amphipod's age/size before decreasing due to the death of heavily infected hosts. Accordingly, we considered the effect of amphipod body size on parasite abundance as second degree polynomial of the form abundance = β₂size² + β₁size + intercept, rather than linear. A significant effect of the interaction between amphipod MOTU and body size would suggest that parasite induced mortality differs significantly among MOTUs. This model was performed using the R package “lme4” (Bates et al., 2015) and subsequent post hoc analyses were performed using the R package “multcomp” (Hothorn et al., 2008). The data contained an excess of uninfected individuals, as indicated by the excess of zeros in the distribution of parasite abundance across MOTUs. In the context of our study, amphipods can be uninfected for two different reasons: (1) they have never encountered any parasite by chance or as a result of strategic avoidance, or (2) they have encountered parasites but resisted their infection. Statistically, the first source of zero is called false zero in contrast to true zeros representing genuine exposure to the phenomenon at the origin of the count process. These two sources of zeros reflect two quite different mechanisms by which individuals avoid being infected. With a fourth analysis, we finally modeled parasite abundance using a zero-inflated mixture model to investigate in further detail the mechanisms of infection avoidance. This was performed using the R package “pscl” (Zeileis et al., 2008) and the function “zeroinfl”. This function allows accounting simultaneously for predictor variables affecting parasite abundance (which is assumed to follow a negative binomial distribution with a log link function) and for predictor variables affecting the probability that the data contains an excess of zeros (i.e. the probability of false zeros, which are modeled as a binomial process with a logit link function). We therefore considered the effect of amphipod MOTU, the polynomial effect of amphipod body size and their interaction on parasite abundance and the effect of amphipod MOTU only on the probability of false zero. In the context of our study, a significant effect of the amphipod MOTU on the probability of false zeros would suggest a significant difference among MOTUs in the mechanisms by which amphipods avoid being infected. For model convergence purposes and to ease interpretations of polynomial fit parameter estimates, we scaled amphipod body size by mean-centering prior to every performed analysis. We also used orthogonal polynomials in models including a polynomial effect of amphipod body size to avoid problems related to multicollinearity.

3. Results

Overall prevalences and abundances varied significantly among MOTUs (prevalence: likelihood ratio test, $\chi^2 = 403.8$, df = 7, $P < 0.001$; abundance: likelihood ratio test, $\chi^2 = 885.2$, df = 7, $P < 0.001$). The invasive *G. roeseli* was significantly less parasitized by local acanthocephalans than native amphipod MOTUs GF-II, GF-III, GF-VI and GF-VIII but showed prevalences similar to those of GF-I and GF-VII MOTUs. *Gammarus roeseli* was even significantly more parasitized than Gp-D amphipods (Fig. 2). Overall, differences in acanthocephalan prevalence among MOTUs did not seem to depend on their level of genetic divergence, as illustrated by the fact that closely related GF-III and GF-I or GF-VI and GF-VII MOTUs harboured substantially different acanthocephalan prevalences (Figs. 1 and 2).

