

Costs of intraspecific and interspecific host sharing in acanthocephalan cystacanths

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SUMMARY

Larval helminths often share individual intermediate hosts with other larval worms of the same or different species. In the case of immature acanthocephalans capable of altering the phenotype of their intermediate hosts, the benefits or costs of host sharing can be evaluated in terms of increased or decreased probability of transmission to a suitable definitive host. Competitive interactions among the immature stages of acanthocephalans within the intermediate host could create additional costs of host sharing, however. The effects of intraspecific and interspecific interactions were measured in 3 sympatric species of acanthocephalans exploiting a population of the amphipod *Echinogammarus stammeri* in the River Brenta, Italy. The strength of interactions was assessed from differences in the size achieved by infective cystacanths in the intermediate host. The size of *Pomphorhynchus laevis* cystacanths was not correlated with host size, whereas the size of *Acanthocephalus clavula* and *Polymorphus minutus* cystacanths increased with host size. Reductions in cystacanth size caused by intraspecific competition were only detected in *P. laevis*, but may also occur in both *A. clavula* and *P. minutus*. When co-occurring in the same amphipod with cystacanths of *A. clavula*, cystacanths of *P. laevis* attained a smaller size than when they occurred on their own. This effect was not reciprocal, with the size of *A. clavula* cystacanths not being affected. This supports earlier suggestions that it is adaptive for *A. clavula* to associate with *P. laevis* in amphipod intermediate hosts, with both species going to the same fish definitive hosts. In contrast, cystacanths of *P. laevis* achieved their largest size when they co-occurred in an amphipod with a cystacanth of *P. minutus*, which has a different definitive host (i.e. birds). These findings suggest that the net benefits of sharing an intermediate host can only be estimated by taking into account both the effects on transmission success and the consequences for cystacanth development.

Key words: *Acanthocephalus clavula*, amphipod, competition, cystacanth development, *Polymorphus minutus*, *Pomphorhynchus laevis*.

INTRODUCTION

One of the many functions of an intermediate host in the complex life-cycle of helminth parasites is to serve as a vehicle taking the parasite larva to its definitive host via predation (Lafferty, 1999). Many helminths, in particular acanthocephalans (Moore, 1984), actively take control of the intermediate host vehicle by manipulating its phenotype in ways that increase predation rates on infected intermediate hosts by suitable definitive hosts (Poulin, 1998). In this context, situations in which the immature stages of different helminths share the same intermediate host have recently been analysed with respect to transmission success (Thomas, Renaud & Poulin, 1998; Lafferty, Thomas & Poulin, 2000). When 2 immature stages of the same species of manipulating parasite share an intermediate host, their probability of transmission to a definitive host will either be the same as if they occurred singly, or it will be increased if their manipulative effects are additive. When

immature stages of different species of helminths share an intermediate host, the costs or benefits in terms of transmission success will depend on whether or not they have the same definitive host. For instance, if a non-manipulative helminth shares an intermediate host with a manipulative helminth, and if they both develop in the same definitive host species, then the non-manipulative species would achieve a higher transmission probability than it would on its own. If both species are capable of manipulation, they could both benefit from increased transmission success if their effects are synergistic. If they have different definitive hosts, however, one of them is bound to die if the other one gets transmitted. Thus the costs and benefits of sharing an intermediate host can be measured in terms of transmission success.

Intermediate hosts serve functions other than transport, however. They provide resources, mainly nutrients and space, to developing helminths. Thus another factor to consider when assessing the net benefits of sharing an intermediate host would be the effects of competition for resources among the immature stages of helminths on their survivorship and rate of development.

