Coexistence patterns of two larval helminth parasites associated with their intermediate host, the mole crab *Emerita analoga* Stimpson, 1857 (Decapoda: Anomura: Hippidae)

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ABSTRACT

Larval helminth parasites that share an intermediate host could be subjected to density-dependent factors, such as crowding effects, as well as demonstrate positive or negative associations between them. We examined intraspecific and interspecific interactions and the nature of species association between an acanthocephalan and a trematode within their crustacean host. The Pacific mole crab *Emerita analoga* (Stimpson, 1857), one of the most abundant decapods in the swash zone of sandy beaches along the Pacific coasts of North and South America, serves as the first intermediate host for the acanthocephalan *Profilicollis altmani* (Perry, 1942) and as the second intermediate host for the trematode *Microphallus nicolli* (Cable & Hunninen, 1938). Both species use shore birds as their definitive hosts. In a large host sample collected from Monterey Bay, California, large female crabs harbored both parasites in significantly greater numbers than the smaller male crabs ($P < 0.001$), indicating that host size and sex are the primary factors governing prevalence of these helminth parasites in the populations of mole crabs. Both larval helminths were noted inside the mole crab's hemocoel; whereas the majority of acanthocephalan cystacanths were located in the medial region, trematode metacercariae were found mostly aggregated around the tubules of the digestive glands in the hemocoel. The degrees of competitive interactions were evaluated by comparing parasite body size (volume) in single *versus* mixed-infections. No evidence of intraspecific or interspecific competition was found, as the volume of cystacanths remained unaffected at various infection intensities or when present with the trematode species. We found a positive association between the two coexisting species of helminth parasites ($r = 0.34$, $P < 0.001$). By sharing both intermediate and definitive hosts, it is likely that their coexistence within their crab host is not accidental, and may benefit both species with regards to transmission to their definitive hosts.

Key Words: Acanthocephala, California, competition, cystacanth, host size and sex, metacercaria, parasitism, species associations, Trematoda

INTRODUCTION

Larvae of helminth parasites utilize their intermediate hosts for resources as well as for trophic transmission to definitive hosts via predation (Lafferty, 1999). An intermediate host may be infected with more than one helminth parasite, raising the possibility of different types of species associations between them. Positive association between two helminths can be expected where both parasites benefit by sharing the same definitive host; however, negative associations, whereby larvae of one species would benefit by avoiding the other species, are likely between larval helminths with conflicting interest, such as having different definitive hosts (Lafferty *et al.*, 2000). For example, trematode metacercariae of *Meiogymnophallus* sp. and *Curtuteria australis* Allison, 1979 in the cockle *Austrovenus stutchburyi* (W. Wood, 1828) share oystercatcher as their definitive hosts, and show positive association (Poulin *et al.*, 2000).

The nematode *Gammarinema gammari* Kinne & Gerlach, 1953 and the trematode *Microphallus papillorobustus* (Rankin, 1940) associated with the amphipod *Gammarus insensitus* (Stock, 1966), however, demonstrate negative association; unlike the nematode, which uses the amphipod as a habitat and source of nutrition, the trematode uses aquatic birds as definitive host (Fauchier & Thomas, 2001).

Species-association patterns can be used to determine the role of interspecific interactions in helminth communities (Lafferty *et al.*, 2000; Poulin, 2001). Coexisting larval helminths may be subjected to competitive interactions within their intermediate host. Such interactions can affect their body size, with important implications. Size may impact parasite fitness by influencing the rate of transmission to definitive hosts, *i.e.*, larger larval sizes in intermediate hosts influence adult success because of enhanced establishment and survivorship of larger individuals (Steinauer & Nickol, 2003), and also affect development (Amin *et al.*, 1980). Measuring parasite size (volume) can be used as indicators of competition (Brown *et al.*, 2003; Fredensborg & Poulin, 2005; Lagrue & Poulin, 2008; Rauque & Semenas, 2011; Caddigan *et al.*, 2017). For instance, reduction in mean cystacanth volume of the acanthocephalan *Pomphorhynchus laevis* (Zoega in Müller, 1776) with conspecifics and in the presence of *Acanthocephalus clavula* Dujardin, 1845 indicated intra- and interspecific competition, respectively (Dezfuli *et al.*, 2001), in their amphipod host, *Echinogammarus stammeri* (Karaman, 1974). In contrast, no significant differences in cystacanth volume of *Pseudocorynosoma* sp. under multiple infections in the amphipod *Hyalella patagonica* (Cunningham, 1871) showed the absence of intraspecific competition (Rauque & Semenas, 2011), and the mean volume of the acanthocephalan *Profilicollis* sp. remaining unaffected by the presence of metacercariae of the trematode *Maritrema* in two species of shore crabs, *Macrophthalmus hirtipes* (Jacquinot in Hombron & Jacquinot, 1846) and *Hemigrapsus crenulatus* (H. Milne Edwards, 1837), suggested lack of interspecific competition (Poulin *et al.*, 2003). Because the Pacific mole crab *Emerita analoga* (Stimpson, 1857) serves as an intermediate host for several larval helminths, this anomuran provides an ideal system to examine competitive interactions as well as evaluate the nature of species association among coexisting helminths.