Parasite-induced mortality varied among MOTUs as suggested by the significant effect of the interaction between amphipod MOTU and the polynomial parameters of amphipod body size on parasite abundance (likelihood ratio test, $\chi^2 = 105.8$, df = 14, $P < 0.001$, Fig. 4). Two main patterns of parasite abundance as a function of amphipod body size (i.e. amphipod age) were found. Individuals from GF-I, GF-II, GF-VI, GF-VII and Gp-D MOTUs showed a bell-shaped relationship between parasite abundance and host body size, with amphipods of intermediate size, and thus age, being more heavily infected than smaller/younger or larger/older ones (Table 1, Fig. 4). Contrastingly, individuals from native GF-III and GF-VIII MOTUs, and the invasive *G. roeseli* showed increasing parasite abundance with increasing body size (i.e. host age; Table 1, Fig. 4). This dichotomy is also well reflected when interpreting parameter estimates $\beta_1$ and $\beta_2$ for the polynomial effect of amphipod body size on parasite abundance. In general, $\beta_1$ is to be interpreted as the rate of change in abundance for mean amphipod body size (i.e. mean age). In other words, positive $\beta_1$ values indicate that from mean amphipod body size (i.e. age) upward, parasite abundance is still expected to increase, whereas it is expected to decrease for negative $\beta_1$ values. $\beta_2$ reflects steepness and curvature of the fitted curve. Positive and negative $\beta_2$ values reflect convex and concave curvatures respectively. Greater absolute $\beta_2$ values indicate steeper curves. Native MOTUs GF-III, GF-VII and the invasive *G. roeseli* showed $\beta_1$ values significantly greater than 0 and greater than $\beta_1$ values of GF-I, GF-II, GF-VI, GF-VIII and Gp-D MOTUs (albeit not significant for the GF-III/GF-VII, GF-VIII/GF-VII and GF-VIII/Gp-D pairs), indicating a steady increase in parasitic abundance with amphipod size across the entire size range (Table 1). Compared to GF-I, GF-II and GF-VI MOTUs in which larger individuals tended to be uninfected, GF-III, GF-VIII MOTUs and the invasive *G. roeseli* amphipods also showed lower absolute $\beta_2$ values (down to $\beta_2 \approx 0$ for G. roeseli) indicating moderate or no decrease in parasitic abundance in larger amphipods (Table 1, Fig. 4).

Including the effects of amphipod MOTU on the probability of false zeros in the zero-inflated mixture model significantly increased the fit of the model, suggesting that the mechanisms responsible for infection avoidance vary among MOTUs (likelihood ratio test, $\chi^2 = 58.86$, df = 26, $P < 0.001$, Table 2). Native MOTUs GF-I, GF-II, GF-III and Gp-D showed rather high estimated probabilities of false zeros (albeit not always significantly different from zero, Table 2), suggesting that uninfected individuals in these MOTUs did not actually encounter parasites rather than resisted infection post-encounter. On the contrary, GF-VI, GF-VII, GF-VIII, and the invasive *G. roeseli* had rather low probabilities of false zero, suggesting that uninfected individuals encountered parasites but resisted their infection (Table 2).

4. Discussion

Despite high genetic divergence values among MOTUs, diagnostic morphological features classically used to distinguish *G. fossarum* from *G. pulex* do not allow reliable discrimination among sympatric MOTUs in the amphipod populations used here (Lagruè et al., 2014). As a result, *G. fossarum/G. pulex* populations...
have long been considered as a single functional unit when it comes to assessing parasite infection levels, parasite-induced mortality and other life history traits, and to comparing them to the invasive *G. roeseli* (Bauer et al., 2000; Rigaud and Moret, 2003; Bollache et al., 2006; Lagrue et al., 2007, 2011; Tain et al., 2007; Kaldonski et al., 2008). However, we showed in a recent study that two thirds of amphipod populations surveyed contained two or three sympatric *G. fossarum/G. pulex* MOTUs (Lagrue et al., 2014).

Furthermore, many sampling sites also contain the invasive *G. roeseli*. It is often expected that invasive species will be less infected than native hosts by local parasites, thus providing the invasive with a competitive advantage and higher invasion success (Clay, 2003; Torchin et al., 2003; Colautti et al., 2004; Prenter et al., 2004; MacNeil and Dick, 2011). However, such a pattern was not clear from parasite prevalences. We found that acanthocephalan prevalences varied greatly among native MOTUs of the *G. fossarum*...


**G. pulex** species complex. Furthermore, the invasive *G. roeseli* displayed local parasite prevalences varying from significantly lower to comparable, up to higher than prevalences recorded in sympatric, native MOTUs. There was also no clear link between genetic divergence among MOTUs and acanthocephalan prevalence; i.e. the level of genetic divergence between two MOTUs did not predict, in any way, potential differences in infection levels by larval acanthocephalans.