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Populations of the amphipod *Echinogammarus stammeri* in the River Brenta, Italy, provide an ideal opportunity to study these effects. The amphipod serves as intermediate host for 3 species of acanthocephalans: *Pomphorhynchus laevis* (prevalence = 19%; Dezfuli, Giari & Poulin, 2000), *Acanthocephalus clavula* (prevalence = 1%) and *Polymorphus minutus* (prevalence = 1%). Both *P. laevis* and *A. clavula* use fish, in particular chub *Leuciscus cephalus*, as definitive hosts, whereas *P. minutus* matures in aquatic birds (Dezfuli & Giari, 1999; Dezfuli *et al.* 1999). All 3 acanthocephalans have the ability to alter the phenotype of their host and its susceptibility to predators. *P. laevis* can change the colouration, phototaxis and activity levels of its amphipod host, as well as its tendency to drift in streams (Rumpus & Kennedy, 1974; Kennedy, Broughton & Hine, 1978; Brown & Thompson, 1986; McCahon, Maund & Poulton, 1991; Bakker, Mazzi & Zala, 1997; Maynard *et al.* 1998). Modifications in behaviour and colouration of intermediate hosts are also commonly induced by members of the genera *Acanthocephalus* (Camp & Huizinga, 1979; Dezfuli *et al.* 1994) and *Polymorphus* (Hindsbo, 1972; Bethel & Holmes, 1973). Cystacanths of all 3 species can occur either singly in an amphipod, with conspecifics, or with cystacanths of other species, with different consequences for their transmission success. Recently, Dezfuli *et al.* (2000) showed that cystacanths of *A. clavula* co-occurred with those of *P. laevis* much more often than expected by chance. They proposed that *A. clavula* may have evolved a strategy of associating with the more common *P. laevis*, since they both have the same definitive hosts. However, even if their manipulations of host phenotype have additive effects on the probability of transmission, this strategy would only be beneficial for *A. clavula* if it does not incur a slower rate of development due to competition.

In this study, we quantify the effects of sharing an intermediate host on the size achieved by cystacanths of all 3 acanthocephalan species. We examine both intraspecific effects, i.e. when conspecific cystacanths share the same amphipod host, and interspecific effects, i.e. when cystacanths of different species share the same host. Combined with the results of our earlier study focusing on transmission strategies (Dezfuli *et al.* 2000), our findings will allow a more accurate assessment of the advantages or costs of host sharing by sympatric helminth species.

MATERIALS AND METHODS

Monthly samples (each consisting of about 200 individuals) of the amphipod *Echinogammarus stammeri* were collected from 1998 to 2000 from the same site in the River Brenta, near Grantorto, Province of Padua, northern Italy. Amphipods were captured along the river bank with a dipnet (mesh

size 3 mm). All specimens were fixed in 6% formaldehyde and returned to the laboratory. Prior to their examination under the microscope, they were sexed, measured (total length, nearest 0.5 mm), and cleared in lactic acid to facilitate the identification and measurement of the parasites in their haemocoel. The thousands of amphipods collected were examined until at least 20 individuals, if possible, were found for each type of single-species and mixed-species acanthocephalan infection that occur in this amphipod population. Multiple infections by cystacanths of the same or different species are relatively rare, however, and in some cases fewer than 20 amphipods could be found. The maximum length and width of each acanthocephalan cystacanth (see below) were measured to the nearest μm under a compound microscope with the aid of an eyepiece micrometer.

The development of acanthocephalan immature stages with amphipods proceeds through 3 stages (Schmidt, 1985; Taraschewski, 2000). First, after an amphipod accidentally ingests an acanthocephalan egg, an acanthor stage hatches from the egg and bores its way out of the host gut and into the haemocoel. There its development proceeds through the acanthella stage toward the cystacanth stage, which is infective to the vertebrate definitive host. At each stage, it grows in size (Schmidt, 1985; Taraschewski, 2000), so that the final size of the immature worm can be used as an index of development and infectivity. Very few acanthellae were found in our samples and they were not measured; all our results concern strictly cystacanths. Cystacanths are encased in an envelope, and, with their proboscis invaginated, their overall body shape is roughly ovoid. The volume of each cystacanth was therefore estimated as the volume of an ovoid, or $(\pi LW^2)/6$, where L is the length of the cystacanth and W is its width. This method may not perform similarly for all 3 parasite species, because of slight variations in shape, but it is the best estimator of cystacanth size available.

Infected amphipods were grouped according to the number and species of acanthocephalan cystacanths they harboured. All analyses were performed on log-transformed cystacanth volume data, to meet the assumptions of parametric tests; values shown in figures are derived from raw data, however. Within each group, if there were a sufficient number of amphipods, the relationship between the volume of cystacanths and amphipod length was assessed separately for each acanthocephalan species using Pearson's product-moment correlation coefficients. Also, for each acanthocephalan species, comparisons of mean cystacanth volume were made among groups using ANOVAs and, if necessary, *a posteriori* pairwise comparisons between means with Fisher's LSD test.

