Ecological impacts of the microsporidian parasite *Pleistophora mulleri* on its freshwater amphipod host *Gammarus duebeni celticus*

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SUMMARY

The microsporidian parasite, *Pleistophora mulleri*, infects the abdominal muscle of the freshwater amphipod *Gammarus duebeni celticus*. We recently showed that *P. mulleri* infection was associated with *G. d. celticus* hosts being more vulnerable to predation by the invasive amphipod *Gammarus pulex*. Parasitized *G. d. celticus* also had a reduced ability to prey upon other co-occurring amphipods. We suggested the parasite may have pervasive influences on host ecology and behaviour. Here, we examine the association between *P. mulleri* parasitism and parameters influencing individual host fitness, behaviour and interspecific interactions. We also investigate the relationship between parasite prevalence and host population structure in the field. In our *G. d. celticus* study population, *P. mulleri* prevalence was strongly seasonal, ranging from 8·5% in summer to 44·9% in winter. The relative abundance of hosts with the heaviest parasite burden increased during summer, which coincided with high host mortality, suggesting that parasitism may regulate host abundance to some degree. Females were more likely to be parasitized than males and parasitized males were paired with smaller females than unparasitized males. Parasitism was associated with reduction in the host's activity level and reduced both its predation on the isopod *Asellus aquaticus* and aggression towards precopula pairs of the invasive *G. pulex*. We discuss the pervasive influence of this parasite on the ecology of its host.

Key words: Amphipod, *Gammarus*, isopod, microsporidian, *Pleistophora mulleri*, trophic.

INTRODUCTION

Parasites are increasingly recognized as potential influences on population and community structure (Price, Westoly and Rice, 1988; Minchella and Scott, 1991; Tompkins, White and Boots, 2003). For example, parasites regulate host populations (Prenter *et al*. 2004) and influence the outcome of interspecific interactions (Hanley, Vollmer and Case, 1995), including those between invading and native species (MacNeil *et al*. 2003*a*). However, while microsporidian parasites are ubiquitous in the animal kingdom (Weiss, 2001; Keeling and Fast, 2002), research has concentrated on parasite taxonomy, structure and pathogenicity, with their potential ecological impacts for natural populations being less well studied.

Recently, we identified a new species of microsporidian, *Pleistophora mulleri* that infects the abdominal muscle of *Gammarus duebeni celticus*, the native freshwater amphipod crustacean in Ireland (Terry *et al*. 2003). The parasite is horizontally transmitted and specific to its *G. d. celticus* host, with laboratory studies indicating that it is not possible to transmit it to sympatric invasive amphipods *Gammarus pulex*, *G. tigrinus* and *Crangonyx pseudogracilis* (MacNeil *et al*. 2003*b*). Recently, we reported that *P. mulleri* infected *G. d. celticus* were more vulnerable to predation by *G. pulex*, which is threatening to replace the native *G. d. celticus* in many Irish rivers (MacNeil *et al*. 2003*a*). However, although predation is usually mutual between *G. d. celticus* and *G. pulex*, no study has considered how parasitism of *G. d. celticus* may alter its predatory impact on *G. pulex*, thereby further altering the biotic resistance of the native to species replacement. Therefore, we address this and investigate the association between infection by this microsporidian parasite and the ecology of its amphipod host *G. d. celticus*.

First, we describe temporal patterns in host abundance and test for links to parasite prevalence and burden. Then we investigate the association between parasitism with respect to gender and parameters linked to host fitness such as reproductive decisions and host activity. *Gammarus* species

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are strongly interactive, functioning as 'keystone species' influencing the population dynamics of other macroinvertebrate species (MacNeil, Dick and Elwood, 1997; Kelly *et al.* 2003). Therefore, we also examine the association between parasitism and the host's interactions with 2 co-occurring macroinvertebrates: a prey species, the isopod *Asellus aquaticus* (L.) and the invasive amphipod *G. pulex*.

MATERIALS AND METHODS

Patterns of parasitism in the field

Adult (males >25 mg, females >12 mg) *G. d. celticus* were sampled monthly from June 2000 to March 2002 along a 10 m stretch of the Downhill River (UK grid ref.: C753363). Each sample comprised 10 random 1-min kick-samples (0·9 mm mesh net), where the river-bed substrate is disturbed by kicking, thereby dislodging animals into the net. Animals were counted in the laboratory and *P. mulleri* infection was identified in live adult *G. d. celticus* using light microscopy, since infected abdominal (pleon) muscle tissue appears opaque (see MacNeil *et al.* 2003*a*, *b*). Individuals were classified as parasitized or unparasitized. Infection intensity was recorded as low burden (infection of one abdominal segment) or high burden (infection of entire pleon). Numbers of dead adult *G. d. celticus* were recorded for each sample, but parasite status could not be determined due to tissue degeneration.

