Journal of Helminthology (2006) **80,** 255–259 DOI: 10.1079/JOH2005330

Spatial covariation between infection levels and intermediate host densities in two trematode species

E.K. Hansen and R. Poulin*

Department of Zoology, University of Otago, PO Box 56, Dunedin, New Zealand

Abstract

Both theoretical arguments and empirical evidence suggest that parasite transmission depends on host density. In helminths with complex life cycles, however, it is not clear which host, if any, is the most important. Here, the relationships between the abundance of metacercariae in second intermediate hosts, and the local density of both the first and second intermediate hosts of two trematode species, are investigated. Samples of the snail *Potamopyrgus antipodarum*, the amphipod *Paracalliope fluviatilis* and the isopod *Austridotea annectens* were collected from ten stations in a New Zealand lake. In the trematode *Coitocaecum parvum*, neither the density of the snail first intermediate host nor that of the amphipod second intermediate host correlated with infection levels in amphipods. In contrast, in the trematode *Microphallus*, infection levels in isopod second intermediate hosts were positively associated with isopod density and negatively associated with the density of snail first intermediate hosts. These relationships are explained by a negative correlation between snail and isopod densities, mediated in part by their different use of macrophyte beds in the lake. Overall, the results suggest that, at least for *Microphallus*, local infection levels depend on local intermediate host densities.

Introduction

Epidemiological models generally rest upon the reasonable assumption that for most types of parasites, transmission success is dependent on host density or local abundance (Roberts *et al.*, 2002). The more hosts there are in one area, the more likely it is that the parasite's dispersal stages will encounter a host. Different lines of evidence support this assumption. For instance, comparative evidence indicates that across populations of the same host species, or across different host species, all else being equal those occurring at higher densities experience higher parasite abundance (Arneberg *et al.*, 1998; Arneberg, 2001; Bagge *et al.*, 2004). Similarly, host species occurring at higher densities are also exploited by a greater diversity of parasite species (Morand & Poulin, 1998; Arneberg, 2002; Takemoto *et al.*, 2005). These large-scale patterns are best explained by the link between the density of host individuals and the probability of parasites finding a host and multiplying.

Although the key role of host density in determining parasite abundance is widely accepted, in the case of parasites with complex life cycles it is not entirely clear which host, if any, is the most important. For example, most trematodes have a three-host life cycle. As adults they live in a vertebrate definitive host, the larvae emerging from their eggs must develop and multiply in a first intermediate host (usually a snail), and the cercarial stages produced in the snail must then encyst as metacercariae in or on a second intermediate host; the life cycle is completed when this second intermediate host is ingested by the definitive host. Which of these three host species is the main determinant of local trematode abundance? Can the density of one host influence the abundance of the parasite in another of the hosts it uses to complete its cycle? In answer to this last question, there is evidence that in avian trematodes, the local density of bird definitive hosts is often positively associated with

*Author for correspondence
Fax: +64-3-479-7584
E-mail: robert.poulin@stonebow.otago.ac.nz

infection levels in both the first (Robson & Williams, 1970; Smith, 2001; Hechinger & Lafferty, 2005) and second intermediate hosts (Marcogliese *et al*., 2001). The spatial scale in these studies is usually of a few kilometres, but even on a scale of a few metres differences in bird density can impact infection levels in snail first intermediate hosts (Smith, 2001).

In the present study, the relationships between the abundance of metacercariae in second intermediate hosts, and the density of both the first and second intermediate hosts of two trematode species, are investigated in a natural system. First intermediate hosts are the source of cercariae that infect second intermediate hosts, and the infection success of cercariae should increase as the density of second intermediate hosts increases. Thus both host populations are potentially affecting how many cercariae will establish, on average, in a second intermediate host. The first trematode species we investigate is *Coitocaecum parvum* (Opecoelidae). It uses the common bully *Gobiomorphus cotidianus*, a small freshwater fish, as its definitive host, the snail *Potamopyrgus antipodarum* as its first intermediate host, and the amphipod *Paracalliope fluviatilis* as its second intermediate host. The second trematode species is an unnamed species of *Microphallus* (Microphallidae); in order, its hosts are various duck species, the snail *P. antipodarum*, and, for second intermediate host, mostly the isopod *Austridotea annectens* but also to a lesser extent the amphipod *P. fluviatilis*. Both trematodes are found in Lake Waihola, 40 km southwest of Dunedin, South Island, New Zealand. The relative mobility of the definitive hosts probably ensures the dispersal of both parasite species throughout the lake (maximum length 5 km, average depth 1.1 m). However, the intermediate hosts are not particularly mobile, and their densities vary across the lake, in particular with respect to the presence of patches of macrophytes, which provide a food base for snails, amphipods and isopods. Therefore, in the lake, there will be patches of high densities of intermediate hosts surrounded by areas of lower densities. Surely the local success of the trematodes, as mirrored in their abundance, will also reflect the immediate availability of intermediate hosts.