No comparisons of mean cystacanth volume were

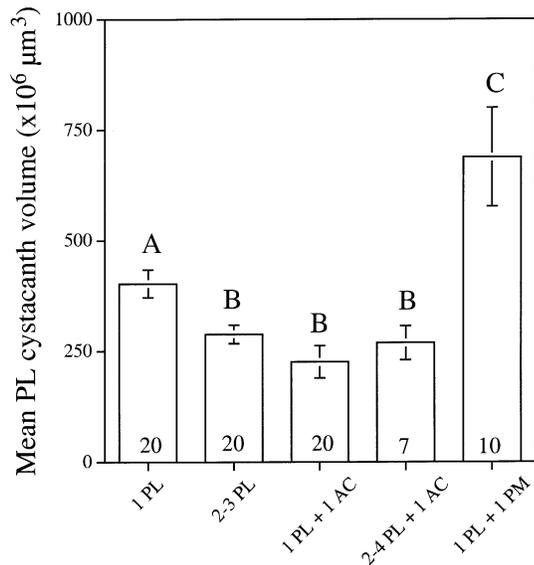


Fig. 1. Mean (\pm s.e.) volume of *Pomphorhynchus laevis* cystacanths in the amphipod intermediate host *Echinogammarus stammeri*, as a function of whether they occurred singly or shared the host with conspecific cystacanths or cystacanths of other species. Numbers of cystacanths per amphipod are given below each bar (abbreviations: PL = *Pomphorhynchus laevis*, AC = *Acanthocephalus clavula*, PM = *Polymorphus minutus*). Numbers of amphipods examined in each group are shown at the base of each bar. Letters above bars indicate significant differences between groups, with similar letters indicating no difference (pairwise comparisons among means, Fisher's LSD test, $\alpha = 0.05$).

made between acanthocephalan species. However, to determine whether cystacanth volumes were more variable in one acanthocephalan species than in the others, we used Bartlett's test for homogeneity of variances (Zar, 1984, pp. 181–183), using only log-transformed data from amphipods harbouring a single cystacanth. The coefficient of variation (ratio of standard deviation to mean, $\times 100$) was used as a descriptor of relative variability corrected for interspecific differences in mean cystacanth volume.

RESULTS

Variances in cystacanth volumes were not homogeneous among the 3 acanthocephalan species in single infections (Bartlett's test, $B_C = 182.6$, $P < 0.001$). Cystacanth volume appears less variable in *Pomphorhynchus laevis* (coefficient of variation = 2.02) than in either *Acanthocephalus clavula* or *Polymorphus minutus* (2.89 and 2.25, respectively), although the differences are rather small.

Volume of *Pomphorhynchus laevis* cystacanths

Cystacanths of *P. laevis* were found either singly, co-occurring with conspecifics, or co-occurring with a single cystacanth of either *A. clavula* or *P. minutus* (a single amphipod was found with a *P. laevis* cysta-

canth co-occurring with more than 1 *A. clavula* cystacanth; it was excluded from further analyses). Among amphipods harbouring only a single *P. laevis* cystacanth, there was no correlation between host length and cystacanth volume ($r = -0.011$, $n = 20$, $P = 0.9638$). There were also no correlations between host length and mean *P. laevis* cystacanth volume among amphipods harbouring 2 *P. laevis* cystacanths only ($r = 0.344$, $n = 18$, $P = 0.1618$), among amphipods harbouring 1 *P. laevis* and 1 *A. clavula* cystacanth ($r = 0.260$, $n = 20$, $P = 0.2677$), and among amphipods harbouring 1 *P. laevis* and 1 *P. minutus* cystacanth ($r = -0.175$, $n = 10$, $P = 0.6293$). There is thus no evidence that the size of *P. laevis* cystacanths is influenced by the size of their amphipod intermediate host.

Among the 5 groups of amphipods infected with *P. laevis* distinguished by the numbers and species of acanthocephalans they harboured (see Fig. 1), there were significant differences in mean *P. laevis* cystacanth volume ($F_{4,72} = 13.07$, $P = 0.0001$). Cystacanths of *P. laevis* that shared an amphipod host with either conspecific cystacanths or an *A. clavula* cystacanth had a significantly smaller volume than *P. laevis* cystacanths that occurred alone in their host (Fig. 1). However, by far the largest cystacanths of *P. laevis* found in our samples were those that shared an amphipod host with 1 *P. minutus* cystacanth (Fig. 1).