To investigate parasite distribution in male and female hosts, in the November 2001 sample, all 185 unpaired adults were sexed and parasite status recorded (low and high burdens combined). *Gammarus* males guard females in precopula pairs for several days before mating occurs (Elwood, Gibson and Neil, 1987). Therefore, to investigate the impact of parasitism on mate choice, males and females from 29 precopula pairs were weighed, male parasite status determined and the male/female pair size ratios compared for parasitized and unparasitized males.

The association of parasitism with changes in host behaviour

In January 2002, Downhill River samples of *G. d. celticus* were collected and maintained in the laboratory in aerated stock tanks ($36\times50\times15$ cm deep) at 12 °C, 9 : 15 h light : dark with source water, flora and fauna. To explore the association between parasitism and host activity, unparasitized, low and high parasite burden *G. d. celticus* ($n=30$ each) were placed individually in circular containers (8 cm diameter) with lines drawn down the middle of the container bottom. Activity level was determined as the number of crosses of the line an individual made during 3 consecutive 2-min periods. Mean numbers of line crossings were examined in a two-factor ANOVA with respect to 'parasite status' and 'time' (repeated measure). Post-hoc tests were Fisher's Protected Least Significant Difference (FPLSD).

To investigate the association between this parasitism and host predation on *A. aquaticus*, in February 2001, *G. d. celticus* and *A. aquaticus* were collected from the Articlave River (UK grid ref.: C785338) and maintained as above. Plastic containers (6 cm diameter) were supplied with 50 ml of filtered Articlave water, substrate (1 cm hollow stone tubes), vegetation (*Elodea canadensis*, elm leaves) and pelleted catfish food. Over 10 days, cannibalism and predation were assessed twice daily in all intra- and interspecific dyadic combinations of adult and juvenile *G. d. celticus* and *A. aquaticus* ($n=30$ for each combination, except no juvenile *G. d. celticus* were found parasitized). Concurrently, mortality of single individuals ($n=30$ each case) was assessed twice daily over 10 days to allow confidence in concluding that cannibalistic/predatory interactions had occurred, as opposed to scavenging following death due to other causes.

To investigate the association between *P. mulleri* infection and behavioural interactions between *G. d. celticus* and the invasive *G. pulex*, we examined how predation of newly moulted *G. pulex* females by *G. d. celticus* males may be influenced by parasitism of the latter. Interspecific predation between these species is strongly influenced by moult stage and sex, with newly moulted females being highly vulnerable to male predation. Mating also coincides with the moult, with females often being attacked and predated despite being paired in precopula with their mate (Dick, Montgomery and Elwood, 1993). In December 2001, *G. pulex* from the Lagan river (UK grid ref.: J299679) were maintained in aerated tanks of source water at 12 °C and a 9 : 15 h light : dark cycle. Precopula pairs were separated by placement on tissue paper until the males released their females, then each was placed into individual plastic containers (8 cm diameter) containing 150 ml of 50% Downhill and 50% Lagan water. Females were inspected twice daily and newly moulted individuals presented to male conspecifics and allowed to form precopula for 30 min. Then, a male *G. d. celticus*, either unparasitized, with low or with high parasite burden ($n=30$ each, matched for size by eye to reduce stress and weighed after experiment to confirm equivalency), was introduced to a pair. Activities of the 3 animals were then recorded for 20 min (see Dick, Elwood and Montgomery, 1995 for details of behaviours recorded), using the Observer Software package (Anon, 1995). Animals were then left together for a further 12 h and any deaths of the amphipods recorded. Data were analysed using one-factor ANOVAs and FPLSD tests.

