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Local diversity reduces infection risk across multiple freshwater host-parasite associations

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SUMMARY

1. In many host–parasite systems, infection risk can be reduced by high local biodiversity, though the mitigating effects of diversity are context dependent and not universal.
2. In aquatic ecosystems, local fauna can reduce the transmission success of parasite free-swimming infective stages by preying on them, acting as decoy hosts, or physically interfering with transmission. However, most prior research has focused on the effect of a single non-host organism at a time and/or has been performed under simplified and artificial conditions.
3. Here, using data on 11 trematode species sampled in different New Zealand lakes, we test whether local biodiversity affects infection risk in target second intermediate hosts, as well as total parasite population size (number of parasites per m^2), under natural conditions. We considered four components of local biodiversity: total biomass of non-host fish species, diversity (Simpson index) of non-host benthic invertebrates, total density of zooplankton and macrophyte biomass. Our analyses also accounted for host density, a known determinant of parasite prevalence, intensity of infection and total parasite population density.
4. The only influence of local biodiversity we detected was a negative effect of the diversity of non-host benthic invertebrates on the prevalence achieved by trematodes in their second intermediate hosts: that is, the proportion of individual hosts that are infected. Interestingly, this effect was discernible in all 11 trematode species considered here, even if very weak within some species.
5. Our findings suggest that higher non-host benthic diversity may generally decrease infection risk for target hosts including snails, arthropods and fish. However, reduced infection success did not automatically mean smaller overall parasite population size, as other factors can maintain the parasite population in the face of high local diversity of non-hosts.

Keywords: benthic diversity, cercariae, dilution effect, New Zealand, trematodes

Introduction

At a time when human activities are reducing biodiversity, multiple studies indicate that the frequency and severity of parasitic diseases in wild and captive populations are increasing (Jones *et al*., 2008). These concurrent trends suggest that biodiversity and diseases are causally linked. Parasites and their hosts do not interact in an ecological vacuum, but as part of complex networks of sympatric species. The species diversity of communities in which they are embedded can potentially affect the transmission of parasites and the prevalence of infectious diseases in several direct and indirect ways (Keesing, Holt & Ostfeld, 2006). For instance, diverse communities may inhibit the abundance of certain parasites by exerting stronger regulation of the populations of key host species, or by reducing the efficiency of parasite transmission. Mounting evidence suggests that high local diversity often decreases infection risk for focal host species, across a range of host and parasite types (Keesing *et al*., 2006, 2010; Ostfeld & Keesing, 2012; Civitello *et al*., 2015). However, the 'diluting' effect of species diversity is not universal and may depend on study scale, the nature of the other species in the community or other idiosyncrasies of particular host–parasite associations (e.g. Randolph & Dobson, 2012; Wood

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& Lafferty, 2012; Salkeld, Padgett & Jones, 2013). Most evidence to date comes from terrestrial host–parasite systems, in particular those involving tick-borne pathogens, though findings from other systems (e.g. Hall *et al.*, 2009; Prinz *et al.*, 2009; Orlofske *et al.*, 2012; Venesky *et al.*, 2014; Rohr *et al.*, 2015) are rapidly accumulating and will allow us to establish the generality of the protection against infection provided by biodiversity (see Civitello *et al.*, 2015).

Research on the impact of local diversity on risk of parasite infection in aquatic ecosystems has focused on parasites transmitted via free-swimming infective stages, as they are the likeliest to be affected by local diversity. The mechanisms by which species diversity can affect the transmission of parasites using mobile infective stages are varied. For instance, other organisms can prey on infective stages, interfere with transmission by creating physical barriers, act as 'decoys' which attract infective stages but in which the latter cannot develop or act as physiologically suitable hosts in which the infective stages can survive but which are dead-ends for further transmission (Thieltges, Jensen & Poulin, 2008a; Johnson & Thieltges, 2010). Most research has centred on trematodes, parasitic flatworms with complex life cycles that are common in aquatic systems (Mouritsen & Poulin, 2002; Morley, 2012; Preston *et al.*, 2013; Rohr *et al.*, 2015). Adult trematodes live in vertebrate definitive hosts, such as fish or birds, from which they release eggs in host faeces (Galaktionov & Dobrovolskij, 2003). Depending on the species, either the eggs are ingested by the first intermediate host, which is almost always a snail, or the eggs hatch and the free-swimming larvae, or miracidia, released from the eggs locate and directly infect the snail. Following asexual multiplication within the snail host, a second type of short-lived, free-living infective stages, known as cercariae, leave the snail to seek and infect the second intermediate host, which may be another mollusc, an arthropod, a fish or some other organism, depending on the trematode species (Galaktionov & Dobrovolskij, 2003). After penetrating this host, the cercariae develop into metacercariae and await passage (through ingestion) to a suitable definitive host.

