

Interactions among four parasite species in an amphipod population from Patagonia

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Abstract

Parasites commonly share their hosts with specimens of the same or different parasite species, resulting in multiple parasites obtaining resources from the same host. This could potentially lead to conflicts between co-infecting parasites, especially at high infection intensities. In Pool Los Juncos (Patagonia, Argentina), the amphipod *Hyaella patagonica* is an intermediate host to three parasites that mature in birds (the acanthocephalan *Pseudocorynosoma* sp. and larval stages of two Cyclophyllidea cestodes), in addition to a microsporidian (*Thelohania* sp.), whose life cycle is unknown, but very likely to be monoxenous. The aim of this study was to describe interactions between these parasite species in their amphipod host population. Amphipods were collected monthly between June 2002 and January 2004 to assess parasite infection. Infection prevalence and mean intensity were greatest in larger male amphipods for all parasite species. We also found a positive association between *Thelohania* sp. and both *Pseudocorynosoma* sp. and Cyclophyllidea sp. 1 infections, though *Pseudocorynosoma* sp. and Cyclophyllidea sp. 1 were negatively associated with each other. We conclude that contrasting associations between parasite species may be associated with competition for both food intake and space in the haemocoel.

Introduction

In freshwater environments, amphipods are frequently host to diverse parasite assemblages (Moore, 2002). Amphipods are also a main food source for a variety of animals, with many trophically transmitted parasites utilizing this as a bridge to reach their definitive host. Co-infections of multiple parasite species in a single amphipod host are common (e.g. Dezfuli *et al.*, 2000; Rauque *et al.*, 2011), making these crustaceans a good model system in which to test interactions between co-infecting parasites.

Parasites face several challenges in their hosts, for example sharing their hosts with specimens of the same or other parasite species. This may cause parasites to adopt various strategies to overcome such challenges

(Lafferty, 1999). Since larval parasites grow and develop in the intermediate host, they use resources that are limited. Therefore, co-infections of relatively large parasites or at high infection intensities could generate potential conflicts for resources between individual parasites. In contrast, when parasite species share their life cycles, using the same intermediate and definitive hosts, it could be beneficial to be associated with other parasite species. Evaluations of such associations between parasite species in shared hosts have been more frequently shown in definitive (Dezfuli *et al.*, 2001) than in intermediate hosts, with both positive and negative associations being noted (Dezfuli *et al.*, 2000; Poulin *et al.*, 2000; Fauchier & Thomas, 2001; Outreman *et al.*, 2002).

In Patagonia, previous studies on the amphipod *Hyaella patagonica* have focused on population dynamics, in terms of the deleterious effects of infection by two acanthocephalan species, *Acanthocephalus tumescens* and *Pseudocorynosoma* sp., and the interactions between these

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acanthocephalan species (Rauque & Semenas, 2007, 2009, 2011). In Lake Mascardi, a large and deep oligotrophic lake, these two acanthocephalans are sympatric in *H. patagonica*, although segregation of infection in time and space occurs to avoid competition, i.e. *A. tumescens* is a fish parasite that predominately infects small juvenile amphipods during winter, while *Pseudocorynosoma* sp. is a bird parasite that mainly occurs in intermediate to large male amphipods during spring and summer (Rauque & Semenas, 2007).

In Pool Los Juncos (Patagonia, Argentina), amphipods are infected by four parasite species; the acanthocephalan *Pseudocorynosoma* sp., two species of cestodes of the order Cyclophyllidea and the microsporidian *Thelohania* sp. *Pseudocorynosoma* sp. and the two species of Cyclophyllidea are located in the haemocoel, and have a typical two-host life cycle, using birds as definitive hosts (Rauque, 2007). *Thelohania* sp. is located in the muscles of the telson (Rauque, 2007), and its life cycle is unknown. Microsporidians can infect almost all animal taxa, and generally their life cycles are monoxenous, ending in the host, with new recruitment only beginning after ingestion by a new host (Tanada & Kaya, 1993). Transmission may be horizontal, through ingestion of contaminated material, or vertical, through the transfer of parasites from one generation of a host to the next (Shou-Chung Cheng, 2004).