![Fig. 4.](image)

**Table 1**

Parameter estimates for the polynomial effect of amphipod host body size on parasite abundance among MOTUs. P-values presented next to $b_1$ and $b_2$ estimates test their significant difference from zero. P-values from pairwise comparisons of parameter estimates among MOTUs are also provided in the right part of the table; differences in $b_1$ and $b_2$ estimates are presented below and above the matrix diagonal, respectively. Significant differences are indicated in bold.

| MOTU   | Parameters Multiple comparisons |
|--------|---------------------------------|
|        | Int (S.E.) $b_1$ size (S.E.) $b_2$ size2 (S.E.) P-value | Gf-I | Gf-II | Gf-III | Gf-VI | Gf-VII | Gf-VIII | Gp-D | Gr |
| Gf-I   | $1.71$ (0.24) $-0.17$ (0.13) $<0.001$ $-0.4$ (0.11) $<0.001$ | 0.99 | $<0.001$ | 0.06 | 0.94 | 0.01 | 0.71 | $<0.001$ |
| Gf-II  | $-0.31$ (0.26) $-0.04$ (0.1) 0.07 $-0.51$ (0.1) $<0.001$ | 0.96 | $<0.001$ | 0.09 | 0.99 | 0.04 | 0.99 | $<0.001$ |
| Gf-III | $-1.21$ (0.24) 0.34 (0.05) $<0.001$ | $-0.19$ (0.05) $<0.001$ | 0.06 | 0.03 | $<0.001$ | 0.06 | 0.61 | 0.03 | 0.91 |
| Gf-VI  | 0.23 (0.3) $-0.51$ (0.18) $<0.001$ | $-0.47$ (0.19) $<0.001$ | 0.99 | 1 | 0.65 | 0.13 | $<0.001$ | 0.02 $<0.001$ |
| Gf-VII | $-2.31$ (0.34) 0.07 (0.34) 0.61 $-0.61$ (0.38) 0.11 | 0.08 | 0.99 | $<0.01$ | 0.98 | 0.08 | 1 | 0.01 |
| Gf-VIII | $-0.09$ (0.41) 0.92 (0.37) $<0.001$ | $-0.26$ (0.28) $<0.001$ | 0.81 | 0.65 | 0.99 | 0.94 | 0.07 | 0.10 | 0.82 |
| Gp-D   | $-0.00$ (0.29) $-0.15$ (0.16) 0.30 | 0.02 (0.09) 0.82 | $<0.01$ | $<0.001$ | 0.14 | 0.14 | $<0.001$ | 0.55 | $<0.01$ |
| Gr     | $-2.00$ (0.24) 0.35 (0.06) $<0.001$ | $-0.01$ (0.04) 0.84 | $<0.001$ | $<0.001$ | 0.02 | 0.08 | $<0.001$ | 0.36 | 0.99 |

Footnote: Int – when Int is transformed to logit, it represents mean parasite abundance for each MOTU and for amphipod host mean body size in each MOTU.

**Table 2**

Probability of false zeros among MOTUs (i.e. the probability that amphipod individuals are uninfected because they did not actually encounter the parasite rather than they resisted infection) and their respective P-values to test their difference from zero. P-values from pairwise comparisons of probability of false zero among MOTUs are also provided in the right part of the table. Significant differences are in indicated in bold.