There are thus either one step (cercariae only) or two steps (miracidia and cercariae) in the life cycle of trematodes where transmission is achieved by mobile larvae. Most research to date has examined how the presence of other organisms affects transmission at the cercarial stage. Various non-host organisms can directly prey on cercariae; these include sea anemones, small crustaceans, larval insects, juvenile amphibians and small fish (Mouritsen & Poulin, 2003; Hopper, Poulin & Thieltges, 2008; Kaplan *et al.*, 2009; Prinz *et al.*, 2009; Orlofske *et al.*, 2012; Welsh *et al.*, 2014; Orlofske, Jadin & Johnson, 2015; Rohr *et al.*, 2015). Either other organisms act as decoy hosts that are unsuitable for the parasite, or they physically interfere with host-finding by cercariae (Leung & Poulin, 2008; Thieltges *et al.*, 2008b; Prinz *et al.*, 2009; Welsh *et al.*, 2014). The presence of alternative host species of lower competence for parasite development can also reduce the infection risk incurred by a focal host species (Johnson *et al.*, 2013). However, except for the latter, most studies have generally focussed on the effect of a single extra organism at a time on parasite transmission, and they have been performed under artificial conditions in aquaria or small mesocosms consisting of highly simplified communities.

Here, we use field data from multiple host–parasite associations from an extensive sampling of the entire littoral communities of four New Zealand lakes to provide a replicated test of the hypothesis that local biodiversity affects infection risk in aquatic systems. Our analysis focuses exclusively on the cercarial transmission step of several trematode species. We use two individual-level measures of infection risk in target second intermediate hosts, prevalence (proportion of hosts that are infected) and mean intensity of infection (mean number of parasites per infected host), as well as a measure of the total population size achieved by the parasite at the post-cercarial stage (numbers of metacercariae per unit surface area). We split local biodiversity into four separate components, to recognise the different mechanisms by which the ambient fauna can affect transmission (Thieltges *et al.*, 2008a). First, we consider the total biomass of fish, per unit area of the zone sampled, that are not host species of a focal parasite. In our systems, larval fish are pelagic and leave the littoral communities, so that only juvenile or adult fish are found. Since these are too large to consume cercariae, they are more likely to act as decoy hosts and/or 'sinks', with cercariae becoming trapped on the fish's skin mucus through accidental contact or their gills during ventilation. Second, we consider the diversity of all benthic or demersal invertebrate species within the area sampled that are not hosts of the focal parasite. Because they are orders of magnitude smaller than fish, many of these invertebrates can prey on cercariae, in addition to affecting their transmission in other ways. Third, we include total density of zooplankton individuals of all species combined, within the area sampled. These are uniformly small bodied and thus capable of direct predation on cercariae. Finally, we consider the total biomass of macrophytes per unit area within the zone sampled, since in marine systems, seaweed can

interfere with cercarial transmission by acting as physical barriers between infective stages and their target hosts (Prinz *et al.*, 2009; Welsh *et al.*, 2014).

Our analysis provides a general test of the diluting effect of biodiversity on infection risk in aquatic systems, replicated across several parasite species and performed under natural conditions. It also highlights how threats to biodiversity, in addition to their likely impact on ecosystem services (Cardinale *et al.*, 2012; Naeem, Duffy & Zavaleta, 2012), may alter disease dynamics in freshwater communities.

Methods

Field sampling and laboratory processing

We sampled all fish, invertebrate and macrophyte species from the littoral zone of four lakes on the South Island of New Zealand. The lakes were small to medium in size, mostly shallow, and at different altitudes and distances from the coast (for name, location and characteristics of each lake, see Appendix S1 in Supporting Information). In each lake, we sampled 4 square areas (15 m × 15 m) with one side of the square along the shore, 123–2250 m distant from each other and selected to represent all habitat types (substrate, riparian vegetation, etc.) present within each lake. This gave us 16 study sites (4 lakes × 4 sampling sites per lake). Each site was sampled in three seasons (September 2012, January and May 2013) to obtain annual averages of infection levels, densities and biomasses of organisms.