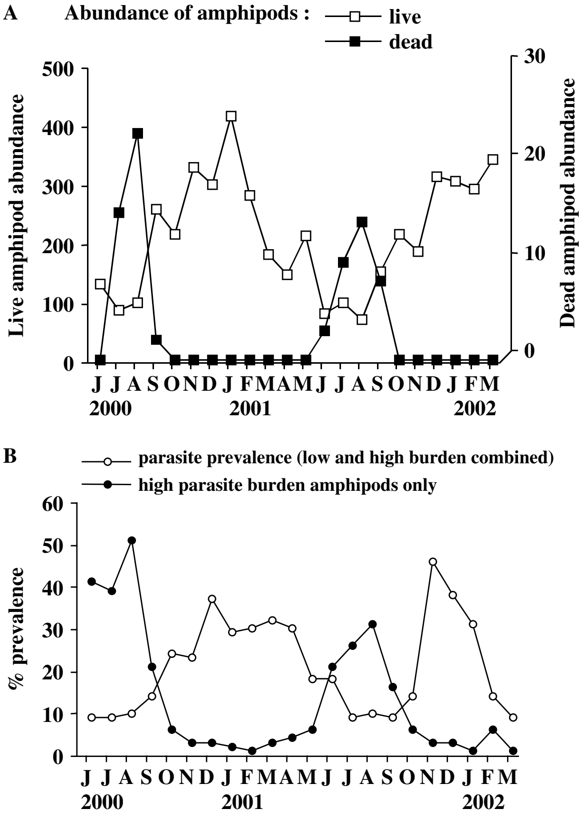

Fig. 1. (A) *Gammarus duebeni celticus* abundance (live and dead animals) and (B) prevalence of parasitized *G. d. celticus* and frequency of high burden parasitized *G. d. celticus* in the Downhill River population over a 22-month period (live animals only).

RESULTS

Parasitism in the field

Live adult *G. d. celticus* abundance ranged from 90 to 425 animals per sample, with lowest abundance in spring/summer and highest in autumn/winter (Fig. 1A). There was a distinct die-off of adults in July/August of both years. Live abundance was negatively correlated with numbers of dead animals recovered in samples (Spearman Rank Correlation $r_s = -0\cdot71$, $n=22$, $P<0\cdot001$; see also Fig. 1A).

Overall parasite prevalence (low and high burdens combined) ranged from 8·5% in summer to 44·9% in winter (Fig. 1B) and this was positively correlated with host live abundance ($r_s=0\cdot44$, $n=22$, $P<0\cdot05$; see also Fig. 1A and B). However, considering the percentage of parasitized *G. d. celticus* that had high parasite burden, there was a negative correlation with *G. d. celticus* live abundance ($r_s=-0\cdot82$, $n=22$, $P<0\cdot001$) and a positive correlation with numbers of dead ($r_s=0\cdot74$, $n=22$, $P<0\cdot001$; see also Fig. 1A and B).

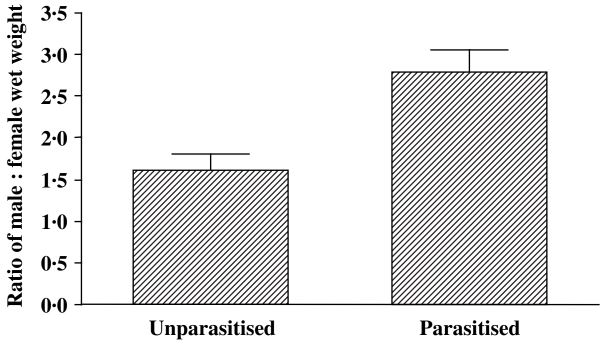

Fig. 2. Mean (+S.E.) ratio of male: female weight in precopula pairs with unparasitized and parasitized guarding males.

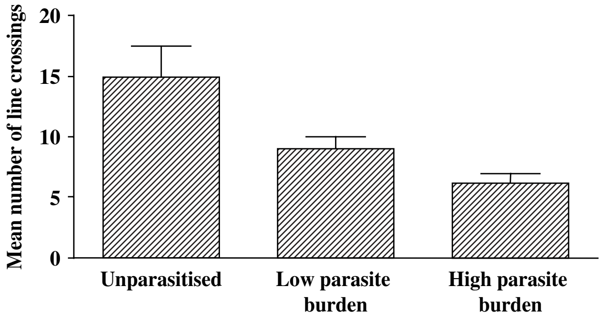

Fig. 3. Mean (+S.E.) number of line crossings of unparasitized, low and high burden parasitized *Gammarus duebeni celticus*.