| MOTU   | Parameters Multiple comparisons |
|--------|---------------------------------|
|        | Int (S.E.) Probability of false zero P-value | Gf-I | Gf-II | Gf-III | Gf-VI | Gf-VII | Gf-VIII | Gp-D | Gr |
| Gf-I   | 0.14 (0.41) 0.54 | 0.73 | $0.04$ |
| Gf-II  | $-0.64$ (0.65) 0.34 | 0.32 | $<0.001$ |
| Gf-III | $-0.02$ (0.41) 0.49 | 0.94 | 0.41 | 0.09 |
| Gf-VI  | $-1.62$ (1.69) 0.17 | 0.34 | 0.23 | 0.47 | 0.28 |
| Gf-VII | $-2.37$ (7.51) 0.08 | 0.75 | 0.40 | 0.85 | 0.74 | 0.92 |
| Gf-VIII | $-1.18$ (1324) 0 | 0.99 | 0.83 | 0.85 | 0.81 | 0.88 | 0.95 |
| Gp-D   | 1.90 (0.39) 0.87 | $<0.001$ | $<0.001$ | $<0.001$ | 0.02 | 0.56 | 0.99 |
| Gr     | $-2.52$ (3.45) 0.07 | 0.46 | 0.39 | 0.51 | 0.43 | 0.74 | 0.98 | 0.99 | 0.15 |
Alternatively or concomitantly, invasive hosts may also be less susceptible than native congeners to local parasite-induced mortality (Hudson and Greenman, 1998; Roche et al., 2010; Blackburn and Ewen, 2016). Accordingly, we found that the invasive G. roeseli amphipod was not affected by parasite-induced mortality (i.e. acanthocephalan parasite abundance increased with host size; Fig. 4) while many native G. fossarum/G. pulex MOTUS showed the bell-shaped curve of parasite abundance across host size, a pattern indicative of parasite-induced mortality in hosts infected with larval helminthes (Crofton, 1971; Amin et al., 1980; Bratney, 1986; Thomas et al., 1995; Brown et al., 2001, 2003; Latham and Poulin, 2002; Outreman et al., 2007; Bates et al., 2010; Heins et al., 2010). Furthermore, our results suggest that G. roeseli is also able to resist infection upon encounter with acanthocephalan parasites better than most native G. fossarum/G. pulex MOTUS. Interestingly however, two cryptic MOTUS (GF-III and GF-VIII) of the native species did not exhibit parasite-induced mortality either. Although variable infection levels among cryptic lineages have been reported previously in G. fossarum (Westram et al., 2011a), this is the first time that such pattern is documented among sympatric cryptic MOTUS and invasive species concomitantly. Like G. roeseli, GF-VIII uninfected individuals seemed to have resisted infection upon parasite encounter rather than avoided parasites (Table 2). The pattern is slightly different in GF-III uninfected individuals who showed rather high probability of false zeros (Table 2). This suggests that when encountering a parasite, GF-III individual do not resist infection, but do not suffer increased mortality from being infected either. These patterns contrast with the common assumption that native host species are generally more susceptible to infection and more affected by local parasites than congeneric invasive species (Clay, 2003; Torchin et al., 2003).

Contrastingly, GF-I, GF-II, GF-VI, GF-VII and Gp-D amphipods did seem to suffer from parasite induced mortality; only uninfected individuals were able to survive to old age (i.e. large size), as indicated by the pattern of decreasing parasite abundance with age/size (Fig. 4). Also, in GF-I, GF-II and Gp-D, uninfected individuals are more likely to have avoided infection by chance rather than by being resistant to it. This pattern is particularly striking in Gp-D individuals which seem to efficiently avoid parasites and are therefore rarely infected. However, when they do acquire parasites, they suffer from parasite-induced mortality. Although acanthocephalan prevalence and mean parasite abundance varied greatly among these MOTUs, similar patterns of relationship between parasite abundance and host size or probability of false zero suggest similar vulnerability to acanthocephalan parasites. Generally, when amphipod individuals of native MOTUS GF-I, GF-II, and Gp-D encounter the parasite, they get infected and die from it; only individuals that do not encounter the parasite reach a large size/old age. Overall, acanthocephalan parasite infection levels and mortality effects on amphipod hosts varied greatly among native MOTUS and may thus be providing some, like GF-III and GF-VIII, with a competitive advantage in inter-MOTU interactions or better resistance to invasion by G. roeseli. Gammarus roeseli may also have a parasite-induced competitive advantage against most native MOTUS, potentially providing the invader with a competitive edge against native, local congeneric.