Fish were sampled using a combination of gear types following a standardised protocol so that samples represented fish diversity and density as accurately as possible (see Appendix S1 for full details). Two fyke nets were set overnight along the edges of the sampling area, perpendicular to the shore, and two 15-m-long multimesh gillnets were deployed in the same place during the day. These were used to capture all fish swimming in and out of the area, that is both residents and visitors to the area. In addition, a standard, fine-mesh purse seine net was dragged across the whole area to capture small and/or sedentary resident fish not captured by passive gear like fyke nets or gillnets. All fish caught were identified to species and counted, and a subsample was returned to the laboratory for dissection.

In each site and in each season, six samples of benthic invertebrates, distributed haphazardly across the sampling area, were taken using a standard Surber sampler net with a 0.1 m² horizontal metal frame fitted with a 250-μm-mesh collecting net. In addition, six samples of demersal invertebrates, living on or near the substrate but not captured in Surber nets, were sampled using a rectangular dip net (30 cm wide and 22 cm high opening) with a 250-μm-mesh net; each sample consisted of a fast, 2-m-long sweep of the net along the lake bottom without dredging the substrate. All invertebrate samples were preserved in ethanol for later identification, counting and dissection.

Four plankton samples were taken per site and per season in each lake. Sampling was performed at night by towing a conical net (25 cm mouth diameter) made of fine nylon mesh (90 μm mesh size) through the water for a 3-m horizontal distance. Samples were distributed haphazardly across the sampling site. Zooplankton was fixed in 70% ethanol for later identification and counting. The volume of water from which zooplankton was obtained was estimated as the product of tow length and the area of the net opening.

Macrophytes were sampled along with benthos using Surber sampler nets (see above). They were rinsed to dislodge invertebrates and sediment, sealed into plastic bags and frozen for later sorting, identification and biomass assessment.

In the laboratory, all individuals were identified to the lowest possible taxonomic level and counted. All fish were weighed to the nearest 0.01 g, allowing calculation of a mean individual mass for each fish species and for each site, from which biomass could be derived as the product of individual body mass and density per m^2. Macrophytes were patted dry to eliminate excess moisture and weighed to determine the fresh weight of each species (all individuals combined) per sample. Finally, large subsamples of each fish and invertebrate species were dissected carefully following a standardised protocol for parasite recovery and identification (see Appendix S1 for full details).

Measuring infection and local biodiversity

The snail *Potamopyrgus antipodarum* serves as the first intermediate host, that is as the source of cercariae, for most trematode species considered here; for a few such as *Tylodelphys* sp., the snail first intermediate host has not yet been identified. Infection pressure, that is the local quantity of cercarial infective stages, is an important determinant of infection risk for target hosts (second intermediate hosts). However, prevalence of infection in snail first intermediate hosts is extremely low. In our data set, larval stages of some species (e.g. *Tylodelphys* sp.) were never found in snails of any species, whereas for all other species recorded, prevalence was often zero

Table 1 Parasite species included in the present analysis, the type of second intermediate hosts targeted by their cercarial infective stages and number of populations (i.e. number of localities) where they occurred

Trematode species	Target host species	Second intermediate host taxon	Number of localities
Apatemon sp.	*Gobiomorphus cotidianus*	Fish	16
Coitocaecum parvum	*Paracalliope fluviatilis*, *Paracorophium excavatum*	Crustacean	9
Maritrema poulini	*Paracalliope fluviatilis*, *Paracorophium excavatum*, *Austridotea annectens*	Crustacean	8
Microphallus sp.	*Paracalliope fluviatilis*	Crustacean	4
Microphalloidea sp.	*Oecetis* sp., *Triplectides* sp.	Insect	4
Pronocephaloid sp. I	*Potamopyrgus antipodarum*	Snail	4
Pronocephaloid sp. IV	*Potamopyrgus antipodarum*	Snail	3
Stegodexamene anguillae	*Gobiomorphus cotidianus*	Fish	15
Telogaster opisthorchis	*Gobiomorphus cotidianus*, *Galaxias maculatus*	Fish	16
Tylodelphys sp.	*Gobiomorphus cotidianus*	Fish	4
Unidentified trematode	*Potamopyrgus antipodarum*	Snail	13

in particular sampling localities and season, or very close to zero. Therefore, we did not include the density of infected snails (proxy for infection pressure) as a predictor in our analyses because values would be either unknown or near zero. In addition, the overall density of the source host, combining infected and uninfected snails, has no real impact on infections in the next host (Lagrue & Poulin, 2015).