Here, for both *C. parvum* and *Microphallus*, the relationships between the densities of first and second intermediate hosts, and the abundance of trematodes in second intermediate hosts, are evaluated across sampling stations in Lake Waihola. Given the restricted mobility of the intermediate hosts, the study was performed on a scale of 100–200 m. The results suggest that spatial patterns in trematode abundance may closely parallel those in intermediate host densities, forming a mosaic of foci where infection levels are high amid larger areas of low infection levels.

Materials and methods

Ten sampling sites were selected along the southern and western shores of the lake, i.e. the part of the lake least disturbed by boating and other human activities. The ten sites were located along the shore, and each site was separated from adjacent sites by about 150 m on average (approximate range 100–200 m). At each sampling site, an effort was made to sample within a macrophyte bed, although high turbidity and thus poor water clarity meant that some samples were taken just outside a patch of macrophytes. Two samples were taken at each site, for a total of 20 samples. At each site, one sample was taken closer to the shore, in water about 1 m deep, and the other was taken further offshore, several metres from the first one, in water about 1.5 m deep; this allowed for a comparison of two water depths. Samples were collected with an apparatus consisting of a benthic grab (opening 20×20 cm, or $0.04\,m^2$), with a 1.8 m long tubular net (250 μm mesh size) attached to the top side of the grab and ending in a collecting tube. Lowered from the side of a boat, the jaws of the grab could be shut once it reached the bottom, cutting the stems of any macrophytes in the sample. This apparatus provided an integrated sample of the water column, the macrophytes, and the upper layers of sediments over an area of $0.04\,m^2$, as well as all snails, amphipods and isopods in the water or on the substrate. Each sample was rinsed into a large container, the water was drained and all remaining material was preserved in 95% ethanol for subsequent sorting. All snails, amphipods and isopods recovered in each sample were counted (there is only one species of each type in the lake). The amphipods and isopods were dissected, and any metacercariae they harboured were counted and identified. For each sample, both the prevalence (percentage of hosts infected) and the abundance (mean number of metacercariae per host) were recorded.

In addition to being separated into inshore and offshore (i.e. 1 m deep or 1.5 m deep), the samples were categorized as coming from within a macrophyte bed or from outside a macrophyte bed, based on the presence or absence of macrophytes in the material collected by the sampler. All density data were log-transformed to meet the assumptions of parametric tests. Since there were relatively few samples, and since some had to be excluded from certain analyses because of zero values (see results), no attempt was made to include all predictor variables in a single multivariate analysis. This would have resulted in few degrees of freedom and low statistical power. Instead, in an attempt to identify existing associations, mostly univariate analyses were used which, although less conservative, still serve to distinguish any patterns.

Results

Overall, 29,587 snails, 3699 amphipods, and 75 isopods were recovered from the 20 samples. Using a two-way ANOVA with inshore-offshore and within-or-outside macrophyte beds as class variables, snail densities were significantly higher within than outside macrophyte beds ($F_{1,16} = 29.17$, $P < 0.001$), but they did not differ between offshore and inshore samples ($F_{1,16} = 1.05$, $P = 0.322$). In contrast, isopod densities tended to be lower within than outside macrophyte beds ($F_{1,16} = 3.51$, $P = 0.079$), but also did not differ between offshore and inshore samples ($F_{1,16} = 0.03$, $P = 0.869$). Amphipod densities did not differ between any type of samples (both $P > 0.116$).

There was no significant interaction term between inshore-outshore and within-outside macrophyte beds for either type of hosts (all $P > 0.444$).

Across all samples, there was a positive relationship between snail density and amphipod density ($r = 0.547$, $N = 20$, $P = 0.013$). A very weak negative association was observed between snail density and isopod density ($r = -0.396$, $N = 20$, $P = 0.084$), and there was no relationship between amphipod density and isopod density ($r = 0.079$, $N = 20$, $P = 0.741$).

The abundance of the trematode Coitocaecum parvum

The overall prevalence of *C. parvum* across all samples was 8.9%, and all infected amphipods harboured a single metacercaria; thus, only analyses involving prevalence data are presented. Given the importance of macrophytes for the density of first intermediate hosts, the prevalence of *C. parvum* in samples taken within macrophyte beds was compared with that in samples taken outside macrophyte beds, but no difference was found (Mann-Whitney U-test, $P = 0.229$). Once an outlier (a sample with an unusually high prevalence) was excluded from the analyses, there was no relationship between the prevalence of *C. parvum* and either snail or amphipod density across all samples (both $P > 0.55$).