Emerita analoga is one of the most abundant decapods found buried in sand in the swash zone of beaches along the Pacific coast of North and South America (Contreras *et al.*, 1999; Jaramillo *et al.*, 2000). These crabs are known to form dense aggregations in the intertidal zone (Dugan *et al.*, 2004). They serve as intermediate hosts for a variety of helminths, including the acanthocephalan *Profilicollis altmani* (Perry, 1942) and the trematode *Microphallus nicolli* (Cable & Hunninen, 1938) (Smith, 2007). Shorebirds such as the surf scoter *Melanitta perspicillata* (Linnaeus, 1758) and the western gull *Larus occidentalis* Audubon, 1896 are definitive hosts for *P. altmani* (Nickol *et al.*, 2002; Smith, 2007) as well as *M. nicolli* (Smith, 2007). When birds release eggs of both helminth species via feces, they are ingested by suitable intermediate hosts. Acanthocephalan eggs are consumed by mole crabs during filter feeding; once inside the intestine, the eggs hatch into acanthella larvae, which burrow through the intestinal wall into the hemocoel and develop into the resting infective cystacanth stage (Karl, 1967). *Microphallus nicolli* uses the snail *Olivella biplicata* (Sowerby I, 1825) as its first intermediate host; it releases free-swimming cercariae, which penetrate and encyst as metacercarial cysts in mole crabs, their second intermediate host (Smith, 2007).

The migration of larval stages to specific sites and their eventual encystment within their intermediate hosts are poorly understood. Few reports exist on the distribution patterns of cystacanths within their intermediate hosts. Nickol & Heard (1973) reported cystacanths inside the hemocoel adjacent to the digestive glands of the isopod *Caecidotea scrupulosa* (Williams, 1970), Muzzall & Rabalais (1975) documented their presence in different regions of the hemocoel of the isopod *Lirceus lineatus* (Say, 1818), and Dezfuli *et al.* (1994) noted their presence within the anterior and posterior halves of the hemocoelic space of the isopod *Asellus aquaticus* (Linnaeus, 1758).

Such distribution patterns raise the possibility of potential interactions between coexisting parasites in their intermediate hosts.

The primary objective of our research was to gain an understanding of coexistence patterns of two helminth parasites, *P. altmani* and *M. nicolli*, in *E. analoga*. We first examined the relationship between host body size and sex, and parasite prevalence and intensity of infection. We then analyzed the spatial distribution pattern of cystacanths and compared their size (parasite volume) inside the host hemocoel, and, using parasite volume as indicators of competition, we looked for evidences of intraspecific competition by comparing cystacanth volume at various infection intensities, and interspecific competition by comparing cystacanth volumes between single and mixed infections, i.e., in the presence of *M. nicolli*. We finally tested for species association patterns between the acanthocephalan and the trematode species. We predicted a positive association because both species share the same definitive hosts.

MATERIALS AND METHODS

Mole crabs were collected with a shovel and sieve in the swash zone of Del Monte Beach, Monterey, California (36.80°N, 121.90° W) in August and September 2014 and 2015. Crabs were manually transferred into an ice cooler, transported to the laboratory, and frozen for future analysis. Freezing had no effect on the encysted helminth larvae or on their measurements.