The aim of the present study was to describe, through field samples, interactions among the four parasite species in an amphipod population of *H. patagonica*.

Materials and methods

Collection and examination of amphipods

Pool Los Juncos (41°03'S; 71°00'W) is a small (4 ha surface area), shallow (1.8 m maximum depth), fish-less freshwater environment with a diverse bird community (Perotti *et al.*, 2005). Specimens of the amphipod *H. patagonica* are found in the pool all year round. Amphipods were collected monthly with a sieve (1 mm mesh size) from the coastal vegetated zone between June 2002 and January 2004. Amphipods were fixed with 5% formalin in the field and transported to the laboratory to be assessed for total length (head to telson) and sex, being categorized as sexual (males and females) and asexual individuals (juveniles and undetermined). Asexual individuals were classified following Rauque & Semenas (2007), as juveniles (less than 5.68 mm) and undetermined (greater than 5.68 mm, but without secondary sex characters or developed gonads that allowed its determination). Amphipods were necropsied under a stereoscopic microscope to seek, determine and count parasite species. Determination was based on morphological criteria such as size, colour, body shape and the possession of relevant structures, such as hooks. Then prevalence, mean intensity and abundance of infection were assessed.

Additionally, to compare parasite relative sizes, a subsample of parasites was measured under a stereoscopic microscope. Parasite volume was calculated as an ovoid [$V = (\pi LW^2)/6$], where L = maximum length and W = maximum width].

Parasite identification

Larvae of *Pseudocorynosoma* sp. are bright orange, measuring 767 μm (L) by 514 μm (W), $n = 23$. Both cestode larvae are colourless with rostellar hooks (Rauque, 2007; Rauque & Semenas, 2007), with Cyclophyllidea sp. 1 measuring 225 μm (L) by 179 μm (W), $n = 20$, and Cyclophyllidea sp. 2 measuring 697 μm (L) by 403 μm (W), $n = 4$. The microsporidian *Thelohania* sp. is found in groups of eight spores surrounded by an envelope measuring 3.3 μm (L) by 1.8 μm (W) (Rauque, 2007).

Data analysis

Standardized and adjusted residuals analysis was used to evaluate differences in infection levels among male, female, juvenile and undetermined amphipods. Generalized linear models (logistic regression), using a binomial error distribution, were used to test the influence of total amphipod length and sex on parasite infection, and the influence of other parasites on the infection of each parasite species separately. G-test *post-hoc* analysis was used to calculate expected mixed infections that indicated the kind of association between parasites (positive or negative). A Kruskal–Wallis test, and multiple comparisons of mean ranks test were used to evaluate differences in amphipod length among infected and uninfected amphipods. All tests were performed with a significance level of 5%.

Results

Of the 6201 amphipods collected, 3493 were female, 1783 male, 541 juveniles and 384 were undetermined. Mean length was 7.9 mm (SD = 1.8 mm) for females, 8.5 mm (SD = 2.2 mm) for males, 4.8 mm (SD = 0.6 mm) for juveniles and 5.6 mm (SD = 1.9 mm) for undetermined amphipods.

Prevalence and mean intensity of the four parasite species recorded was 6.4% and 1.0 for *Pseudocorynosoma* sp., 8.3% and 24.4 for Cyclophyllidea sp. 1, 0.8% and 1.3 for Cyclophyllidea sp. 2, and 3.3% for *Thelohania* sp. Only simultaneous infections by two species (mixed infections) were recorded. All parasites species were involved in mixed infections, with a total of 156 amphipods involved in them (prevalence = 2.5%). Parasite mean volume