For unpaired adults, the sex ratio was 1:2 male: female, with a significantly lower frequency of males parasitized than females (27% *vs* 62%, $\chi^2=43\cdot9$, D.F.$=1$, $P<0\cdot0001$). Parasitized males carried relatively smaller females ($t=3\cdot7$, D.F.$=27$, $P<0\cdot001$; Fig. 2).

The association of parasitism with changes in host behaviour

Host activity was significantly lower with increasing levels of parasitism ($F_{2,87}=12\cdot1$, $P<0\cdot0001$, all pairwise FPLSD comparisons $P<0\cdot001$; Fig. 3).

Mortality of single *G. d. celticus* and *A. aquaticus* was zero. We thus conclude that any deaths in dyadic combinations were due to intra- or interspecific interactions. No animals moulted during the experiment. There was no cannibalism in *A. aquaticus* and only juvenile *G. d. celticus* were cannibalized by adults (3/30 by unparasitized adults, 0/30 by parasitized adults). There was no *A. aquaticus* predation of *G. d. celticus*. However, unparasitized adult *G. d. celticus* killed adult *A. aquaticus*, albeit at a low frequency (3/30), whereas parasitized adult *G. d. celticus* did not kill any adult *A. aquaticus*. Predation of juvenile *A. aquaticus* by unparasitized adult *G. d. celticus* was severe and significantly more frequent than that by parasitized *G. d. celticus* (16/30 compared to only 2/30, $P<0\cdot0001$).

There was no significant difference in weight between unparasitized, low and high parasite burden male *G. d. celticus* ($F_{2,87}=1\cdot4$, N.S.). Parasitized

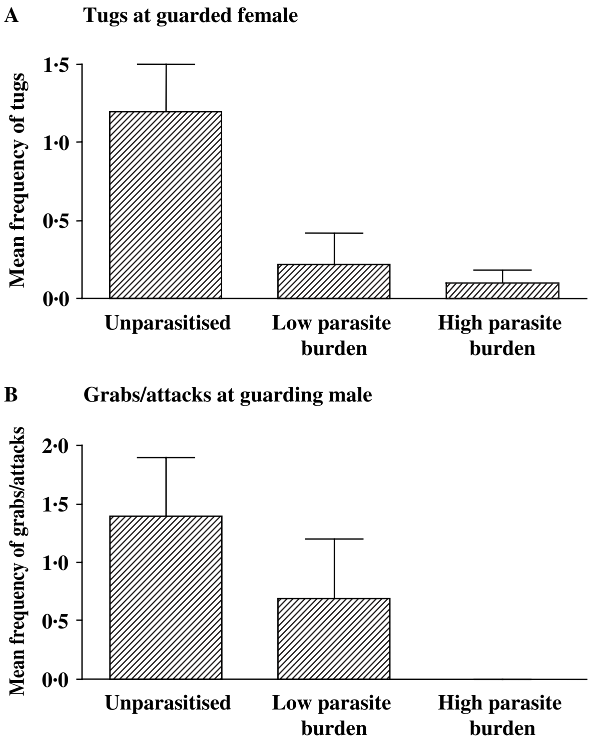

Fig. 4. Mean (+S.E.) frequency of (A) tugs at the guarded female *Gammarus pulex* by the attacking male *Gammarus duebeni celticus* and (B) grab/attacks at the guarding male *G. pulex* by the attacking male *G. d. celticus*.

G. d. celticus made significantly fewer antennal contacts with precopula pairs of *G. pulex* than unparasitized individuals ($F_{2,87} = 58{\cdot}9$, $P < 0{\cdot}0001$) and this decreased significantly with parasite load (FPLSD, all $P < 0{\cdot}001$). Parasitized *G. d. celticus* tugged significantly less at the female and made significantly fewer grab/attacks at the guarding male ($F_{2,87} = 6{\cdot}8$, $P < 0{\cdot}001$, Fig. 4A and $F_{2,87} = 8{\cdot}7$, $P < 0{\cdot}0001$, Fig. 4B), but there were no differences between *G. d. celticus* with low and high parasite burden. The frequencies of defensive kicks by guarding *G. pulex* males and females was significantly lower when parasitized *G. d. celticus* were present ($F_{2,87} = 27{\cdot}1$, $P < 0{\cdot}0001$ and $F_{2,87} = 4{\cdot}8$, $P < 0{\cdot}01$) and this decreased as parasite burden of attacking *G. d. celticus* increased for guarding males (all $P < 0{\cdot}05$) but not for guarding females.