The abundance of the trematode Microphallus

The overall prevalence and abundance of *Microphallus* in isopod hosts across all samples were, respectively, 42.7% and 1.8 metacercariae per host. Because these values were extremely low in amphipod hosts (0.4% and 0.004 metacercaria per host), here we only focus on *Microphallus* in isopods.

Both the prevalence (Mann-Whitney U-test: $Z = 2.814$, $N = 20$, $P < 0.005$) and abundance ($Z = 2.808$, $N = 20$, $P < 0.005$) of *Microphallus* in isopods were higher outside than within macrophyte beds. There were positive relationships between isopod density and both *Microphallus* prevalence and, to a lesser extent, abundance, when including only samples in which isopods were present (prevalence: $r = 0.559$, $N = 17$, $P = 0.019$; abundance: $r = 0.440$, $N = 17$, $P = 0.077$). Thus, in areas where isopods occur at higher densities, they also tend to display higher infection levels (fig. 1). In contrast, there were strong negative relationships between snail density and both *Microphallus* prevalence ($r = -0.851$, $N = 17$, $P = 0.0001$) and abundance ($r = -0.862$, $N = 17$, $P = 0.0001$). These results indicate that where snails occur at higher density, nearby isopods display lower infection levels (fig. 2).

Discussion

The role of parasites in host population dynamics and community structure is now undeniable (Combes, 1996; Mouritsen & Poulin, 2002). However, the impact of parasites on a given host population is not necessarily uniform in space or time, and we need to better understand the patterns and processes underlying heterogeneity in parasite abundance. From the parasite's perspective, transmission does not occur homogeneously across the entire host population, but instead probably happens mostly in a certain number of foci within the host population. In helminth parasites with complex life cycles, previous studies have indicated that the density of definitive hosts can determine the local abundance of parasites in intermediate hosts (Marcogliese *et al.*, 2001; Smith, 2001; Latham & Poulin, 2003; Hechinger & Lafferty, 2005). Here, we tested for relationships between the abundance of trematode parasites in their intermediate hosts and the latter's densities. In the trematode *Coitocaecum parvum*, neither the density of the snail first intermediate host nor that of the amphipod second intermediate host correlated with infection levels in amphipods. In contrast, in the trematode *Microphallus*, infection levels in isopod second intermediate hosts were positively associated with isopod density and negatively associated with the density of snail first intermediate hosts. These results indicate that at least for some trematodes, local infection levels depend on local intermediate host densities.

The present findings suggest that macrophyte beds play an important role in both the population structure of invertebrates and in host–parasite dynamics in the lake. Snails concentrate on macrophytes, whereas isopods tend

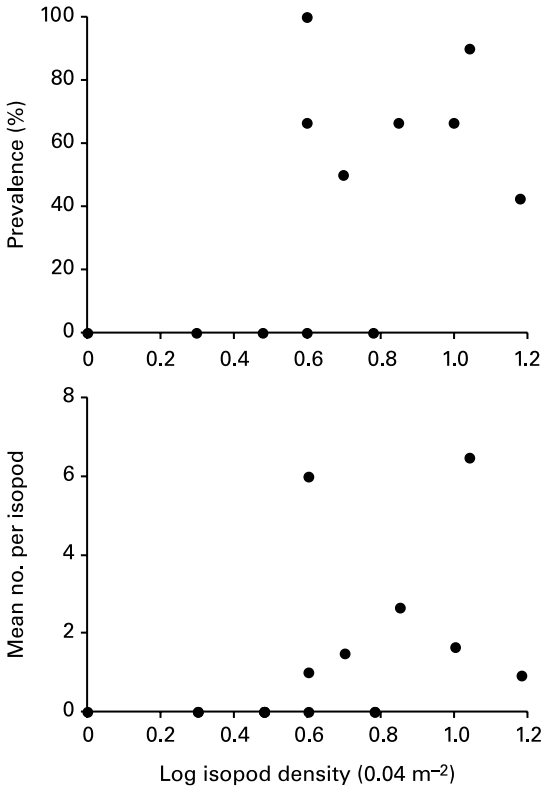

Fig. 1. Relationship between isopod density and both prevalence (top) and mean number of metacercariae per host (bottom) of the trematode *Microphallus* in the isopod *Austridotea annectens*, among 20 samples from Lake Waihola, New Zealand. Note: because infected isopods are absent from some samples, some points are stacked along the x-axis of both plots.