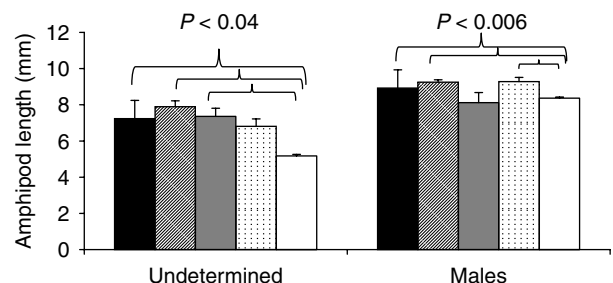


Fig. 1. Mean length \pm SE of male and undetermined amphipods in Pool Los Juncos, uninfected (white bars) and infected by *Pseudocorynosoma* sp. (black bars), Cyclophyllidea sp. 1 (hatched bars), Cyclophyllidea sp. 2 (shaded bars) and *Thelohania* sp. (dotted bars).

Table 1. Prevalence and Standardized and Adjusted Residual (STAR) value, of parasites in sexes and developmental stages of *Hyalella patagonica* in Pool Los Juncos. STAR values higher than 1.96 are considered significant. The highest positive STAR values (observed – expected) are indicated in bold case.

<i>Hyalella patagonica</i>	Pool Los Juncos			
	Prevalence (STAR value)			
	<i>Pseudocorynosoma</i> sp.	Cyclophyllidea sp. 1	Cyclophyllidea sp. 2	<i>Thelohania</i> sp.
Females	6.3% (–0.2)	7.2% (–3.6)	0.4% (–3.9)	1.7% (–7.9)
Males	7.3% (2.0)	11.7% (6.3)	1.5% (3.8)	6.6% (9.4)
Juveniles	2.0% (–4.3)	7.6% (–0.6)	0.4% (–1.2)	1.8% (–1.9)
Undetermined	8.6% (1.8)	3.4% (–3.6)	1.8% (2.2)	4.2% (1.0)

differed significantly between species ($H = 35.4$, $n = 47$, $P < 0.001$), ranging from 0.11 mm^3 for *Pseudocorynosoma* sp., 0.07 mm^3 for Cyclophyllidea sp. 2 and 0.004 mm^3 for Cyclophyllidea sp. 1. In undetermined ($H = 92.1$, $n = 358$, $P < 0.001$) and male amphipods ($H = 57.1$, $n = 1744$, $P < 0.001$), infected individuals were significantly longer than uninfected individuals (fig. 1), with the exception of undetermined amphipods infected by *Thelohania* sp. and male amphipods infected by Cyclophyllidea sp. 2. No differences were found in host length between uninfected and infected individuals for juvenile or female amphipods ($H = 11.1$, $n = 534$, $P > 0.05$; $H = 13.5$, $n = 3463$, $P > 0.05$, respectively). Male amphipods showed significantly higher prevalence for all parasite species (table 1). Sex, length and their second-order interactions were significant for infection by *Pseudocorynosoma* sp., Cyclophyllidea sp. 1 and *Thelohania* sp. In contrast, both predictors were not significant for Cyclophyllidea sp. 2, although their second-order interactions were significant (table 2).

In male and female amphipods, two associations, *Pseudocorynosoma* sp. + Cyclophyllidea sp. 1 and *Pseudocorynosoma* sp. + *Thelohania* sp. were significant (table 3), with *Pseudocorynosoma* sp. infections being positively associated with *Thelohania* sp. infections and negatively associated with Cyclophyllidea sp. 1 infections (table 4). In undetermined amphipods, infection by Cyclophyllidea sp. 1 was positively associated with *Thelohania* sp. infections (tables 3 and 4). For juveniles, no models were performed because no mixed infections were recorded.