Each crab was measured using a digital caliper (total carapace length, in mm) and sex was determined by locating the pleopods, with females having three pairs, whereas pleopods are absent in males. Crabs were dissected under a binocular stereo microscope (Olympus 10X dissecting microscope; Olympus, Tokyo, Japan). The hemocoel and internal organs were carefully examined for parasites; once encountered, they were identified using taxonomic keys (Schlechte, 1969) and counted. The placement of cystacanths in the host hemocoel (anterior, medial, and posterior) was noted in order to examine spatial distribution. The anterior region was situated in the hemocoel surrounding the foregut, the medial area around the midgut, and the posterior region constituted the area around the hindgut. Cystacanths were transferred to a petri dish filled with distilled water and left for 10 min to evert their probosces. The length of cystacanths (from the proboscis tip to the posterior base of the trunk) and width were measured using a reticule (in microns). Trematode cysts were found mostly aggregated in the hemocoel region around the tubules of the digestive glands.

Prevalence (percentage of infected crab hosts in sample) and mean intensity (number of parasites per infected hosts) were calculated (Margolis *et al.*, 1982). Fisher's exact test was used to examine differences in prevalence between crab sexes. A two-way analysis of variance (ANOVA) was performed to examine the effects of host sex and infection status (uninfected *versus* infected hosts), as well as their interaction, on body size. Two-way ANOVA was also used to study the effects of host sex, infection intensity, and their interaction, on body size. A Chi-square test was used to test for spatial distribution of the cystacanths among three locations in the hemocoel. One-way ANOVA was employed to compare volumes of cystacanths found in the three hemocoel microhabitats, followed by post hoc comparisons using the Tukey HSD test.

As species interactions can result in unequal body sizes, larval volumes were calculated for *P. altmani* alone and in the presence of *M. nicolli*. A cystacanth is roughly oval; hence, its size was estimated as the volume of an ovoid, or $(\pi LW^2)/6$, where L and W are the length and width, respectively (Dezfuli *et al.*, 2001; Rauque & Semenas, 2011). As the metacercarial cysts of *M. nicolli* are spherical, their body surface was calculated using πR^2, where R is the radius of the cyst (Lagrue & Poulin, 2008). A one-way analysis of covariance (ANCOVA) was conducted to determine a significant difference between infection intensity on parasite volume, controlling for crab size, with intensity as the independent

variable, parasite volume as the dependent variable, and crab size as the covariate. A t-test was employed to account for any significant difference between volumes of cystacanths in single and mixed infections (in the presence of metaceracrial cysts). As the number of metacercariae can vary substantially among crabs (Violante-Gonzalez *et al.,* 2015), and their presence as well as their numbers that can affect cystacanth volumes, a linear regression analysis was performed between the average cystacanth volume per crab *versus* the number of metacercariae per crab.

Any change in numbers of parasite individuals during mixed infections compared with what is observed in single infections can provide evidence of positive and/or negative associations between co-occurring helminth parasites in their intermediate host (Poulin, 2001). Association of the two parasites was therefore verified using Pearson product moment correlation coefficient (*r*) adapted to binary data, *i.e.*, presence-absence data (Janson & Vegelius, 1981). All statistics were performed in SPSS 24 statistical software (SPSS, Chicago, IL, USA), and probability (*P*) values < 0.05 were considered significant.

RESULTS

Of 520 mole crabs examined, 401 (77.12%) were females and 119 (22.88%) males. This resulted in a significant sex ratio bias (3.37:1, $\chi^2 = 152.93$, $P < 0.001$). Infection parameters of *E. analoga* are presented in Table 1. Of all crabs inspected, 360 (69.2%) were infected with at least one parasite and 185 (35.6%) crabs harbored two or more parasites. The most common parasites encountered were the acanthocephalan *P. altmani* and the trematode *M. nicolli.* Trypanorhynch tapeworms were found on two occasions. We also
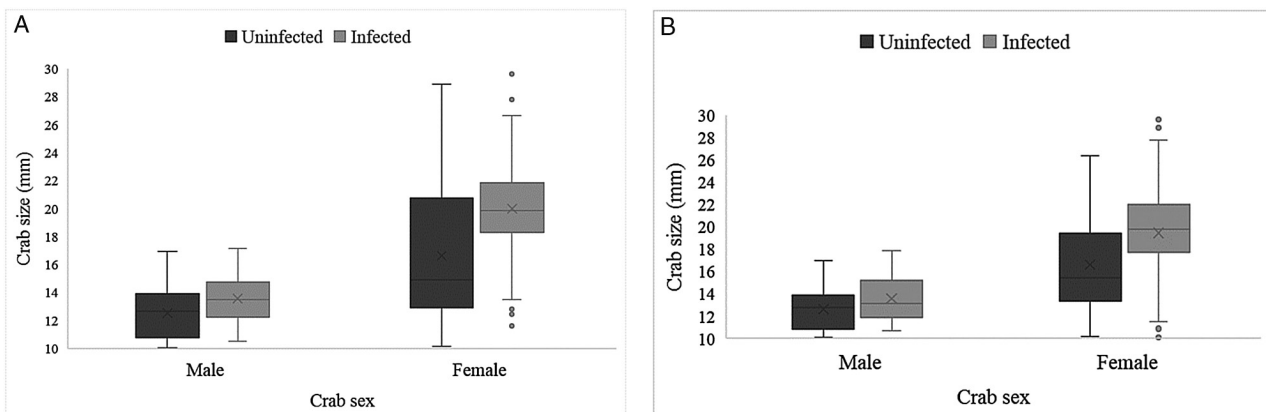
found few bivalve molluscs inside the hemocoel, an unusual finding described by Bhaduri *et al.* (2017).