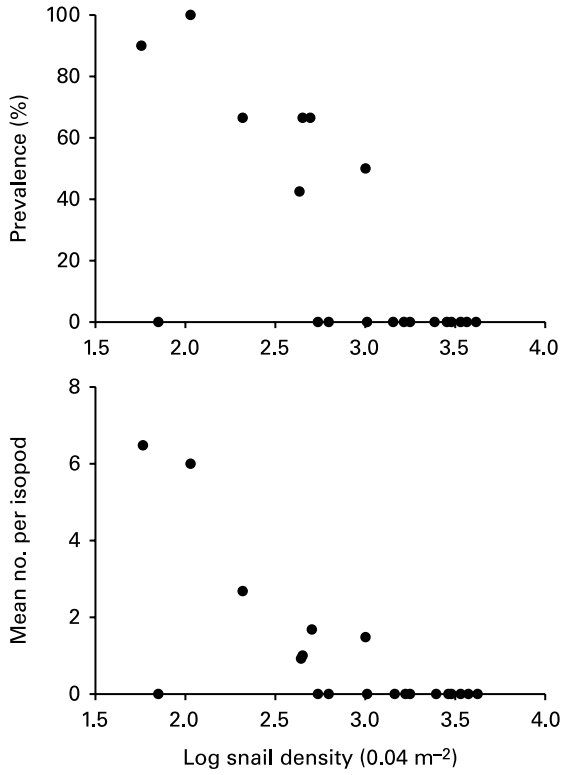

Fig. 2. Relationship between density of the snail *Potamopyrgus antipodarum*, and both prevalence (top) and mean number of metacercariae per host (bottom) of the trematode *Microphallus* in the isopod *Austridotea annectens*, among 20 samples from Lake Waihola, New Zealand.

to occur outside macrophyte patches. While snails feed on epiphytic microalgae growing on macrophytes, the isopods are mostly sediment dwellers feeding on decomposing plant matter (Chadderton *et al.*, 2003). Since *Microphallus* infections in isopods are more common at high isopod densities, and since isopod densities are generally higher outside macrophyte beds, it is not surprising that infections are more common outside macrophyte beds. Macrophytes therefore indirectly influence infection by *Microphallus* by directly affecting isopod densities.

Experimental studies have documented the greater infection success of cercariae at higher densities of second intermediate hosts in a range of trematode species (e.g. McCarthy, 1990). All else being equal, the probability that a cercaria will find a host increases with host density. This explains our finding that infection levels by *Microphallus* correlate spatially with isopod densities. Assuming an equal number of cercariae per surface area or water volume, their infection success would have to increase exponentially, and not linearly, with host density for the mean abundance of metacercariae per host to increase. Alternatively, a greater number of infected snails where isopod densities are high could also generate this result; however we actually found a negative association between isopod and snail densities.

The negative correlation between the density of the snail first intermediate host and *Microphallus* infection levels in the isopod second intermediate host is more intriguing. Assuming a roughly equal prevalence of infection in snails among samples, more cercariae should be produced and released where snails are most abundant. Our finding may simply be a by-product of the weak negative relationship between snail density and isopod density: infection levels in isopods are low where snails are numerous simply because this is also where isopods occur at low densities. If this interpretation is correct, it would mean that the densities of second intermediate hosts are more influential than those of first intermediate hosts. In other words, infection levels in second intermediate hosts may be more sensitive to the probability of cercarial infection success than to the local production of cercariae. Of course, other explanations are also possible, and further work will be necessary to elucidate the processes controlling infection levels in this system.

There are at least three reasons why no significant patterns were observed between intermediate host densities and infection levels for the trematode *C. parvum*. Firstly, the spatial variation in infection levels among samples was very limited, and thus it may be difficult to disentangle the determinants of this variation from background noise without obtaining many more samples. Secondly, the fish definitive host of *C. parvum* is most likely not as mobile as the avian hosts of *Microphallus*. During their extended reproductive season, male common bullies are territorial whereas females remain within the vicinity of nesting males (McDowall, 1990). Therefore the local availability of definitive hosts may vary and play a greater role than that of intermediate hosts, while the mobility of waterfowl definitive hosts of *Microphallus* would cancel out any local effect they may have on infection levels by this trematode. Thirdly, although transmission from the snail first intermediate host to the crustacean second intermediate host is achieved by cercariae in both *Microphallus* and *C. parvum*, only in the former species are the cercariae capable of swimming and modest dispersal. In *C. parvum*, cercariae do not possess a tail, and search amphipods by crawling on the substrate (Macfarlane, 1939). Perhaps their limited dispersal weakens the link between local host densities and infection levels.