The second intermediate hosts, or target hosts, varied among trematode species and included snails, insects, crustaceans and fish (Table 1). In most cases, a parasite targeted a single host species in a given site. However, in cases where the parasites used two or more host species, the main host was defined as the species harbouring the most parasite individuals. If the second most important host species harboured at least a quarter of the number of parasites found in the main host, it was combined with the main host to calculate prevalence and mean intensity of infection for that locality. There was no case where the third most important host species in a locality harboured even one-tenth of the number of parasites found in the second most important host species, and thus, we never considered more than two host species per locality to calculate prevalence and mean intensity of infection. As the parasite's main host and secondary hosts typically were taxonomically close and ecologically similar (i.e. similar body sizes), each individual host was treated as equivalent in the calculations of prevalence and mean infection intensity. For the calculation of parasite density (total number of metacercariae per m^2), all host species in which a parasite species occurred were included.

We treated each sampled locality (four sites in each of 4 lakes = 16 localities) separately; therefore, for each parasite species, we have up to 16 data points, depending on their frequency of occurrence among localities (localities from which a parasite was absent were not included for that parasite). Measures of prevalence, mean intensity and local parasite density, as well as the five predictors (see below), were computed by pooling data taken from the three seasonal sampling series, to obtain annual averages for each locality.

In an earlier study (Lagrue & Poulin, 2015), the local density (individuals per m^2) of hosts was identified as an important determinant of mean intensity of infection and parasite population density in trematodes. Therefore, it was included here as a main predictor to account for its effects while assessing the impact of local biodiversity on infection risk. For each site and each sampling season, we first averaged the density values of each host species across all samples. Then, we calculated the mean density per locality across all three seasonal sampling series. Parasites using two or more host species were treated as above, that is each individual host was treated as equivalent in the calculations of mean host density.

The other four predictors of infection risk were fish biomass, benthic invertebrate diversity, zooplankton density and macrophyte biomass per locality. Fish biomass was computed as the total mass of fish (g per m^2) that are not host species of a focal parasite, averaged across all three seasonal sampling series. Benthic invertebrate diversity was computed by pooling data across the three seasonal sampling series for all benthic and demersal invertebrate species that are not hosts of the focal parasite. Using their density values (individuals per m^2) as measures of relative abundance, we computed the Simpson index of diversity for each locality, as follows:

$$D = \frac{1}{\sum_{i=1}^{i=S} p_i^2},$$

where p_i is the relative abundance of the ith species in a community of S species of demersal and benthic

invertebrates. Relative abundances were calculated based on the contribution of each species to total density of organisms per m^2. The reciprocal of the Simpson index, used here, has been shown to be an unbiased estimator of diversity that is influenced by species evenness and not just species richness (DeJong, 1975; Mouillot & Leprêtre, 1999), although it is somewhat weighted towards the most abundant species. Zooplankton density was simply the sum of the local densities of all zooplankton species, converted to density per m^2 by projecting the number of individuals contained in 1 m^3 of lake water onto the flat surface necessary to contain that volume according to water depth at each sampling site (all sites are littoral areas with depth <1 m). Macrophyte biomass was taken as the total macrophyte biomass per m^2 across all species, averaged among all samples per site.

Statistical analysis

Parasite prevalence values were arcsine-transformed, and mean intensity of infection and parasite density values were log-transformed prior to all analyses. These three measures of infection were not statistically independent from each other (pairwise correlations: prevalence versus intensity: $N = 96$, $r = 0.610$, $P < 0.0001$; prevalence versus density: $N = 96$, $r = -0.374$, $P = 0.0002$; intensity versus density: $N = 96$, $r = 0.105$, $P = 0.3074$). They will nevertheless be considered as distinct response variables in the following analyses.

