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PARASITE VOLUME AS AN INDICATOR OF COMPETITION: THE CASE OF *ACANTHOCEPHALUS TUMESCENS* AND *PSEUDOCORYNOSOMA* SP. (ACANTHOCEPHALA) IN THEIR INTERMEDIATE HOST

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ABSTRACT: In Lake Mascardi (Patagonia), 2 acanthocephalan species, *Acanthocephalus tumescens* and *Pseudocorynosoma* sp., share an amphipod intermediate host but have different definitive hosts. Because both acanthocephalan species are potentially capable of manipulating amphipod behavior, one of the parasites may, therefore, have no opportunity to complete its life cycle; accordingly, negative interactions between them can be expected. The purpose of the present work was to examine the possibility of competition in the intermediate host through a comparison of *A. tumescens* and *Pseudocorynosoma* sp. cystacanth volume. Specimens of the amphipod *Hyalella patagonica* were collected monthly over almost 2 yr. Amphipods were measured (total length), necropsied, and cystacanths collected. Cystacanths were also measured, and their volume was calculated. Size of both acanthocepalan species was positively associated with amphipod total length. Competition, during 3 different infection periods, was assessed: high level of *Pseudocorynosoma* sp. infection (HP), high level of *A. tumescens* infection (HA), and high level of mixed infection (HM). In *Pseudocorynosoma* sp., intra-specific competition in HM was the only interaction found. In contrast, in *A. tumescens*, inter-specific competition in HP, intra-specific competion in HA, and intra- and inter-specific competition in HM were found. We suggest that *Pseudocorynosoma* sp. is a non-plastic species mostly found in single infections, while *A. tumescens* is a more variable species occurring more frequently in co-infections.

Larvae of acanthocephalans are transmitted by predation of the intermediate host by the definitive host. In some cases more than 1 larva of the same, or another, species may share an intermediate host, establishing conditions for positive, or negative, interactions between them. For parasite species transmitted via similar trophic pathways, i.e., sharing intermediate and definitive hosts, association between parasites can be expected and has been reported for the acanthocephalans *Pomphorhynchus laevis* and *Acanthocephalus clavula* in the amphipod *Echinogammarus stammeri* and for the acanthocephalan *Profilicollis* spp. and the trematode *Maritrema* sp. in the crab *Macrophthalmus hirtipes* (Dezfuli et al., 2000; Poulin, Nichol, et al., 2003). However, if 2 parasite species are manipulators of host behavior and infect different definitive hosts, a potential for conflict between them could happen, and negative interactions, such as competition in the intermediate host, could occur. Parasite species can develop mechanisms to prevent co-infections; e.g., microhabitat partitioning in the acanthocephalans *Leptorhynchoides thecatus* and *Pomphorhynchus bulbocolli* allows these species to infect different subsets of the same amphipod populations (Barger and Nickol, 1998). Despite this segregation, negative interactions in the amphipod intermediate host between the 2 parasite species (such as a slower development in co-infections) have also been reported (Barger and Nickol, 1999).

Intermediate hosts are obviously required as transport for parasite transmission but also as a supply from which parasites acquire resources for their development (Crompton and Nickol, 1985). Thus, the size of parasites can be potentially affected by their interactions with other individuals. Parasite volume previously has been used as an indicator of competition. For instance, intra-specific competition was observed when parasite volumes were smaller in individual acanthocephalans found in multiple infections compared to those found in single infections (Poulin, Nichol, et al., 2003; Cornet, 2011). Additionally, reduced volumes of *P. laevis* in infections with conspecifics, and with individuals of *A. clavula,* were perceived as evidence for intra- and inter-specific competition (Dezfuli et al., 2001).

In Lake Mascardi (Patagonia), the amphipod *Hyalella patagonica* is parasitized by 2 acanthocephalan species, *Acanthocephalus tumescens* and *Pseudocorynosoma* sp., which use freshwater fishes and aquatic birds as definitive hosts, respectively (Rauque and Semenas, 2007). At this site, both acanthocephalans are segregated by season, size, sex, and developmental stages of amphipods (Rauque and Semenas, 2007). Considering that in co-infections one species has no chance to complete its life cycle, competitive interactions are likely.

