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Effects of conspecifics and heterospecifics on individual worm mass in four helminth species parasitic in fish

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Abstract Intraspecific and interspecific effects on the growth and body size of helminths are rarely studied in natural situations, yet knowing what determines helminth sizes and thus fecundity is crucial to our understanding of helminth ecology and epidemiology. The determinants of average individual worm mass were investigated in four common species of helminths parasitic in trout, *Salmo trutta*. In the acanthocephalan *Echinorhynchus truttae*, there was a negative relationship between the intensity of infection by conspecifics and average individual worm size. However, in the acanthocephalans *Pomphorhynchus laevis* and *Acanthocephalus anguillae* and in the cestode *Cyathocephalus truncatus*, the relationship was positive: individual worms were larger on average when co-occurring with many conspecifics than when co-occurring with very few. In addition, the average mass of individual *C. truncatus* in a host decreased as the total mass of other helminth species in the same host increased. This interspecific effect involves the whole helminth community, as the combined effect of all other helminth species is a better predictor of reduced mass in *C. truncatus* than the mass of any other species taken on its own. These results illustrate the importance of considering helminth interactions and helminth growth in a natural setting.

Introduction

The body size of an individual animal is usually its single most important feature, often being the main determinant of its reproductive success relative to that of its conspecifics (Peters 1983; Stearns 1992). The same is true of parasitic animals (Poulin 1998). In species of helminth parasites, however, the intraspecific variation in body size observed within the same population is much greater than what is normally seen in free-living animals. For instance, Shostak and Dick (1987) found 20-fold differences in body mass among mature, gravid adults of the cestode *Triaenophorus crassus* in fish hosts. Similarly, Szalai and Dick (1989) observed 90-fold differences in body mass among adult females of the nematode *Raphidascaris acus* in fish. The causes of this extreme variation in helminth body sizes are not yet fully understood.

Density-dependent regulation, through some form of intraspecific competition, is often invoked to explain such effects (Keymer 1982; Shostak and Scott 1993). Increases in worm burden are often accompanied by reductions in either mean worm length or mean egg output per worm (e.g., Szalai and Dick 1989; Richards and Lewis 2001; Dezfuli et al. 2002). In natural systems where many helminth species co-exist within the same hosts, interspecific influences can also affect the size attained by helminths. The number of worms of one species can have impacts on the growth, fecundity or even survival of worms of another species sharing the same host. These effects have often been observed in laboratory experiments (e.g., Moqbel and Wakelin 1979; Silver et al. 1980; Dash 1981; Holland 1984). Despite their clear-cut results, these studies have some weaknesses that render their interpretation tentative. First, they use infection intensities that are higher than the ones occurring on average in nature, and the infection procedures do not generally allow for the gradual accumulation of worms. Second, these earlier studies focused on simple two-species laboratory models, thus ignoring the potential effects of multi-species interactions found in natural helminth communities. Very few studies (e.g. Dezfuli et al. 2002) have examined intra- and interspecific effects on helminth growth in a natural context.

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We investigated these effects in four helminth species commonly found in trout, *Salmo trutta*, in northern Italy: the acanthocephalans *Pomphorhynchus laevis*, *Acanthocephalus anguillae* and *Echinorhynchus truttae*, and the cestode *Cyathocephalus truncatus*. These four helminths co-occur frequently in the same individual fish (Dezfuli et al. 2001), making it possible to look at their effects on each other's growth. Our specific objective was to determine whether the average mass of an individual worm is influenced by (1) the number of conspecifics with which it shares a fish host, and (2) the total mass of other helminths also occurring in the host. We examined patterns of worm sizes within a natural context, an approach that may reveal trends that would not be apparent in a more artificial two-species scenario.

Materials and methods

A total of 167 trout were obtained from tributaries of the River Brenta, Province of Padua, northern Italy. The fish were captured by electric fishing between November 1998 and December 1999. Each fish was anaesthetized following capture and then killed by a blow to the head; its total length was recorded prior to dissection. The entire digestive tract of each fish was removed, fixed in 8% formaldehyde and opened subsequently in the laboratory. All helminths found in the intestine of each individual trout were identified and counted. In addition, the total mass (wet weight, to the nearest 0.0001 g) of all helminths of the same species from the same fish host was recorded. Thus, for each parasite species in each host, we obtained the total mass and, by dividing the total mass by the number of helminths, the average mass of individual worms.