The three measures were analysed using three separate mixed-effects models with Gaussian error structure implemented in JMP version 11.0 (SAS Institute Inc., Cary, NC, U.S.A.). Gaussian distributions were most appropriate for our response variables, which are local mean values and not individual data points (e.g. individual intensity values would be best fitted by a negative binomial, but the distribution of local mean values is different). Our main goal was to test the effects of the various components of local biodiversity on local infection risk for second intermediate hosts, while accounting for the influence of target host density. Therefore, target host density, fish biomass, benthic invertebrate diversity, zooplankton density and macrophyte biomass (all log-transformed) were included as fixed factors in the models. Among these five predictors, there was only weak positive correlations between benthic invertebrate diversity and fish biomass ($N = 96$, $r = 0.208$, $P = 0.0421$) and between benthic invertebrate diversity and macrophyte biomass ($N = 96$, $r = 0.250$, $P = 0.0142$), with all other pairwise correlations among predictors being non-significant (all $P > 0.10$). There is therefore no confounding colinearity among the predictors.

Sampling site (nested within lake) was included as a random factor in the models. This accounts for idiosyncrasies of particular lakes and for the non-independence and correlated spatial structure of the data arising from the fact that multiple data points come from the same site. In addition, parasite species as well as the higher taxon of the target host (three categories: snail, arthropod or fish) were also included as nested random factors to account for any phylogenetic influences. We calculated the proportion of the total variance unexplained by the fixed effects that could be accounted for by each random effect (Nakagawa & Schielzeth, 2013).

Results

The data set consisted of 96 data points on local infection risk and parasite densities, representing 11 trematode species using 8 host species (Table 1). Within trematode species, there was much variability among local populations in infection parameters, with intensity of infection varying from less than 2-fold to more than 40-fold in some species, and density varying from about 2-fold to well over 100-fold in several species. The values for the four components of local biodiversity associated with the various trematode populations also showed substantial variation (Fig. 1).

The analysis of factors affecting measures of infection and parasite population size revealed that, as expected, the density of the target host is a key determinant of both intensity of infection and total parasite density per m^2 (Table 2). The only influence of any aspect of local biodiversity was a negative effect of local diversity of non-host benthic invertebrates on the prevalence achieved by trematodes in their second intermediate hosts (Table 2). Interestingly, this effect applied to various degrees to all 11 trematode species considered in our study (Fig. 2) and amounted to a 3–25% reduction in local prevalence with increasing benthic invertebrate diversity.

The random factors included in the analysis explained a large portion of the remaining variance (Table 2). In particular, the taxonomic affiliation of the target second intermediate host accounted for much variance, which is not surprising as different taxa are typically infected to different extents. For instance, trematodes almost invariably achieve higher prevalence and intensity of infection in fish than in invertebrates (e.g. see Fig. 2). In contrast, lake identity and site within lake explained only 1–2%