Since both *A. tumescens* and *Pseudocorynosoma* sp. are potential manipulators of host behavior and use *H. patagonica* as an intermediate host, yet employ different definitive hosts, the purpose of the present study was to examine intra- and inter-specific interactions in the amphipod host using cystacanth volume as an indicator of competition.

MATERIALS AND METHODS

Specimens of *H. patagonica* were collected monthly with sieves along the shoreline of Lake Mascardi, Patagonia, Argentina (41°17′S, 71°38′W). Amphipods were fixed in the field in 5% formalin and taken to laboratory. Crustaceans were measured (total length) and necropsied using a dissecting microscope. Fully developed larvae, as judged by their inverted proboscis (cystacanths), were measured using light microscopy; their volume was estimated using the formula for an ovoid, $V = (\pi L W^2)/6$, where L = maximum length and W = maximum width.

Two different comparisons were made. The first contrasted cystacanth volume during all mo sampled, while the second compared parasite volume in 3 different infection periods, i.e., (HP) high level of *Pseudocorynosoma* sp. infections (December 2002–February 2003), (HA) high level of *A. tumescens* infections (March 2003–September 2003), and (HM) high level of mixed infections (October 2003–February 2004) (Fig. 1).

A Spearman Correlation Rank Test was use to evaluate co-variations between amphipod total length and cystacanth volume. A Mann-Whitney *U* test, Kruskall Wallis, and Multiple Comparisons of Mean Ranks Test were used to compare cystacanth volume in single, multiple, and mixed infections. Tests were performed with a significance level of 5%.

RESULTS

Comparison of *Pseudocorynosoma* sp. cystacanths

A total of 804 cystacanths (mean 0.104 mm^3; SE = 0.001 mm^3) was collected from October 2002 to May 2004. Cystacanth

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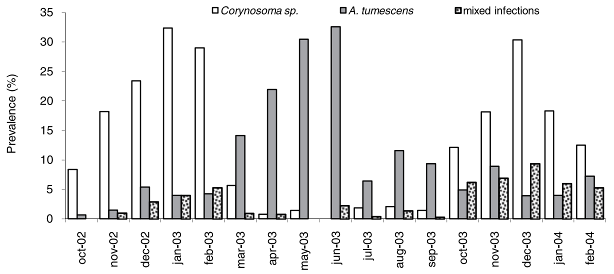

FIGURE 1. Proportion of specimens of *Hyalella patagonica* infected with *Pseudocorynosoma* sp. and *Acanthocephalus tumescens* during high levels of *Pseudocorynosoma* sp. infections (October 2002–February 2003), of *A. tumescens* infections (March 2003–September 2003), and of mixed infections (October 2003–February 2004).

volume showed a weak positive correlation with amphipod total length ($r = 0.09$; $P = 0.009$; n = 804) (Fig. 2). Cystacanths were classified into 3 categories of infection, i.e., 1 *Pseudocorynosoma* sp. cystacanth per amphipod (single infection), between 2 and 3 *Pseudocorynosoma* sp. cystacanths per amphipod (multiple infections), and 1 *Pseudocorynosoma* sp. larvae co-occurring with *A. tumescens* (mixed infections). No significant differences in cystacanth volume were found among these categories ($H = 1.77$; $P = 0.41$; n = 804) (Fig. 3A).

Comparison of *Pseudocorynosoma* sp. cystacanths in the 3 infection periods

In the period of high infection levels of *Pseudocorynosoma* sp., no significant differences in cystacanth volume were found among single, multiple, and mixed infections ($H = 3.85$; $P = 0.15$; n = 281). In the period of high infection levels of *A. tumescens*, no multiple infections were recorded, and no significant differences in volume were found between single and multiple infections ($Z = 0.14$; $P = 0.89$; n = 20). In the period of high levels of mixed infections, however, cystacanth volume was significantly lower in multiple infections than in single infections ($H = 6.59$; $P = 0.04$; n = 421) (Fig. 4A).