Prevalences of *P. altmani* and *M. nicolli* were significantly higher in females than in male crabs (Fisher's exact test, $P < 0.001$). Results of the two-way ANOVA examining the effects of sex and infection status on body size showed there was an effect of sex for *P. altmani* ($F = 174.13$, $P < 0.001$), infection status ($F = 30.56$, $P < 0.001$), and interaction between sex and infection status ($F = 8.41$, $P = 0.004$; Fig. 1A). There was an effect of sex for *M. nicolli* ($F = 131.17$, $P < 0.001$), infection status ($F = 19.89$, $P < 0.001$), and interaction between sex and infection status ($F = 4.77$, $P = 0.029$; Fig. 1B). These results indicate that prevalence was significantly influenced by sex. Female crabs were larger than males, and infected crabs were larger than uninfected crabs. There was a positive correlation between infection intensity and host size in both *P. altmani* ($R^2 = 0.141$, $P < 0.05$) and *M. nicolli* ($R^2 = 0.137$, $P < 0.05$). With regards to the effects of sex and the intensity of infection on body size, the main effects of sex ($F = 44.55$, $P < 0.001$) and infection intensity ($F = 2.43$, $P = 0.02$) were significant in *P. altmani*; the main effects of sex was significant ($F = 4.14$, $P = 0.04$) but not for intensity in *M. nicolli* ($F = 0.98$, $P = 0.57$). Interaction effects between sex and infection intensity were not significant for both *P. altmani* ($F = 0.26$, $P = 0.90$) and *M. nicolli* ($F = 1.36$, $P = 0.244$).

A total of 721 cystacanths were retrieved from 257 crabs (mean 2.8 per host), which included 49 cystacanths from male ($N = 27$) and 672 from female crabs ($N = 230$). Intensities of infections by cystacanths and metacercarial cysts are shown in Figures 2A and 2B, respectively. The majority of crabs (> 70%) had 1–3 cystacanths and < 10% hosted 6 or more cystacanths. Approximately 65% of the crabs carried fewer than 10 metacercariae and ~5% crabs had greater than 40 metacercariae. One crab with 310 metacercariae had cysts distributed throughout the hemocoel and also within the tubules of the digestive gland.

Spatial distribution patterns within the hemocoel revealed that 493 cystacanths (68.4%) were located in the medial region, 183 (25.4%) in the posterior region, and 45 (6.2%) in the anterior region ($\chi^2 = 228.74$, $P < 0.001$). Distribution pattern affected parasite volume; those cystacanths located in the anterior region had significantly higher volume than those found in the medial or posterior region of the host (Fig. 3; one-way ANOVA, $F = 3.07$, $P < 0.05$). A Bonferroni post-hoc test did not reveal any difference in volumes of cystacanths found in medial and posterior regions.

There was a positive correlation between host size and the volume of both *P. altmani* ($R^2 = 0.153$, $P < 0.05$; Fig. 4A) and *M. nicolli* ($R^2 = 0.128$, $P < 0.05$; Fig. 4B). Because females were significantly larger than males, and to examine the likelihood that host body length could restrict the growth of the parasite, mean

Table 1. Descriptive statistics for the mole crab *Emerita analoga* infected with cystacanths of the acanthocephalan *Profilicollis altmani* and metacercarial cysts of the trematode *Microphallus nicolli*. Numbers in parentheses represent total hosts sampled ($N = 520$). Mean intensity is reported as mean ± 1 SE.