Discussion

Co-infecting parasites share resources from their hosts and, on some occasions, these parasites can be found

in high infection intensities and in large sizes relative to their hosts, so some negative interactions could be predicted between such parasites. For example, in crabs, high infection intensities reduce the volume of *Proflicollis* sp. (Poulin *et al.*, 2003). When presence/absence of parasites or the number of parasites of one particular species in a host population is studied, association patterns between species are often seen. Associations may be positive (e.g. *Pomphorhynchus laevis* and *Acanthocephalus clavula*, or *Meiogymnophallus* sp. and *Curtuteria australis*), random (e.g. *Polymorphus minutus* and *P. laevis*), or negative (e.g. *Gammarinema gammari* and *M. papillorobustus*) (Dezfuli *et al.*, 2000; Poulin *et al.*, 2000, Fauchier & Thomas, 2001; Outreman *et al.*, 2002). Differences in associations between parasites are thought to be explained by their life cycles, with positive associations when parasite species have similar life cycles, and either random or negative associations when they differ (Outreman *et al.*, 2002; Poulin *et al.*, 2003; Lagrue & Poulin, 2008).

In Pool Los Juncos, Cyclophyllidea sp. 1 was the dominant species in terms of infection prevalence and intensity, while *Pseudocorynosoma* sp. and *Thelohania* sp. were less abundant, and Cyclophyllidea sp. 2 was rare. *Pseudocorynosoma* sp. showed a negative association with Cyclophyllidea sp. 1. Because both of these species have the same life cycle, this pattern could indicate that competition for resources may be occurring, and suggests that mixed infections of both parasite species could have high energy resource demands, since *Pseudocorynosoma* sp. is the larger of the two parasite species, while Cyclophyllidea sp. 1 tends to be found in higher infection intensities. A possible method to avoid co-infections could involve mechanisms preventing the establishment and growth of new parasites (Barger & Nickol, 1999), or to change the behaviour of the infected host. Acanthocephalans are known to be manipulators of host behaviour,

Table 2. Generalized linear models of single parasite infections controlling for sex and length effects in Pool Los Juncos. Significant differences are indicated in bold case.

	<i>Pseudocorynosoma</i> sp.		Cyclophyllidea sp. 1		Cyclophyllidea sp. 2		<i>Thelohania</i> sp.	
	Wald stat.	P	Wald stat.	P	Wald stat.	P	Wald stat.	P
Intercept	33.7	<0.001	68.8	<0.001	8.6	0.003	40.2	<0.001
Total length	8.7	0.003	5.6	0.018	1.2	0.266	8.7	0.003
Sex	14.7	0.002	27.3	< 0.001	7.6	0.054	12.1	0.007
Total length–sex	20.9	0.0001	41.8	< 0.001	14.0	0.003	15.9	0.001

Table 3. Generalized linear models to detect associations between the four parasite species in amphipods from Pool Los Juncos. Significant differences are indicated in bold case. *P* values are given in the upper diagonals, and chi-square values in the lower diagonals.

		<i>Pseudocorynosoma</i> sp.	Cyclophyllidea sp. 1	Cyclophyllidea sp. 2	<i>Thelohania</i> sp.
Females	<i>Pseudocorynosoma</i> sp.	–	0.0002	No mixed infections	0.0012
	Cyclophyllidea sp. 1	13.94	–	0.436	0.54
	Cyclophyllidea sp. 2	1.96	0.61	–	No mixed infections
	<i>Thelohania</i> sp.	10.50	0.37	0.45	–
Males	<i>Pseudocorynosoma</i> sp.	–	0.0009	No mixed infections	0.0355
	Cyclophyllidea sp. 1	10.89	–	0.15	0.48
	Cyclophyllidea sp. 2	3.81	2.10	–	0.78
	<i>Thelohania</i> sp.	4.42	0.49	0.07	–
Undetermined	<i>Pseudocorynosoma</i> sp.	–	0.09	0.52	0.05
	Cyclophyllidea sp. 1	2.92	–	0.17	0.00001
	Cyclophyllidea sp. 2	0.41	1.91	–	No mixed infections
	<i>Thelohania</i> sp.	3.69	19.07	1.18	–