Comparison of *Acanthocephalus tumescens* cystacanths

A total of 362 cystacanths (mean 0.268 mm^3; SE=0.005 mm^3) was collected between December 2002 and May 2004. Cystacanth volume was positively correlated with amphipod total length ($r = 0.27$; $P < 0.001$; n = 362) (Fig. 2). Cystacanths were classified into 3 categories, i.e., single *A. tumescens* larva per amphipod (single infections), between 2 and 4 *A. tumescens* larvae per amphipod (multiple infections), and between 1 and 2 *A. tumescens* larvae co-occurring with *Pseudocorynosoma* sp. (mixed infections). Cystacanth volume was significantly lower in multiple infections compared with single infections ($H = 12.49$; $P = 0.001$; n = 362) (Fig. 3B).

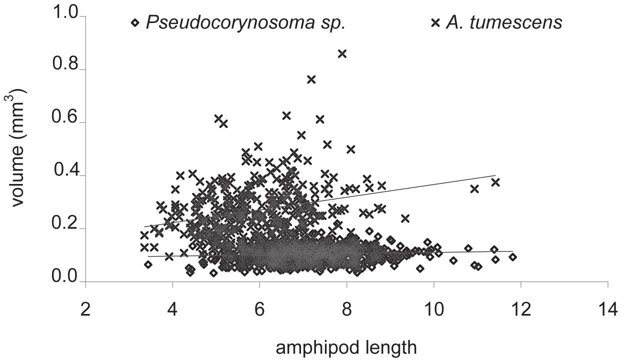

FIGURE 2. Relationship between cystacanth volume and total length of amphipods for *Pseudocorynosoma* sp. and *A. tumescens*.

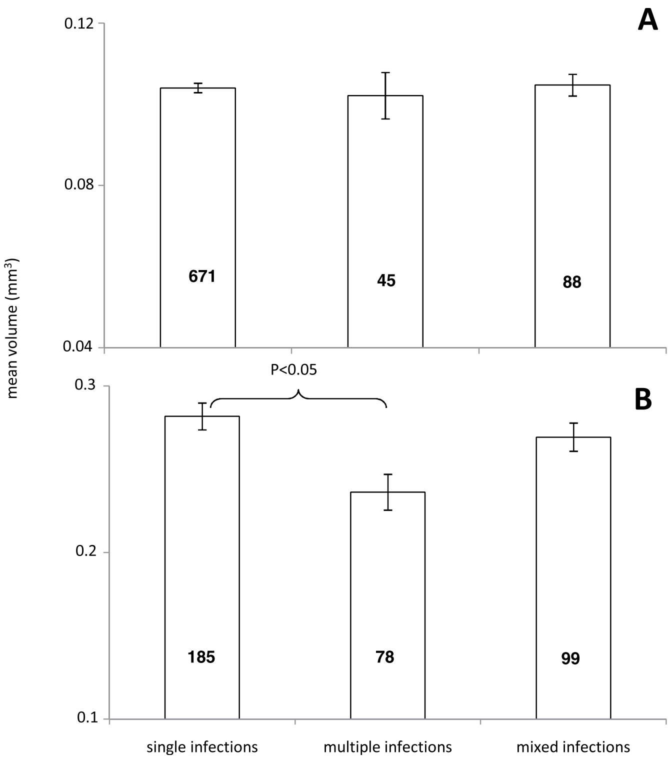

FIGURE 3. Mean (± SE) cystacanth volume of overall cystacanths of (**A**) *Pseudocorynosoma* sp. and (**B**) *A. tumescens* among categories of infection. Numbers of cystacanths per amphipods are given below bars.