The exact causes of the differences in host parasite-induced mortality among sympatric amphipod hosts documented here remain unclear however. The respective roles of increased predation on infected hosts due to host-manipulation by acanthocephalan and/or direct pathogenic effects of the infection, and potential inter-MOTU variations in their respective effects, are yet to be tested (Benesh, 2011). Additional experiments testing for the exact causes of mortality are thus needed but should also account for other factors influencing potential variability in predation-linked mortality rates among cryptic MOTUs and invasive species. This for instance includes microhabitat segregation among MOTUS, body size differences and/or variable behavioural and morphological defences (Bollache et al., 2000, 2006; Kaldonski et al., 2008; Galipaud et al., 2015b). Furthermore, the “snapshot” nature of our sampling did not allow for testing the temporal stability of the infection patterns observed or the mechanisms involved, or even the stability of co-occurrence of MOTUS in amphipod populations. In sympatric MOTU populations, parasites may promote stable coexistence of cryptic MOTUS or eventually drive all but one to extinction through competitive advantage to the less affected MOTU (Chesson, 2000). However, we cannot draw conclusion on the potential effects of parasites on long term co-occurrence of sympatric MOTUS from our results. Still, results suggest that some morphologically cryptic MOTUS may possess behavioural and/or immunological mechanisms preventing and/or reducing their vulnerability to acanthocephalan parasites while others are highly vulnerable. Cryptic diversity should thus be considered as an additional layer of complexity and accounted for when assessing infection patterns in host populations as sympatric, cryptic lineages of the same morphological species may display highly contrasting patterns of vulnerability to infection and parasite-induced mortality. High genetic divergence among amphipod MOTUS, and the occurrence of populations composed of sympatric MOTUS, may have profound ecological and/or evolutionary implications on observed infections patterns or host-parasite co-evolution and local adaptation (Cothran et al., 2013a, b). It is becoming obvious that amphipod species present high levels of cryptic diversity (Müller, 2000; Sutherland et al., 2010; Westram et al., 2011b; Cothran et al., 2013a; Lagrue et al., 2014; Weiss et al., 2014). Evidence also suggests that different MOTUS of G. fossarum have variable sensitivities to contaminants (Feckler et al., 2012). Here, we show that vulnerability to macroparasites may also be highly variable and not directly linked to genetic divergence levels.

In conclusion, our data showed contrasting levels of infection by acanthocephalan parasites among G. fossarum/G. pulex MOTUS, but also compared to the invasive G. roeseli. Highly variable parasite prevalence and abundance among sympatric MOTUS and between native and invasive amphipods may be due to a combination of factors including MOTU-specific vulnerability to infection and/or parasite induced-mortality. Overall these results, and that of other recent studies (Lagrue et al., 2014; Galipaud et al., 2015a, b), indicate not only that cryptic MOTUS of the native G. fossarum/G. pulex complex are commonly found in sympatry, but also that they can display widely different vulnerability to local parasites. Although the mechanistic causes of such differences are still unclear, they potentially have large effects in the outcome of inter-MOTUS competition and co-occurrence (Chesson, 2000). Furthermore, our results show that parasite effects on interactions between native and invasive amphipods is likely MOTU specific. Cryptic diversity thus adds an extra layer of complexity in intra and interspecific interactions with potentially deep implications in invasion dynamics that cannot, and should not be ignored.

Acknowledgments

C. Lagrue was funded by a post-doctoral grant from the regional council of Burgundy. We thank A. Besson and C. Dubreuil for help in the field and during laboratory processing. This study was partly funded by an ANR grant (grant ANR-13-BSV7-0004, “MultiStress”). We are also grateful to Andrew Thompson and two anonymous reviewers for very constructive comments on a previous version of the manuscript.
Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijppaw.2017.04.005.

References

Amin, O.M., Burns, L.A., Redlin, M.J., 1980. Ecology of Acanthocephalus parksidei Amin, 1975 (Acanthocephala, Echinorhynchidae) in its isopod intermediate host. Proc. Helminthol. Soc. Wash. 47, 37–46.

Anderson, R.M., Gordon, D.M., 1982. Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortality. Parasitology 85, 773–789.

Arundell, K., Dunn, A., Alexander, J., Shearman, R., Archer, N., Ironside, J.E., 2015. Enemy release and founder genetic effects in invasive killer shrimp populations of Great Britain. Biol. Invasions, 1439–1451.

van Balen, M.V., 1988. Coevolution of recovery ability and virulence. Proc. R. Soc. Lond. B Biol. Sci. 265, 317–325.

Bates, A.E., Poulin, R., Lamarre, M.D., 2010. Spatial variation in parasite-induced mortality in an amphibian: shore height versus exposure history. Oecologia 163, 651–669.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48.

Bauer, A., Haine, E.R., Perrot-Minnot, M.J., Rigaud, T., 2005. The acanthocephalan Acanthocephalus lucii (Acanthocephala) in the isopod Gammarus roeseli (Amphipoda). J. Invertebr. Pathol. 79, 256–268.