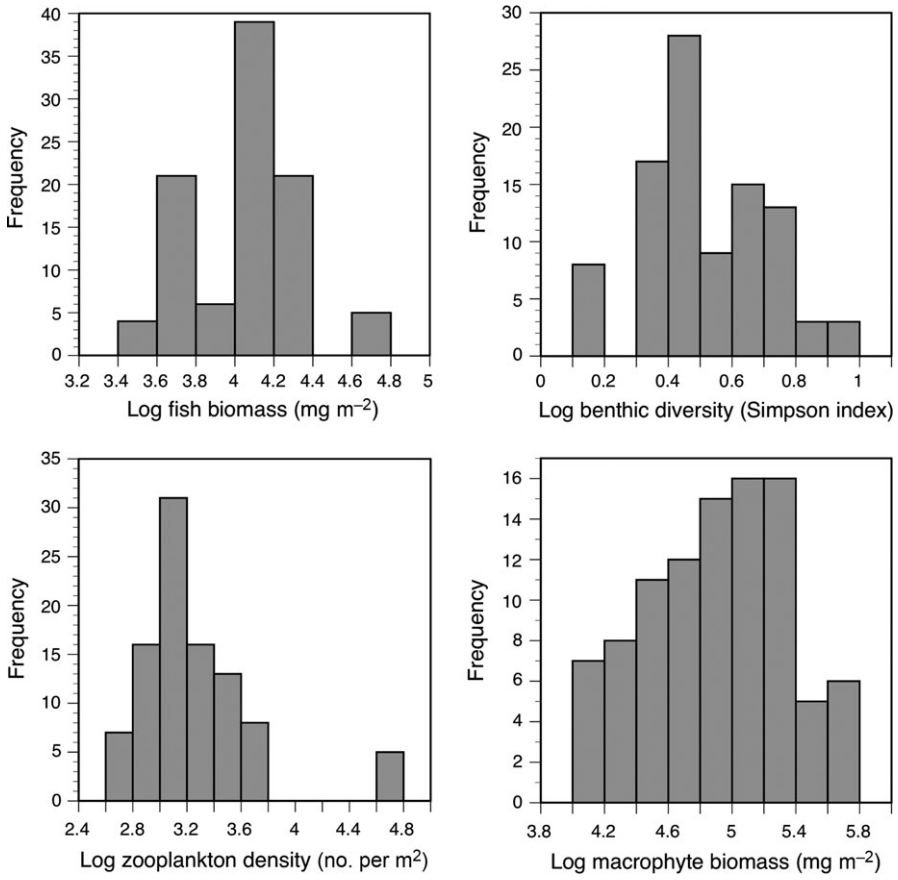

Fig. 1 Frequency distribution of values (N = 96) for non-host fish biomass, benthic (incl. demersal) invertebrate diversity, zooplankton density and macrophyte biomass in the New Zealand lake sites sampled for trematode infection levels and population densities.

of the variance in any of the response variables considered.

Discussion

With biodiversity under increasing threat, and emerging diseases becoming more frequent in aquatic ecosystems (Lafferty, Porter & Ford, 2004; Johnson & Paull, 2011), we urgently need to better understand how changes in diversity affect disease risk. Here, we found that higher diversity of non-host benthic invertebrates was associated with lower prevalence of infection by trematodes in their target hosts, in the littoral zone of New Zealand lakes. Although the effect was only marginal for some species, the negative prevalence–diversity trend was observed in all 11 trematode species considered in our study. No other aspect of infection or parasite population size was affected, and no other component of local biodiversity had a significant influence on infection. Nevertheless, our finding provides a rare example of the negative effect of aquatic biodiversity on disease risk under totally natural conditions.

Many earlier studies on the effect of non-host organisms on cercarial transmission were performed on marine host–parasite systems and involved non-hosts that were either filter feeders, such as barnacles or mussels, or indiscriminate particle feeders, such as anemones (e.g. Mouritsen & Poulin, 2003; Hopper *et al.*, 2008; Welsh *et al.*, 2014). These types of feeders are ideal candidates as potential predators of cercariae, but they are mostly absent from freshwater benthic invertebrate communities. However, given the sizes of individual cercariae (body length, excluding tail, 100–1000 μm, depending on the species), it is conceivable that they are taken as prey by many other types of invertebrate predators (Orlofske *et al.*, 2015). Cercariae can be an abundant resource for consumers capable of exploiting them (Thieltges *et al.*, 2008c; Johnson *et al.*, 2010; Morley, 2012). For instance, annual cercarial productivity has been estimated around 1–2 kg ha^{-1} in freshwater systems (Preston *et al.*, 2013) and 10–40 kg ha^{-1} in coastal marine systems (Kuris *et al.*, 2008). Furthermore, cercarial penetration of unsuitable hosts and subsequent parasite death may also result in local reduction of infection risk for suitable target hosts. During dissection of non-hosts in the present study, no dead metacercariae were found (encapsulated or melanised), suggesting either that cercariae do not often penetrate an unsuitable host

Table 2 Results of the mixed-effects models with either parasite prevalence, mean intensity of infection or parasite density as the response variable, showing the effects of the main predictors and the proportion of the remaining variance accounted for by the random factors