In 88 of the 90 observations, aggressive interactions were observed between the native male and the invader pair, although after a further 12 h, none of the female *G. pulex* had been predated by the *G. d. celticus*.

DISCUSSION

The *G. d. celticus* population monitored in this study showed a distinct cycle of abundance that could be attributed to many causes. That the parasite may be one causal factor, however, is indicated by the decrease in parasite prevalence as host numbers declined. That is, parasitized animals seem more likely to die. In addition, marked declines in the host population coincided with large proportions of the parasitized animals harbouring the heaviest burdens. Schrag and Rollinson (1994) found that the weakest individuals in a population were most likely to acquire parasites. However, MacNeil *et al.* (2003*b*) found that *P. mulleri* infection could be experimentally produced in *G. d. celticus*, with healthy animals being easily infected, so the evidence suggests infection occurs to any individual. Clearly, co-incidental rises in temperatures, change in food supply and predator activity may all influence host abundance, but it is reasonable to conclude that *P. mulleri* is partially involved in structuring its host population. Indeed, crangonid sand shrimps infected with *Pleistophora crangoni* experience a winter and spring fall in prevalence associated with the related deaths of adult hosts (Breed and Olson, 1977). Seasonality of microsporidian parasites has also been attributed to environmental stresses on fish hosts, such as elevated summer water temperature and associated decreases in dissolved oxygen concentration (Chen and Power, 1972; Cali and Takvorian, 1991) and to opportunities for horizontal transmission to intermediate hosts in mosquitoes (Andreadis, 2002).

The study population also had a strongly female biased sex ratio. *Gammarus duebeni* harbours vertically transmitted microsporidia that cause feminization of broods (Dunn and Smith, 2001) and this may be the case here. In addition, however, a higher proportion of females than males harboured *P. mulleri*. This may reflect a higher susceptibility of females to infection or a higher mortality of infected males. Parasitism was associated with changes in parameters that influence host fitness. For instance, males do appear to incur a fitness cost of parasitism, since they carried smaller and hence less fecund females in precopula than did unparasitized males (see also Elwood *et al.* 1987). This may be due to the direct pathogenicity of the parasite in the abdominal musculature (Terry *et al.* 2003), impairing the physical exertion of precopula pairing, reduced mate searching abilities or female choice. Parasitism was associated with a significant reduction in the activity of the host suggesting that the parasites may cause muscle damage that inhibits locomotion (Terry *et al.* 2003). Indeed, we found experimentally that as parasite burden increased, host activity levels decreased. Similarly, reduced mobility and partial paralysis has been observed in the prawn *Penaeus indicus* infected with cotton shrimp disease (Ramasamy, Jaykumar and Brennan, 2000). Muscle fibres became crystallized and were eventually destroyed and replaced by spores of the invading microsporidian, *Thelohania* sp.

Gammarus spp. are predators of many other macroinvertebrates (e.g. chironomids, mayflies and mysids; MacNeil *et al.* 1997) and as such can influence community structure (Kelly *et al.* 2003). Our data indicate that predation pressure by *G. d. celticus* on *A. aquaticus* is moderated by *P. mulleri* infection, with infected *G. d. celticus* less able to prey upon *A. aquaticus* juveniles. Reduced aggression and predation have also been reported in the predatory mite, *Phytoseiulus persimilis*, infected with the microsporidian, *Microsporidium phytoseiuli* (Bjornson and Keddie, 1999).

Populations of *G. d. celticus* are actively replaced by invading *G. pulex*, principally through differential mutual predation that appears to be mediated by parasitism (MacNeil *et al.* 2003*a*). The present study suggests that parasitism by *P. mulleri* is associated with a reduction in the capacity of *G. d. celticus* to predate guarded females of the invader *G. pulex*. This may again be a direct result of the effects of the parasite on the host abdominal musculature. Further, since populations of *G. d. celticus* show wide fluctuations in density that may be partially driven by the parasite, the invasion success of *G. pulex* may be facilitated through decreased biotic resistance from the native at certain times of year. In addition, since *P. mulleri* does not transmit to invading *G. pulex* (MacNeil *et al.* 2003*a*, *b*), the parasite clearly disadvantages the native over the invader and may indeed speed the replacement of the former. We thus encourage further studies of the pervasive influence of microsporidia in mediating interspecific interactions and hence broader features of community organization and trophic function.

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