Bollache, L., Rigaud, T., Cezilly, F., 2002. Impact of a microphagid trematode on the behaviour and survival of its isopod intermediate host: phylogenetic inheritance? Parasitol. Res. 87, 242–246.

Bower, C., 1980. Life-history and population biology of larval Acanthocephalus lucii (Acanthocephala). J. Mar. Biol. Assoc. U.K. 60, 745–752.

Bramble, D.M., 2003. Evolution and behaviour in an amphibian. Evolution 58, 455–469.

Brattey, J., 1986. Life-history and population biology of larval Acanthocephalus lucii (Acanthocephala). J. Mar. Biol. Assoc. U.K. 60, 745–752.

Buchanan, D.P., 1971. Intensity-dependent host mortality: what can it tell us about larval growth strategies in complex life cycle helminths? Parasitology 138, 913–925.

Buchanan, D.P., Valtonen, E.T., 2007. Effects of Acanthocephalus lucii (Acanthocephala) on intermediate host survival and growth: implications for exploitation strategies. J. Parasitol. 93, 735–741.

Blackburn, T.M., Ewen, J.G., 2016. Parasites as drivers and passengers of human-mediated biological invasions. EcolHealth. http://dx.doi.org/10.1002/etc3.1092-6.

Blaxter, M., Mann, J., Chapman, T., Thomas, F., Whitton, C., Floyd, R., Abebe, E., 2002. Adaptive Dynamics of Pathogen-host Interactions. Cambridge University Press, Cambridge.

Dunn, A.M., Dick, J.T.A., 1998. Parasitism and epibiosis in native and non-native gammarids in freshwater in Ireland. Ecography 21, 593–598.

Ebert, D., 1994. Virulence and local adaptation of a horizontally transmitted parasite. Trends Ecol. Evol. 9, 159–165.

Ebert, D., 1997. Virulence and local adaptation of a horizontally transmitted parasite. Trends Ecol. Evol. 13, 387–394.

Ebert, D., 1999. The evolution of trophic transmission. Parasitol. Today 15, 583–586.

Emblidge Fromme, A., Dybdahl, M.F., 2006. Resistance in introduced populations of the freshwater gastropod Littorina littorea. Mol. Mar. Biol. Biotechnol. 3, 741–748.

Goater, C.P., Ward, P.I., 1982. Negative effects of Schistocephalus solidus (Nematomorpha) on the fecundity and pairing status of female Gammarus stimpsoni. Arch. für Hydrobiol. 147, 547–558.

Grabowski, M., Rewicz, T., Bacia-Spychalska, K., Konopacka, A., Mamos, T., Jazdzewski, K., 2012. Cryptic invasion of Baltic lowlands by freshwater amphipod of Pontor origin. Aquat. Invasions 7, 337–346.

Hansen, E.K., Poulin, R., 2005. Impact of a microphagid trematode on the behaviour and survival of its isopod intermediate host: phylogenetic inheritance? Parasitol. Res. 97, 242–246.

Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R., 2003. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B Biol. Sci. 270, 313–321.

Heins, D.C., Birden, E.L., Baker, J.A., 2010. Host mortality and variability in epizootics of Schistosoma solidum infecting the threespine stickleback, Gasterosteus aculeatus. Parasitology 137, 1681–1686.

Hogg, I.D., Stevens, M.I., Schnabel, K.E., Chapman, M.A., 2006. Deeply divergent lineages of the widespread New Zealand amphipod Paracalliope fluvialis revealed using allozyme and mitochondrial DNA analyses. Freshw. Biol. 51, 236–248.

Holmes, J.C., Bethel, W.M., 1972. Modification of intermediate host behaviour by parasites. J. Zoological Soc. London. 151, 123–140.

Kaltz, O., Shykoff, J., 1998. Local adaptation mediated by parasites: theoretical progress. Trends Ecol. Evol. 13, 387–390.