Volume of *Acanthocephalus clavula* cystacanths

Cystacanths of *A. clavula* were found either singly, sharing an amphipod with conspecifics, or sharing an amphipod with 1 or more cystacanths of *P. laevis*. Amphipod length correlated positively with the volume of *A. clavula* cystacanths among amphipods harbouring a single cystacanth ($r = 0.439$, $n = 20$, $P = 0.0526$) and among amphipods harbouring 1 *A. clavula* and 1 *P. laevis* cystacanths ($r = 0.653$, $n = 20$, $P = 0.0018$). There was some noise in the data (Fig. 2), but in general larger amphipods tended to harbour larger *A. clavula* cystacanths.

Among the 4 groups of amphipods infected with *A. clavula* that were created, based on the numbers and species of acanthocephalans they harboured (see Fig. 3), there were no statistically significant differences in mean *A. clavula* cystacanth volume ($F_{3,46} = 1.876$, $P = 0.147$). Sharing an amphipod host with conspecifics appeared to result in lower mean volume among *A. clavula* cystacanths (Fig. 3), and small sample sizes may be the reason why no significant difference was detected among our specimens. Similarly, the largest *A. clavula* cystacanths tended to be those that shared their host with more than 1 *P. laevis* cystacanth, another comparison hampered by a small sample size. The contrast between *A. clavula* cystacanths occurring singly and those sharing the host with a single *P. laevis*

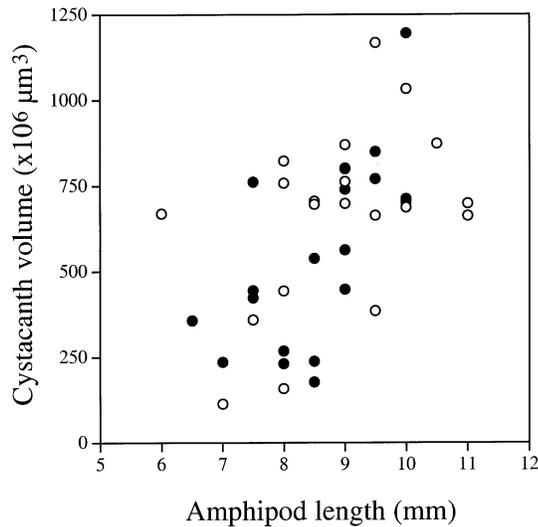


Fig. 2. Relationship between the volume of *Acanthocephalus clavula* cystacanths and the length of their intermediate host, the amphipod *Echinogammarus stammeri*. Data are shown separately for *A. clavula* cystacanths occurring singly in an amphipod (○), and for those sharing an amphipod with 1 *Pomphorhynchus laevis* cystacanth (●).

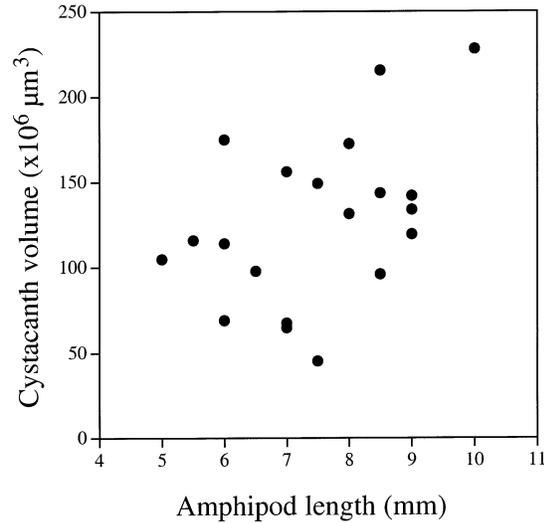


Fig. 4. Relationship between the volume of *Polymorphus minutus* cystacanths and the length of their intermediate host, the amphipod *Echinogammarus stammeri*.