Comparison of *Acanthocephalus tumescens* cystacanths in the 3 infection periods

In the period of high infection levels of *Pseudocorynosoma* sp., no multiple infections were recorded, and cystacanth volume was significantly lower in mixed infections ($Z = -2.05$; $P = 0.04$; n = 32) (Fig. 4B). In the period of high infection levels of *A. tumescens*, cystacanth volume was significantly lower in multiple infections compared with single infections ($H = 13.89$; $P = 0.001$; n = 151) (Fig. 4C). In the period of high levels of mixed infections, cystacanth volume was significantly lower in multiple infections ($H = 13.58$; $P = 0.02$; n = 154) and in mixed infections ($H = 13.58$; $P = 0.001$; n = 154) than in single infections (Fig. 4D).

DISCUSSION

Although competition has been observed in several freshwater host-parasite systems and even between different stages of the same species (Cézilly et al., 2000; Sparkes et al., 2004; Lagrue and Poulin, 2008), no information regarding temporal variation of these interactions has been compiled. In our study, when cystacanth volume of *Pseudocorynosoma* sp. larvae was analyzed

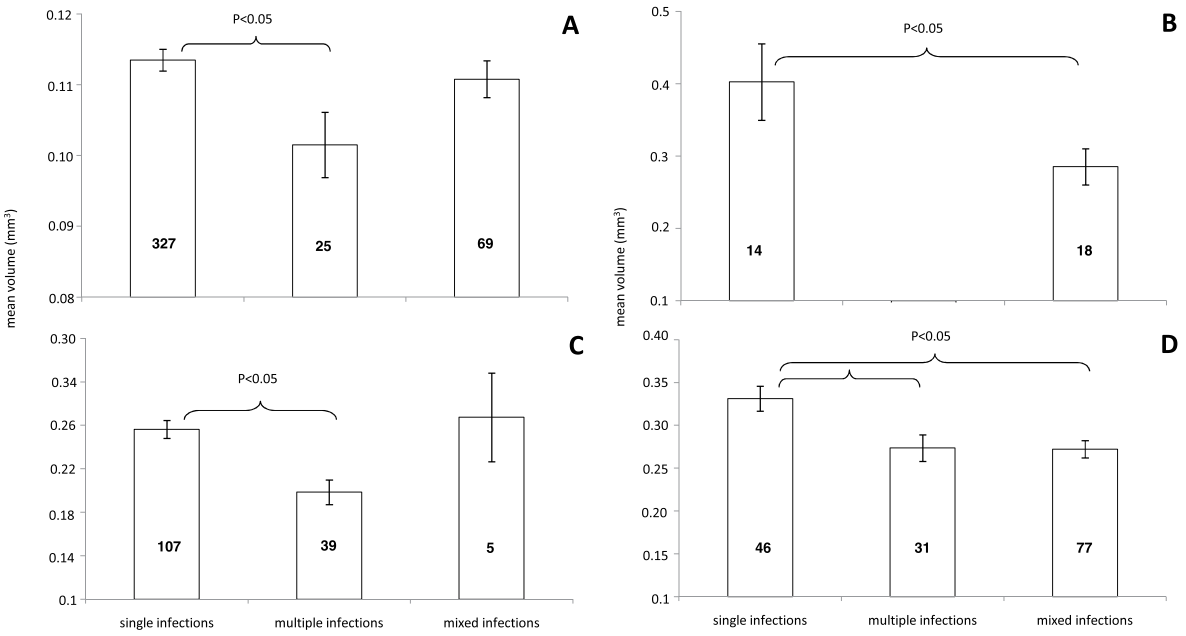

FIGURE 4. Mean (± SE) cystacanth volume of (**A**) *Pseudocorynosoma* sp. in HM period, and *A. tumescens* in (**B**) HP period, (**C**) HA period, and (**D**) HM period. Numbers of cystacanths per amphipods are given below bars.