Kaldonski, N., Lagrue, C., Motreuil, S., Rigaud, T., Bollache, L., 2008. Habitat segregation mediated by predation threat: increased refuge use by pre-established individuals. J. Mar. Biol. Assoc. U.K. 88, 668–672.

Kaldonski, N., Lagrue, C., Motreuil, S., Rigaud, T., Bollache, L., 2008. Habitat segregation mediated by predation threat: increased refuge use by pre-established individuals. J. Mar. Biol. Assoc. U.K. 88, 668–672.

Kamler, J., 2003. Parasites lost. Nature 421, 585–586.

Kamler, J., 2003. Parasites lost. Nature 421, 585–586.

Kamler, J., 2003. Parasites lost. Nature 421, 585–586.

Kamler, J., 2003. Parasites lost. Nature 421, 585–586.

Kamler, J., 2003. Parasites lost. Nature 421, 585–586.

Kamler, J., 2003. Parasites lost. Nature 421, 585–586.

Kamler, J., 2003. Parasites lost. Nature 421, 585–586.

Kamler, J., 2003. Parasites lost. Nature 421, 585–586.

Kamler, J., 2003. Parasites lost. Nature 421, 585–586.

Kamler, J., 2003. Parasites lost. Nature 421, 585–586.

Kamler, J., 2003. Parasites lost. Nature 421, 585–586.