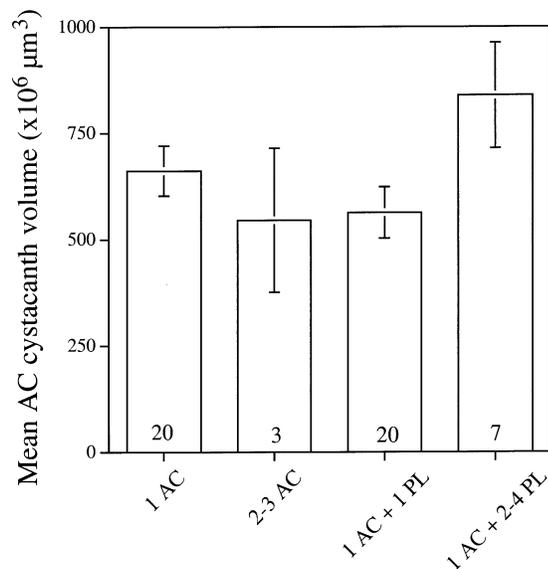


Fig. 3. Mean (\pm s.e.) volume of *Acanthocephalus clavula* cystacanths in the amphipod intermediate host *Echinogammarus stammeri*, as a function of whether they occurred singly or shared the host with conspecific cystacanths or cystacanths of other species. Numbers of cystacanths per amphipod are given below each bar (abbreviations are as in Fig. 1). Numbers of amphipods examined in each group are shown at the base of each bar.

cystacanth, however, is more robust (Fig. 3), and does not indicate any reduction in the volume of *A. clavula* associated with sharing with the other species.

Volume of Polymorphus minutus cystacanths

Cystacanths of *P. minutus* were found either singly,

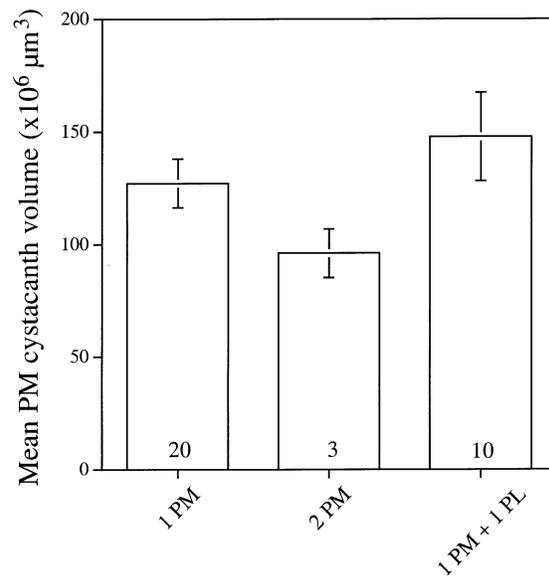


Fig. 5. Mean (\pm s.e.) volume of *Polymorphus minutus* cystacanths in the amphipod intermediate host *Echinogammarus stammeri*, as a function of whether they occurred singly or shared the host with conspecific cystacanths or cystacanths of other species. Numbers of cystacanths per amphipod are given below each bar (abbreviations are as in Fig. 1). Numbers of amphipods examined in each group are shown at the base of each bar.

sharing an amphipod with 1 conspecific, or sharing an amphipod with 1 cystacanth of *P. laevis*. Amphipod length correlated positively with the volume of *P. minutus* cystacanths among amphipods harbouring a single cystacanth ($r = 0.449$, $n = 20$, $P = 0.0473$; Fig. 4), but not among amphipods harbouring 1 *P. minutus* and *P. laevis* cystacanths ($r = -0.277$, $n = 10$, $P = 0.4386$).

Three groups of amphipods infected with *P. minutus* could be distinguished based on the numbers

and species of acanthocephalans they harboured (see Fig. 5); among these groups, there were no statistically significant differences in mean *P. minutus* cystacanth volume ($F_{2,30} = 1.271$, $P = 0.2953$). Sharing an amphipod host with 1 conspecific appeared to result in lower mean volume of *P. minutus* cystacanths (Fig. 5), but again small sample sizes may have prevented the detection of any existing difference among our specimens.