therefore *Pseudocorynosoma* sp. may alter the behaviour of *H. patagonica* to reduced its chance of acquiring mixed infections, such as by causing infected amphipods to occupy habitats where there is less chance of ingesting eggs from other parasite species. Our data do not allow us to infer the mechanism of segregation, and experimental infections and behavioural studies are necessary. In our study, we also found a positive association between *Thelohania* sp. and both *Pseudocorynosoma* sp. and Cyclophyllidea sp. 1. As this microsporidian obtains resources from muscles, and not from the haemocoel like the other parasites, it may be assumed that this parasite uses a different ecological niche, thereby avoiding competition not only for space but also for resources. Alternatively, this positive association could be the side-effect of the microsporidian infection, since this parasite can potentially impair the function of the amphipod's telson muscles, which would alter normal swimming movements (Ryan & Kohler, 2010) and may lead to microsporidian-infected hosts being located in habitats with higher risk of infection by other parasite species. This mechanism has been invoked for the association of the copepod *Pseudomyicola spinosus* with echinostome cercariae in the cockle *Austrovenus stutchburyi* (Leung & Poulin, 2007), for example. Here we have to be cautious, because a positive association between *Thelohania* sp. and Cyclophyllidea sp. 1. was only recorded in undetermined amphipods, so this pattern seems to be restricted only to a small fraction of the amphipod population. Why were no associations between Cyclophyllidea sp. 2 and any other parasites observed? This cestode is a relatively large

parasite, and was uncommon in terms of prevalence and mean infection intensity. Interestingly, almost no mixed infections were found between this parasite and *Pseudocorynosoma* sp. or *Thelohania* sp. (see table 3). Therefore we suspect that selection to avoid infected hosts (negative association) may be acting between Cyclophyllidea sp. 2 and the other parasite species.

In Pool Los Juncos, sex and length of hosts were associated with parasite infections, and male amphipods, in particular, were more commonly infected by all parasite species. Many parasite species are commonly found infecting males; this pattern in mammals has been attributed to behavioural, genetic or immunity differences (mediated by immunosuppressant effects of testosterone) between sexes (Zuk & McKean, 1996; Klein, 2004). However, because no testosterone is found in male invertebrates, sex-biased infection is an unusual finding (Sheridan *et al.*, 2000). So, differences between sexes recorded here can be attributed to genetics or behaviour of males. For example, it has been suggested that higher infections in males could be associated with an increased food intake, and accumulation of parasites in larger males (Outreman *et al.*, 2002; Rauque & Semenas, 2007; Rauque *et al.*, 2011). However, experimental infections have shown that male copepods are more susceptible to infection than females, and this is thought to be explained by the more intensive sexual selection pressures acting on males, which would result in less energy being available for an immune response against parasites (Wedekind & Jakobsen, 1998). Finally, in this study female amphipods were more abundant than

Table 4. Observed and expected mixed infections (using G-test) in males, females and undetermined amphipods in Pool Los Juncos.

Amphipods	Parasites	Mixed infections		Type of association
		Observed (expected) with <i>Pseudocorynosoma</i> sp.	Observed (expected) with <i>Thelohania</i> sp.	
Males	Cyclophyllidea sp. 1	5 (12.9)	–	Negative
	<i>Thelohania</i> sp.	15 (7.1)	–	Positive
Females	Cyclophyllidea sp. 1	4 (12.3)	–	Negative
	<i>Thelohania</i> sp.	11 (2.7)	–	Positive
Undetermined	Cyclophyllidea sp. 1	–	8 (3.3)	Positive

males. Although this pattern has been recorded in other environments in Patagonia (Rauque, 2007; Rauque & Semenas, 2007), no information is available for the possible feminizing effects of microsporidians, which are known to provoke such alterations (Rodgers-Gray *et al.*, 2004).

In conclusion, in Pool Los Juncos we found a combination of positive and negative associations between mixed parasite infections in *H. patagonica*, and that infection patterns are likely to be attributed to competition for both food intake and space in the haemocoel of the amphipod.

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