Data on the numbers of helminths per host and on the average mass of individual worms were log-transformed to meet the assumptions of parametric tests. Data on the total mass of worms of different species per fish host (see below), however, could not be normalised by a log-transformation, and instead a rank transformation was applied to these data. Because our objective was to determine whether both intraspecific and interspecific effects can influence worm sizes in natural communities, we performed four separate multiple regressions, one for each of the four helminth species studied, using the average mass of individual worms as the dependent variable. The three independent variables were (1) the number of conspecific worms per host, (2) the total mass of all other helminth species combined, as an index of the competitive pressure imposed by all other parasites in the host, and (3) fish size, which may affect parasite sizes directly or indirectly. The multiple regressions allowed us to assess the independent effect of these three variables on the average size attained by *P. laevis*, *A. anguillae*, *E. truttae* and *C. truncatus* inside trout.

Results

Of the 167 trout obtained, 119 were infected by at least one helminth species. The mean total length of the 119 fish was 25.4 cm, with a range of 11.5–53.0 cm. All helminths recovered from these fish were adult worms. The acanthocephalan *Pomphorhynchus laevis* and the cestode *Cyathocephalus truncatus* were found in more fish, and at higher intensities of infection and total mass than the acanthocephalans *Acanthocephalus anguillae* and *Echinorhynchus truttae* (Table 1). In addition to these four common helminth species, another four species were found in a few fish. These were the acanthocephalans *Acanthocephalus clavula* (found in three fish), *A. lucii* (in three fish) and *Neoechinorhynchus rutili* (in three fish), and the nematode *Raphidascaris acus* (in four fish). They always occurred at low intensities of infection (1–2 worms per fish, except for one fish with 13 *R. acus*), and when they occurred, their mass was included in the total mass of other helminths found in fish hosts.

In the acanthocephalans *P. laevis*, *A. anguillae* and *E. truttae*, there was a relationship between the number of conspecific worms per fish and the average mass of individual worms, albeit the relationship was only marginal in the latter two species (Table 2). Surprisingly, however, the relationship is positive for both *P. laevis* and *A. anguillae*. There is much scatter in the data, and the relationship is essentially due to the fact that the smallest average worm sizes are recorded only in fish hosts harbouring few worms of these two species (Fig. 1). Neither the combined mass of the other helminth species present, nor the size of the fish host, had any effect on the average mass attained by any of the three acanthocephalans in their fish host (Table 2).

As in two of the acanthocephalan species, a positive relationship was found between the number of conspecific worms per fish and the average mass of individual worms in the cestode *C. truncatus* (Table 2). Again, this results from the fish harbouring the fewest cestodes also having the smallest ones on average (Fig. 1). In contrast to the acanthocephalans, however, there was a relationship between the combined mass of other helminth species present in the host and the average mass of individual *C. truncatus*: the larger the mass of other worms, the smaller the average size of the cestodes (Table 2). In fact, there was a significant difference (two-tailed *t*-test: $t=2.553$, $df=79$, $P=0.0126$) between the average mass of *C. truncatus* in fish with no other helminths and that in fish with other helminth species. On average, individual *C. truncatus* that share their fish host with other helminth species are approximately 25% smaller than *C. truncatus* that do not co-exist with other helminths (Fig. 2). Finally, there was no effect of fish length on the mass of individual cestodes (Table 2).

Table 1 Number of trout, *Salmo trutta*, infected, mean number of parasites per fish, and mean total biomass for each helminth parasite species (ranges in parentheses)

Parasite species	No. fish infected	Mean no. worms per infected fish	Mean total biomass per infected fish (g)
Pomphorhynchus laevis	70	25.1 (1–248)	0.019 (0.0003–0.120)
Acanthocephalus anguillae	31	3.3 (1–15)	0.005 (0.0004–0.029)
Echinorhynchus truttae	41	4.0 (1–36)	0.004 (0.0002–0.019)
Cyathocephalus truncatus	81	6.1 (1–85)	0.023 (0.0002–0.513)

Table 2 Influence of three independent variables on the average individual mass of worms from four species of helminth parasites in trout, *Salmo trutta*. Values are partial regression coefficients from multiple regressions in which average individual worm mass was the dependent variable (the number of fish included in each regression is also shown)