DISCUSSION

When one or more helminth species use the same intermediate host population, and if one of them can manipulate the phenotype of intermediate hosts, selection should favour non-random associations among these species (Lafferty *et al.* 2000). This assumes that there are no costs of competition within the intermediate host. Here we report that such costs exist, at least for certain species combinations. There are two particularly interesting features of our results. First, some species of acanthocephalans may be more sensitive than others to intraspecific competition within the intermediate host. In our study, the size attained by cystacanths of *P. laevis* was influenced by the presence of other cystacanths, whereas the sizes of *A. clavula* and *P. minutus* cystacanths were not significantly affected by other cystacanths sharing their amphipod host. Second, the outcome of competition between acanthocephalan cystacanths of different species can be asymmetrical, with only 1 species incurring negative effects. When *P. laevis* and *A. clavula* share the same host, cystacanths of *P. laevis* achieve a smaller size whereas those of *A. clavula* are not affected. Such asymmetrical effects among competing helminths are not uncommon (Holmes, 1961; Poulin, 1998).

Why is *P. laevis* more sensitive than the other 2 species to competition from other cystacanths when sharing an intermediate host? One reason may be that under given conditions, its development may be more constrained and less plastic than that of the other 2 acanthocephalan species. The significant heterogeneity in the variance of cystacanth volumes among the 3 species, and the error bars on Figs 1, 3 and 5, suggest that the size of *P. laevis* cystacanths is less variable than that of either *A. clavula* or *P. minutus* cystacanths in similar situations. Also, the size of *P. laevis* cystacanths does not covary with host size, whereas cystacanths of the other two species (one of which is on average smaller, and one of which is on average larger than *P. laevis*) show larger sizes in larger amphipods. If the development of *P. laevis* cystacanths is less plastic, it may be incapable of adjusting to the presence of other cystacanths.

Evidence for negative effects of intraspecific competition was only found for cystacanths of *P. laevis*, but the trend was also apparent for both *A. clavula* and *P. minutus*. The reason no significant

effects were found for those 2 species may be the small number of amphipods with more than 1 cystacanth of these species that were available for analysis. This means that for *P. laevis* and possibly the other 2 species, selection may favour any mechanism that would reduce the chances of 2 conspecific cystacanths co-occurring in the same amphipod host. If their manipulations of the host had small additive effects on the probability of transmission to the definitive host, this increased transmission would be offset by a lower development rate and a longer time to reach infectivity. The fact that multiple infections by conspecific cystacanths do occur could mean that the additive effects on transmission are substantial enough to compensate for the cost of competition, or that infection of amphipods by conspecific cystacanths is a stochastic event. Since additional cystacanths of *P. laevis* (or *P. minutus*) do not amplify the behavioural changes induced in amphipods by a single cystacanth (Cézilly, Grégoire & Bertin, 2000), the second scenario appears more likely.

The findings of costs and benefits associated with interspecific host sharing are most interesting. Cystacanths of *P. laevis* attain a smaller size when sharing an amphipod with 1 or more cystacanths of *A. clavula* than they do when they occur singly in an amphipod. On the contrary, the size of *A. clavula* cystacanths is not influenced significantly by the presence of *P. laevis* cystacanths. Perhaps the relative size of the two different cystacanths, with *A. clavula* being much larger than *P. laevis*, can explain this finding. In any case, this asymmetrical effect seems to validate the suggestion that the rarer *A. clavula* has evolved mechanisms to enhance its chances of co-infecting an amphipod with the more common *P. laevis* (Dezfuli *et al.* 2000), with the 2 manipulative parasites going toward the same definitive host. This hypothesis assumes that *A. clavula* incurs no competitive costs, as observed here.

Even more intriguing is the observation that cystacanths of *P. laevis* achieve a larger size when they share an amphipod with a cystacanth of *P. minutus* than when they occur alone in an amphipod. This positive effect is surprising and quite pronounced. The way in which acanthocephalan cystacanths absorb nutrients and pigments could provide a possible proximate explanation for this phenomenon. Nutrients and carotenoids are obtained from the host's haemolymph through pores on the parasite's tegument; the reddish or orange colour of the parasite cystacanths results from the accumulation of carotenoids within the thickness of the parasite's body wall. Different species of acanthocephalans apparently prefer different types of carotenoids (Barrett & Butterworth, 1968, 1973), but can convert other carotenoids into their preferred type before absorption (Taraschewski, 2000). Cystacanths of *P. minutus* have a much brighter red colour than