Fixed factors	Estimate	Std error	*t*-value	*P*	Random factors	% variance
RESPONSE: prevalence of infection						
Intercept	0.6264	0.6534	0.96	0.3539	Site [lake]	1.8
Log target host density	−0.0420	0.0309	1.36	0.1783	Parasite species	20.5
Log fish biomass	0.0570	0.0950	0.60	0.5584	Higher host taxon	69.7
Log benthic invertebrate diversity	**−0.3265**	**0.1369**	**2.38**	**0.0227**		
Log zooplankton density	0.0161	0.0577	0.28	0.7861		
Log macrophyte biomass	0.0115	0.0637	0.18	0.8597		
RESPONSE: intensity of infection						
Intercept	1.4769	1.4429	1.02	0.3318	Site [lake]	0.9
Log target host density	**0.4560**	**0.0543**	**8.39**	**<0.0001**	Parasite species	14.3
Log fish biomass	−0.0149	0.1851	0.08	0.9369	Higher host taxon	82.0
Log benthic invertebrate diversity	0.2404	0.2570	0.94	0.3562		
Log zooplankton density	−0.1474	0.1129	1.31	0.2241		
Log macrophyte biomass	−0.1308	0.1248	1.05	0.3216		
RESPONSE: parasite density						
Intercept	−0.6397	1.8793	0.34	0.7436	Site [lake]	0.2
Log target host density	**1.5036**	**0.0874**	**17.19**	**<0.0001**	Parasite species	8.8
Log fish biomass	0.2008	0.2209	0.91	0.3818	Higher host taxon	87.3
Log benthic invertebrate diversity	0.1842	0.3492	0.53	0.6034		
Log zooplankton density	−0.0674	0.1319	0.51	0.6198		
Log macrophyte biomass	−0.0981	0.1446	0.68	0.5139		

Significant effects are shown in bold.

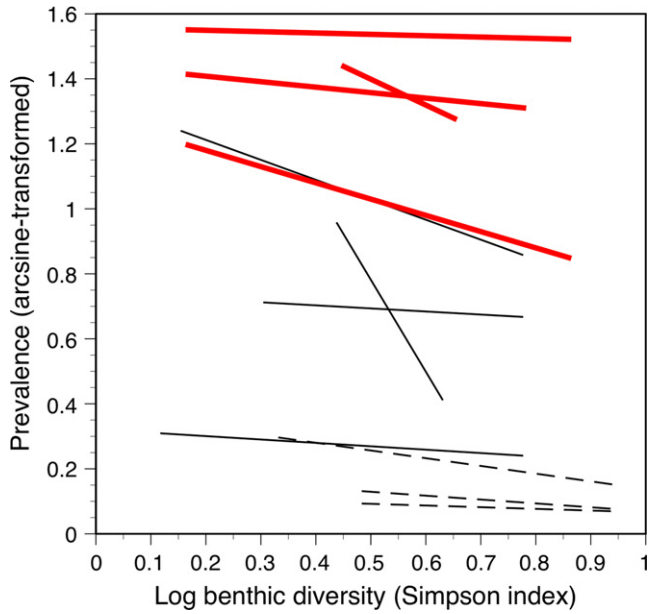

Fig. 2 Relationships between prevalence of infection and local benthic (incl. demersal) invertebrate diversity across different sampling sites, for 11 trematode species in New Zealand lakes. The second intermediate hosts targeted by these trematodes are either snails (thin broken lines), arthropods (thin black lines) or fish (thick red lines). Only best-fitting trend lines are shown; for number of sites included in each case, see Table 1.

in which they are subsequently killed, or that they are killed quickly leaving no trace. Therefore, the most plausible mechanisms by which diverse benthic communities mitigate infection risk for freshwater target hosts are, firstly, by direct predation on cercariae and, secondly, by failed infection of non-hosts.

It remains to be determined which benthic invertebrates are responsible for the observed negative relationship between non-host diversity and prevalence. The most likely culprits, based on their high average densities (from a few 100s to several 1000s per m^2), include many species of invertebrates in which we never found parasites, such as oligochaetes (Tubificidae), crustaceans (*Tenagomysis*), insects (Chironomidae, Ceratopogonidae) and bivalves (Sphaeridae). The small body size of these organisms also increases their likelihood of preying on cercariae (Orlofske *et al.*, 2015). For instance, sphaerid bivalves could filter-feed on the smallest cercariae (*Maritrema poulini*, *Microphallus* sp., Microphalloidea sp.) as these would fit through their siphons. In contrast, tubificid oligochaetes are deposit feeders unlikely to eat cercariae, and if they interfere with transmission to target hosts, it is more likely to be as unsuitable decoy hosts. In addition, there are several parasitised species that could, due to their high abundance, nonetheless affect the transmission success of parasites targeting other hosts; they include crustaceans (Amphipoda, Isopoda), insects (Trichoptera) and snails (*Potamopyrgus*). Snails achieve the highest densities of any benthic species at our study sites, yet these grazers appear incapable of eating cercariae even when placed with them for long