over the 3 infection groupings (high infection of *Pseudocorynosoma* sp., high infection of *A. tumescens*, and high infection of mixed infections), no variation in the first 2 states was observed, but a reduced volume was seen in multiple infections, indicating intra-specific competition in high mixed infections. This difference in cystacanth volume during only 1 infection state suggests a lower variability of *Pseudocorynosoma* sp. In contrast, when cystacanth volume of *A. tumescens* was assessed during the same 3 infection groupings, larvae were more variable in size, suggesting competition. In the period of high infection by *Pseudocorynosoma* sp., *A. tumescens* exhibited inter-specific competition. In the period of high infection by *A. tumescens*, the species exhibited intra-specific competition, while in the period of high levels of mixed infection, *A. tumescens* reflected intra- and inter-specific competition. These patterns point to high vulnerability of *A. tumescens*, with its cystacanth being negatively affected by interactions that reduced their volume. This species, less prevalent than *Pseudocorynosoma* sp., was more common in multiple and mixed infections than *Pseudocorynosoma* sp., indicating a more intense competition in the amphipod intermediate host. The reasons of these variations of competition in *A. tumescens* are unclear, but an influence of a small sample size could be involved considering the absence of *A. tumescens* cystacanths in multiple infections during the period of high infection levels of *Pseudocorynosoma* sp. and the low numbers of cystacanths (only 5) in mixed infections during the period of high infection levels of *A. tumescens*.

In analyses including the total number of cystacanths collected, *Pseudocorynosoma* sp. showed no change in cystacanth volume, whereas *A. tumescens* exhibited a decrease in cystacanth volume, a pattern consistent with intra-specific competition. The results show that competition is the driving force for size variation in *Pseudocorynosoma* sp. and *A. tumescens* and that this competition varies according to the environmental abundance of individuals of the same and of the other species.

The greater variation in size of *A. tumescens* may suggest a higher allocation of resources to growth, as was previously pointed out for *Acanthocephalus lucii* females (Benesh and Valtonen, 2007).

In our study, *A. tumescens* were found to share their hosts frequently, whereas *Pseudocorynosoma* sp. occurred more commonly in single infections. How can this pattern be explained? Several scenarios seem plausible. First, there could be an aggressive strategy by *Pseudocorynosoma* sp., including effects preventing the establishment and growth of other parasites as were described for *Pomphorhynchus bulbocolli* and *Leptorhynchoides thecatus* in *Hyalella azteca* (Barger and Nickol, 1999). Alternatively, failing establishment when other parasites are previously infecting the amphipods is a possibility. Our research, however, fails to give us enough information to choose one scenario over another. Nonetheless, avoiding conspecifics seems to be an effective strategy for a common parasite such as *Pseudocorynosoma* sp., but not for less prevalent ones such as *A. tumescens*.

In Lake Mascardi, both *A. tumescens* and *Pseudocorynosoma* sp. cystacanth volume increased with amphipod total length. Similar results have been recorded in acanthocephalans from other freshwater systems (Dezfuli et al., 2001). These findings can be attributed to the greater availability of space for development and to increased food resources for parasites in larger amphipods. Comparing both parasite species, *A. tumescens* showed a stronger correlation with amphipod total length than *Pseudocorynosoma* sp. (Fig. 2). This could be associated with the greater *A. tumescens* cystacanth volume and agrees with our previous suggestion of elevated variability in volume and superior allocation to growth in *A. tumescens* compared to *Pseudocorynosoma* sp. Variations in cystacanth volume could have an strong impact on the fitness of individual parasites; i.e., it has been suggested that cystacanth volume could influence the transmission rate to definitive hosts (Steinauer and Nickol, 2003), determine the size of adults (Poulin, Wise, et al., 2003), affect the speed of maturation (Amin et al., 1980), and increase the numbers of eggs as was reported for some digeneans (Fredensborg and Poulin, 2005).

In Lake Mascardi, interactions between the 2 acanthocephalan species vary according to the conditions of infection, indicating different parasite strategies: *A. tumescens* has more variability and is more frequently found in co-infections, while *Pseudocorynosoma* sp. shows less variation in size, being found mainly in single infections. To the best of our knowledge, this is the first study to show temporal variation of competition among parasites in an intermediate host.

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