The study design did not allow us to evaluate how temporal patterns might combine with the observed spatial heterogeneity in parasite abundance. The patches of macrophytes sampled are not permanent; for instance, they are uprooted by occasional storms, and new beds grow in slightly different areas. Although important, this temporal variation does not detract from the main finding of the study: within the lifespan of these macrophyte patches, they and the areas in between them are inhabited by various densities of snails, amphipods and isopods, and these densities may influence the average parasite abundance experienced by these animals. These results show that any regulation of host populations exerted by parasites may be dependent on host densities, and importantly, that the densities of one host can influence infection levels in populations of the next host in the parasite's life cycle.

Acknowledgements

This work was partially supported by a grant from the Marsden Fund of New Zealand to RP.

References

Arneberg, P. (2001) An ecological law and its macroecological consequences as revealed by studies of relationships between host densities and parasite prevalence. *Ecography* **24**, 352–358.

Arneberg, P. (2002) Host population density and body mass as determinants of species richness in parasite communities: comparative analyses of directly transmitted nematodes of mammals. *Ecography* **25**, 88–94.

Arneberg, P., Skorping, A., Grenfell, B. & Read, A.F. (1998) Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society of London B* **265**, 1283–1289.

Bagge, A.M., Poulin, R. & Valtonen, E.T. (2004) Fish population size, and not density, as the determining factor of parasite infection: a case study. *Parasitology* **128**, 305–313.

Chadderton, W.L., Ryan, P.A. & Winterbourn, M.J. (2003) Distribution, ecology, and conservation status of freshwater Idoteidae (Isopoda) in southern New Zealand. *Journal of the Royal Society of New Zealand* **22**, 529–548.

Combes, C. (1996) Parasites, biodiversity and ecosystem stability. *Biodiversity and Conservation* **5**, 953–962.

Hechinger, R.F. & Lafferty, K.D. (2005) Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. *Proceedings of the Royal Society of London B* **272**, 1059–1066.

Latham, A.D.M. & Poulin, R. (2003) Spatiotemporal heterogeneity in recruitment of larval parasites to shore crab intermediate hosts: the influence of shorebird definitive hosts. *Canadian Journal of Zoology* **81**, 1282–1291.

Macfarlane, W.V. (1939) Life cycle of *Coitocaecum anaspidis* Hickman, a New Zealand digenetic trematode. *Parasitology* **31**, 172–184.

Marcogliese, D.J., Campagna, S., Bergeron, E. & McLaughlin, J.D. (2001) Population biology of eye-flukes in fish from a large fluvial ecosystem: the importance of gulls and habitat characteristics. *Canadian Journal of Zoology* **79**, 1102–1113.

McCarthy, A.M. (1990) The influence of second intermediate host dispersion pattern upon the transmission of cercariae of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae). *Parasitology* **101**, 43–47.

McDowall, R.M. (1990) *New Zealand freshwater fishes: a natural history and guide*. 553 pp. Auckland, Heinemann Reed.

Morand, S. & Poulin, R. (1998) Density, body mass and parasite species richness of terrestrial mammals. *Evolutionary Ecology* **12**, 717–727.

Mouritsen, K.N. & Poulin, R. (2002) Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology* **124**, S101–S117.

Roberts, M.G., Dobson, A.P., Arneberg, P., de Leo, G.A., Krecek, R.C., Manfredi, M.T., Lanfranchi, P. & Zaffaroni, E. (2002) Parasite community ecology and biodiversity. pp. 63–82 *in* Hudson, P.J., Rizzoli, A., Grenfell, B.T., Heesterbeek, H. & Dobson, A.P. (*Eds*) *The ecology of wildlife diseases*. Oxford, Oxford University Press.

Robson, E.M. & Williams, I.C. (1970) Relationships of some species of Digenea with the marine prosobranch *Littorina littorea* (L.). I. The occurrence of larval Digenea in *L. littorea* on the North Yorkshire coast. *Journal of Helminthology* **44**, 153–168.

Smith, N.F. (2001) Spatial heterogeneity in recruitment of larval trematodes to snail intermediate hosts. *Oecologia* **127**, 115–122.

Takemoto, R.M., Pavanelli, G.C., Lizama, M.A.P., Luque, J.L. & Poulin, R. (2005) Host population density as the major determinant of endoparasite species richness in floodplain fishes of the upper Paraná River, Brazil. *Journal of Helminthology* **79**, 75–84.

(Accepted 28 October 2005)