Kamler, J., 2003. Parasites lost. Nature 421, 585–586.
2011. Interspecific differences in drift behaviour between the native Gammarus pulex and the exotic Gammarus roeselii and possible implications for the invader’s success. Biol. Invasions 13, 1409–1421.
Lagrange, C., Kaldonski, N., Perrot-Minnot, M.J., Motreuil, S., Bollache, L., 2007. Modification of hosts’ behaviour by a parasite: field evidence for adaptive manipulation. Ecology 88, 2839–2847.
Lagrange, C., Wattier, R., Galipaud, M., Gauthey, Z., Rullmann, J.P., Dubreuil, C., Rigaud, T., Bollache, L., 2014. Confrontation of cryptic diversity and mate discrimination within Gammarus pulex and Gammarus fossarum species complexes. Freshw. Biol. 59, 2555–2570.
Latham, A.D.M., Poulin, R., 2002. Field evidence of the impact of two acanthocephalan parasites on the mortality of three species of New Zealand shore crabs (Brachyura). Mar. Biol. 141, 1131–1139.
Lefèvre, T., Donaudy, C.J., Malard, F.M., Gibert, J., 2007. Testing dispersal and cryptic diversity in a widely distributed groundwater amphipod (Niphargus rhenus-rhodanensis). Mol. Phylogenetics Evol. 42, 676–686.
Levy, H.C., Garcia-Maruniak, A., Maruniak, J.E., 2002. Strain identiﬁcation of hosts’ behavior by a parasite: ﬁeld evidence for adaptive manipulation. Ecology 88, 2839–2847.
May, R.M., Anderson, R.T., 1983. Epidemiology and genetics in the coevolution of parasites and hosts. Proc. R. Soc. Lond. B. Biol. Sci. 219, 281–313.
Mede, V., Bollache, L., Beisel, JN., 2006. Host manipulation of a freshwater crustacean (Gammarus roeselii) by an acanthocephalan parasite (Polyopoma minus-tus) in a biological invasion context. Int. J. Parasitol. 36, 1351–1358.
Moret, Y., Bollache, L., Wattier, R., Rigaud, T., 2007. Is the host or the parasite the most locally adapted in an amphipod—acanthocephalan relationship? A case study in a biological invasion context. Int. J. Parasitol. 37, 637–644.
Morgan, A.D., Gandon, S., Moret, Y., 2003. Differential phenoloxidase activity between native and their consequences for host fitness in two sexual brachiopods: alien Artemia franciscana and native A. salina from sympatric-populations. PeerJ 3, e1073.
Rigaud, T., Moret, Y., 2003. Differential phenoloxidase activity between native and invasive gammarids infected by local acanthocephalans: differential immuno-suppression? Parasitology 127, 571–577.
Roche, D.G., Leung, B., Mendoza Franco, E.F., Torchon, M.E., 2010. Higher parasite richness, abundance and impact in native versus introduced cichlid ﬁshes. Int. J. Parasitol. 40, 1525–1536.
Rousset, F., Thomas, F., De Meûts, T., Renaud, F., 1996. Inference of parasite-induced host mortality from distributions of parasite loads. Ecology 77, 2203–2211.
Sánchez, M.J., Rode, N.O., Flaven, E., Redón, S., Amaì, F., Vassileva, G.P., Lenormand, T., 2012. Differential susceptibility to parasites of invasive and native species of Artemis in living in sympathy: consequences for the invasion of A. franciscana in the Mediterranean region. Biol. Invasions 14, 1819–1829.
Sutherland, D.L., Hogg, I.D., Waas, J.R., 2010. Phylogeny and species discrimination in the Paracallophlebus miniatus species complex (Crustacea: Amphipoda): can morphologically similar heterospeciﬁcs identify compatible mates? Biol. J. Linn. Soc. 99, 190–205.
Tain, L., Perrot-Minnot, M.J., Cézilly, F., 2006. Altered host behaviour and brain serotonin activity caused by acanthocephalans: evidence for speciﬁcity. Proc. R. Soc. Lond. B. Biol. Sci. 273, 3039–3045.
Tain, L., Perrot-Minnot, M.J., Cézilly, F., 2007. Differential inﬂuence of Pomphro-thynus larvis (Acanthocephala) on brain serotonin activity in two congeneric host species. Biol. Lett. 3, 69–72.
Thomas, F., Renaud, F., Rousset, F., Cézilly, F., De Meûts, T., 1995. Differential mor-tality of two closely related host species induced by one parasite. Proc. R. Soc. Lond. B. Biol. Sci. 260, 349–352.
Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J., Kuris, A.M., 2003. Introduced species and their missing parasites. Nature 421, 628–630.
Tromp, P., Fiser, C., 2009. Cryptic species diversity should not be trivialised. Syst. Biodivers. 7, 1–3.
Vale, P.F., Little, T.J., 2009. Measuring parasite ﬁtness under genetic and thermal variation. Heredity 103, 102–109.
Vioante-González, J., Monis, S., Quiterio-Rendon, G., García-Ibáñez, S., Larumbe-Morán, E., Rojas-Herrera, A.A., 2016. Life on the beach for a sand crab (Emerita rathbunae)(Decapoda, Hippoidea): parasite-induced mortality of females in populations of the Paciﬁc sand crab caused by Microphallus nici (Microphal-ldae). Zoosystematics Evol. 92, 153–161.
Weiss, M., Leese, F., 2016. Widely distributed and regionally isolated! Drivers of genetic structure in Gammarus fossarum in a human-impaired landscape. BMC Evol. Biol. 16, 153.
Weiss, M., Macher, J.N., Seefeldt, M.A., Leese, F., 2014. Molecular evidence for further overlooked species within the Gammarus fossarum complex (Crustacea: Amphipoda). Hydrobiologia 721, 165–184.
Westram, A.M., Baumgartner, C., Keller, I., Jokela, J., 2011a. Are cryptic host species also cryptic to parasites? Host speciﬁcity and geographical distribution of acanthocephalan parasites infecting freshwater Gammarus. Infection. Genet. Evol. 11, 1083–1090.
Westram, A.M., Jokela, J., Baumgartner, C., Keller, I., 2011b. Spatial distribution of cryptic species diversity in European freshwater amphipods (Gammarus fossa-rum) as revealed by pyrosequencing. PLoS One 6, e23879.
Witt, J.D.S., Hebert, P.D.N., 2000. Cryptic species diversity and evolution in the amphipod genus Hyalella within central glacialized North America: a molecular phylogenetic approach. Can. J. Fish. Aquatic Sci. 57, 687–698.
Witt, J.D.S., Threlfell, D.L., Hebert, P.D.N., 2006. DNA barcoding reveals extraordinary cryptic species diversity in an amphipod genus: implications for desert spring conservation. Mol. Ecol. 15, 3073–3082.
Zeileis, A., Kleiber, C., Jackman, S., 2008. Regression models for count data in R. J. Stat. Softw. 27, 1–25.