Variable	*P. laevis*	*A. anguillae*	*E. truttae*	*C. truncatus*
No. of fish	70	31	41	81
No. conspecific worms per host	0.246**	0.376*	–0.307*	0.228**
Total mass of other worm species	–0.083	–0.010	0.144	–0.355***
Length of host fish	–0.044	–0.207	0.084	–0.098

$^{*}P < 0.10$; $^{**}P < 0.05$; $^{***}P < 0.005$

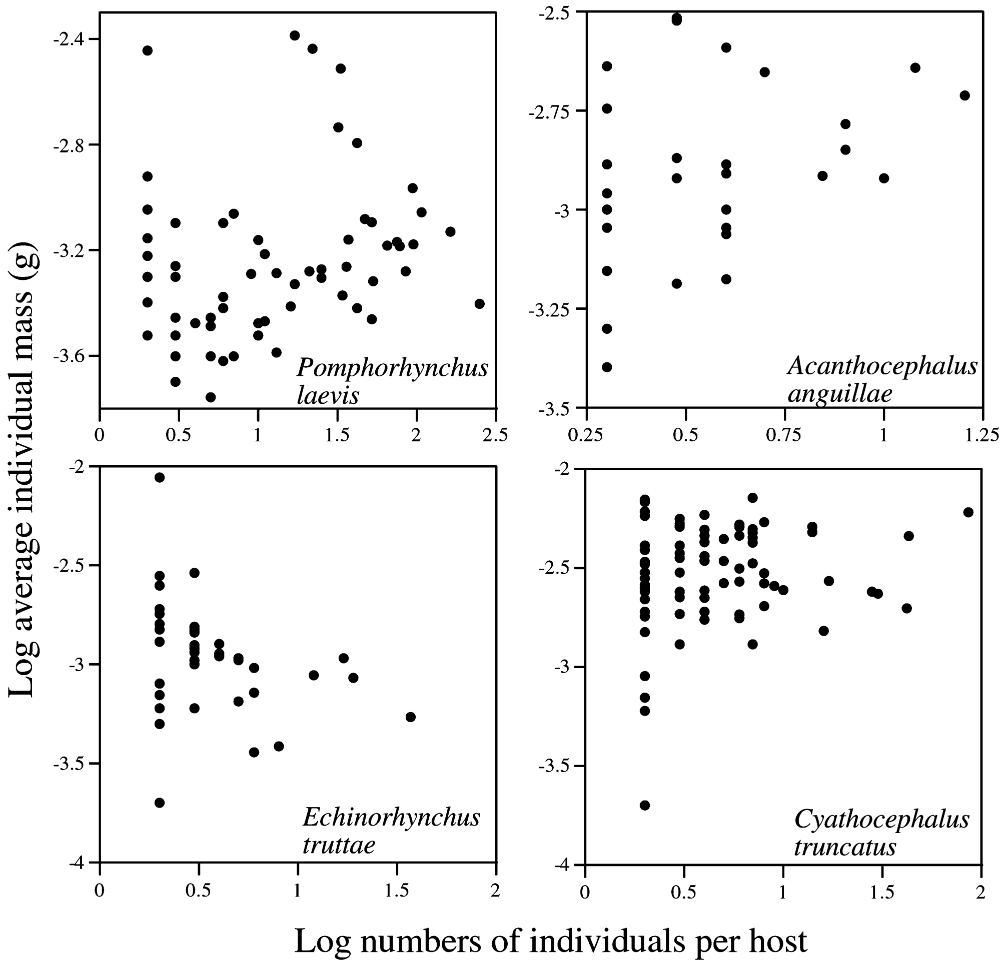

Fig. 1 Relationship between the average mass of individual worms in each host and the number of worms of that species per host, for each of four helminth species from trout, *Salmo trutta*

In order to find out which of the three acanthocephalan species, if any, had the most pronounced influence on the individual sizes of *C. truncatus*, the multiple regression for the cestode was repeated using the total mass per fish of each acanthocephalan species as separate independent variables instead of the combined mass of all species. Only the total mass of *P. laevis* in a fish host had an influence on the average individual mass of *C. truncatus*, but only marginally ($P = 0.045$). Thus the combined mass of all other helminth species in a host remains a better predictor of *C. truncatus* sizes than the mass of any given species.