periods in Petri dishes (C. Lagrue, personal observation). Experimental studies in small mesocosms will be necessary to confirm whether species from this list are responsible, either singly or in combination with others, for the reduction in prevalence seen when benthic invertebrate diversity increases, and also to confirm the precise mechanisms (e.g. predation on cercariae, decoy hosts, physical interference, etc.) involved. Experimental studies would also allow us to control infection pressure, which we could not do in our field study because of the extremely low prevalence of infection in snail first intermediate hosts, often too low for detection.

A remarkable feature of our results is that, although not always very pronounced, the negative prevalence–diversity relationship was discernible in all 11 trematode species studied here, regardless of the nature of their target host. Within individual trematode species, the relationship is mostly non-significant, but to a large extent because most of these species occurred at relatively few sites and thus the number of observations is small. Species-specific properties of cercariae (i.e. size, swimming behaviour, etc.) probably also make some trematodes more subject to the influence of local non-host benthic diversity than others. Nevertheless, even if not truly applicable to all species individually, the overall negative trend across the different species remains detectable. This may be explained by the fact that the main fish used by all trematodes targeting a fish as second intermediate host was the common bully, *Gobiomorphus cotidianus* (Eleotridae), a bottom-dwelling fish that spends much of its time in contact with the substratum. Trematode cercariae are notoriously well adapted to locate their target host by reacting to stimuli that increase their spatial overlap with the host (Combes *et al*., 1994). Thus, whether they targeted fish, snails or arthropods, cercariae of all 11 species were probably photophobic bottom-huggers that also overlapped with non-host benthic invertebrates. This may explain why the density of zooplankton had no impact on infection risk, despite interactions between plankton and photophilic cercariae being common in other systems (Morley, 2012). Also, apart from *G. cotidianus*, either other fish species in our study lakes are pelagic (e.g. Galaxiidae) or they occur at very low abundance (e.g. *Anguilla* spp.), suggesting they are unlikely to impact the transmission of bottom-hugging cercariae. In contrast, macrophyte biomass could in principle affect transmission of cercariae between the benthic snail hosts from which they emerge and the benthic snail, fish or arthropod they seek. In marine systems, seaweed, as well as non-living remains of animals, such as mollusc shells, can also impede the ability of cercariae to locate and infect suitable hosts (Prinz *et al*., 2009; Neal & Poulin, 2012; Welsh *et al*., 2014). The general morphology of the main macrophyte taxa found in our system (genera *Myriophyllum*, *Ranunculus*, *Ruppia*, *Potamogeton* and *Elodea*) is not very different from that of marine macroalgae (genera *Sargassum*, *Fucus* and *Corallina*) shown to diminish cercarial transmission. One possibility is that macrophytes in our sampling sites did not reach the threshold density above which interference with cercarial transmission would have been detectable. This may change in the near future, however, as some of our study sites are experiencing eutrophication.

Importantly, the negative effect of local non-host benthic diversity we uncovered applies strictly to prevalence of infection, that is the proportion of individuals in a host population that are parasitised. Intensity of infection, or the mean number of parasites per infected host, and total parasite density per m^2 were unaffected by benthic diversity. These observations can be reconciled when considering the spatial variation in host density, which is a key determinant of intensity of infection and parasite density (Lagrue & Poulin, 2015) and which differs from one sampling locality to the next. Thus, local non-host benthic diversity may reduce the risk of infection for individual hosts without causing a reduction in total parasite population size. A rich community of non-hosts may allow more hosts to avoid infection and thereby affect the distribution of parasites among hosts, without necessarily changing the overall parasite population. In our data set, there were no significant correlations between host density and any of the four components of local non-host diversity we considered (see Methods). In other cases, such correlations could mask any negative effect of biodiversity on disease risk or parasite population size, suggesting that future tests of the diversity–disease relationship in natural systems will likely be subject to strong idiosyncrasies. It will be crucial to consider how resource availability (host abundance) covaries with biodiversity to properly determine whether the impact of diversity extends to individual-level disease risk only, total parasite population size or both.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Detailed methods and details regarding sampling localities.

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