those of *P. laevis* (B. Dezfuli, personal observations). When cystacanths of the 2 species co-occur, perhaps *P. minutus* depletes the host's haemolymph of its carotenoids, leaving *P. laevis* cystacanths with mainly nutrients to absorb. Whatever mechanism is involved, however, this apparent growth benefit may have little significance overall. Cystacanths of *P. laevis* and *P. minutus* have completely different definitive hosts (fish and birds, respectively). Since *P. minutus* can modify amphipod colouration and behaviour in ways that increase its transmission to birds (Hindsbo, 1972; Bethel & Holmes, 1973), co-occurring *P. laevis* would sometimes end up in the wrong definitive host. A recent study, however, shows that when cystacanths of *P. laevis* and *P. minutus* co-occur in the same amphipod, the resulting alterations in host behaviour are closer to those normally induced by *P. laevis* alone than those caused by *P. minutus* (Cézilly *et al.* 2000). Thus sharing an amphipod with cystacanths of *P. minutus* would not necessarily be disadvantageous for *P. laevis* cystacanths.

A central assumption in the present study is that cystacanth volume is a fitness component for acanthocephalans. Although there has been no direct demonstration of this, several lines of evidence suggest that size may be an important determinant of infectivity, establishment success in the definitive host, and later fecundity. First, activation of the cystacanth following its ingestion by the definitive host appears to be a highly energy-consuming process (Taraschewski, 2000). Glycogen reserves become depleted quickly and several metabolic systems become suddenly operational after the cystacanth reaches the gut of its definitive host (Horvath, 1971; Lackie, 1974; Polzer & Taraschewski, 1994). When many conspecific cystacanths arrive simultaneously inside the host's gut, several of them typically fail to become established. This is true for *P. laevis* (Brown, 1986) as well as many other species (Taraschewski, 2000). Larger cystacanths, with greater energy reserves and a larger proboscis, may have an edge over smaller ones during this initial phase inside the definitive host. Second, fecundity correlates positively with body size in parasites and most other invertebrates (Poulin, 1998). Surely, beginning life in the definitive host with a slightly larger size can only benefit the parasite in terms of life-time reproductive success. It is therefore reasonable to assume that cystacanth size is a component of acanthocephalan fitness.

To our knowledge, this is only the second time that competitive interactions among acanthocephalans in their intermediate hosts have been demonstrated. Barger & Nickol (1999) showed that the presence of immature stages of a species of *Pomphorhynchus* decreased the likelihood that immature stages of another acanthocephalan species reached the infective cystacanth stage in the same

amphipod. Here we only focused on immature parasites at the cystacanth stage. However, because the size of immature stages increases during acanthocephalan development (Schmidt, 1985; Taraschewski, 2000), our index of cystacanth size provides a similar comparative measure. Earlier, Awachie (1967) had observed no interactions between co-occurring cystacanths of *P. minutus* and of another species in experimental infections. Interactions among larval helminths within the intermediate host are common in taxa other than acanthocephalans. For instance, the establishment success of larvae of the cestode *Hymenolepis diminuta* in its insect intermediate host is greatly reduced by the presence of larvae of another cestode species, *Raillietina cesticillus* (Gordon & Whitfield, 1985). Interestingly, this interaction between larval cestodes was found to be asymmetrical: it is as one-sided as the interaction reported here between cystacanths of *P. laevis* and *A. clavula*. Asymmetrical antagonistic interactions are also very common among species of larval digeneans in their snail intermediate hosts (Sousa, 1992; Kuris & Lafferty, 1994). Unlike systems involving acanthocephalans, the snail intermediate hosts of digeneans serve mainly as sources of nutrients and only rarely as vehicles to the definitive host, and intense competition is therefore expected in such systems.

Many questions remain to be answered regarding the advantages or costs of sharing an intermediate host. For example, are the competitive effects reported here subject to the temporal order of infection, i.e. is development of *P. laevis* slowed down only when it infects an amphipod already harbouring *A. clavula* cystacanths? The issue of whether or not immature parasites that share an intermediate host would also compete as adults inside the definitive host remains to be addressed. Nevertheless, the results of Dezfuli *et al.* (2000) and those presented here indicate that there exist both non-random associations and non-reciprocal interactions among these acanthocephalan species having different effects on their development and transmission.

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