Discussion

Natural parasite communities are more complex and rich in different species than the standard laboratory model systems. We need to study interactions among helminth parasites in a natural context if we are to understand how helminth communities are structured (Poulin 2001). The factors regulating adult worm sizes are particularly important, given that helminth adult sizes are the key determinant of their egg output (see Poulin 1998). Here, we investigated how the individual

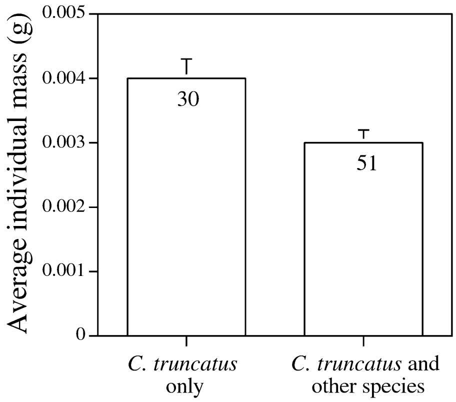

Fig. 2 Average mass (± SE) of individual cestodes, *Cyathocephalus truncatus*, in fish hosts harbouring only this cestode and in fish harbouring other helminth species as well. Numbers of fish in each group are given on the *bars*

worm mass was affected by all other worms in the host, whether conspecifics or not, using naturally infected hosts. We found evidence of both intra- and interspecific effects that are not in full agreement with the results of other studies, especially many laboratory studies.

First, we found that intraspecific, density-dependent effects were observed for all four helminth species investigated. However, for three of the species, the effect was *positive*. Although statistically weak in two of these three species, the fact that it emerges from analyses that took into account both the presence of other helminths and host size suggests that the positive trend is a real one. Thus, for *P. laevis*, *A. anguillae* and *C. truncatus*, the more conspecific worms there are in a host, the larger their average size. This does not mean, of course, that all worms in a host were large. As in typical 'crowding' situations (Read 1951; Bush and Lotz 2000), the large average mass was the result of a few rather large worms being present with many smaller ones. Still, the increase in average worm size with increasing intensity of infection is a result that clashes with the typical findings of studies on density dependence (Keymer 1982; Shostak and Scott 1993).

How could this positive relationship be explained? One potential mechanism involves differences in foraging success or prey types among fish hosts, and its impact on nutrient availability for intestinal helminths. The four helminth species investigated here use either amphipods or isopods as intermediate hosts (Dezfuli et al. 1994, 1999; Dezfuli and Scholz 1995). Fish with higher intensities of infection have presumably ingested these crustaceans at higher rates than fish harbouring few worms. Differences between fish in infection levels by acanthocephalans and cestodes can therefore also reflect differences in diet. Because adult acanthocephalans and cestodes in their vertebrate hosts feed by absorbing nutrients from the host gut through their body surface, the quality of the food they obtain is directly related to what the host eats. It could be that in this system density-dependent competitive interactions at high intensities of infection are offset by a greater availability of certain nutrients that promote helminth growth. Other explanations are possible, of course. Our results simply illustrate the need for studies of helminth ecology performed under natural conditions.

Second, we found evidence for interspecific influences on worm growth. The average individual size of the cestode *C. truncatus* was negatively affected by the presence of other helminth species in the host. Although the total mass of the acanthocephalan *P. laevis* correlated negatively with the average mass of individual *C. truncatus*, the combined mass of all other helminth species proved a better predictor of *C. truncatus* sizes. It is therefore the entire helminth community that must be taken into account: the interaction is not limited to a pair of species. Another interesting feature of our results is the asymmetrical nature of the interspecific effect which we observed. Whereas the size of *C. truncatus* is affected by the presence of other helminths, the sizes of these other helminths is not influenced by the presence of *C. truncatus*. This occurred despite the fact that *C. truncatus* are larger, both in terms of individual mass and with respect to total mass per host, than other helminth species. Such one-sided effects have been documented before (see Poulin 1998), but they are not emphasised enough given how common they appear to be.

Our study has demonstrated the importance of examining intra-and interspecific effects on helminth growth under natural conditions, an approach only adopted by few earlier studies (e.g. Dezfuli et al. 2002). We have demonstrated a counterintuitive density-dependent effect in some of the helminth species investigated, as well as an interspecific effect in which all species in the helminth community had a combined effect on the sizes attained by one of them. These findings illustrate that the growth of helminth parasites must be investigated in a natural, multi-species context.

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