Parasite	Number	Prevalence (%)	Mean intensity	Range
Profilicollis altmani		49.42	2.77 ± 0.12	1–15
Male host	27 (119)	22.69	1.85 ± 0.21	1–3
Female host	230 (401)	57.36	2.88 ± 0.12	1–15
Microphallus nicolli		56.73	16.93 ± 3.02	1–310
Male host	32 (119)	26.89	8.75 ± 1.64	1–30
Female host	263 (401)	65.59	17.93 ± 3.49	1–310

Figure 1. Body size and the sex of mole crabs, uninfected and infected with cystacanth of *Profilicollis altmani* (**A**) and metacercariae of *Microphallus nicolli* (**B**). Box plots represent medians, interquartile ranges, and minimum-maximum ranges.
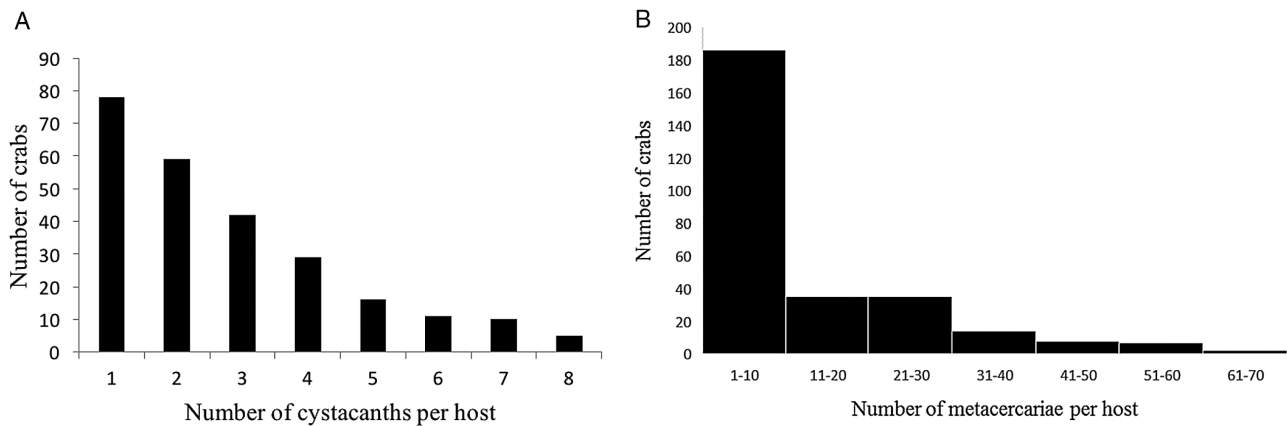
Figure 2. Relationship of the number of *Emerita analoga* and the number of cystacanths of *Profilicollis altmani* (**A**) and metacercariae of *Microphallus nicolli* (**B**).

cystacanth volumes among male and female crabs were compared (0.158 mm^3 and 0.162 mm^3, respectively) and found not to be significant ($t = -1.487$, $P > 0.05$). To examine intra- and interspecific competition, mean cystacanth volume at eight different infection intensities were compared. Infection intensity ranged from one to eight parasites per host and mean volumes ranged from 0.155 mm^3 to 0.176 mm^3 among these intensities. No significant effect of infection intensity on parasite volume was found after controlling for host body size (1-way ANCOVA, $F = 0.801$, $P > 0.05$), which indicated the absence of intraspecific competition. No significant difference was found when mean cystacanth volume during single infection (0.160 mm^3) was compared with those in mixed infections, *i.e.*, in the presence of *M. nicolli* (0.164 mm^3; $t = -1.727$, $P > 0.05$). This ruled out evidence of interspecific competition. Similarly, mean volumes of metacercarial cysts did not differ between single infection (0.0062 mm^3) and mixed infection, *i.e.*, in the presence of *P. altmani* (0.0066 mm^3; $t = -0.123$, $P > 0.05$). The average cystacanth volume per crab *versus* the number of metacercariae per crab were not related (linear regression, $R^2 = 0.0015$, $P > 0.05$) when only using crabs infected with both parasites.

For examining species association between coexisting parasites using presence-absence data, when prevalence of infection between single and mixed infections were compared, the majority of crabs were found to harbor significantly greater proportions of both parasites, of up to 36% ($\chi^2 = 61.12$, $P < 0.001$; Fig. 5). A significant positive association was shown to exist between *P. altmani* and *M. nicolli* in *E. analoga* ($r = 0.336$, $P < 0.001$).

DISCUSSION

We evaluated the coexistence of larvae of *P. altmani* and *M. nicolli* and found both parasites co-occurring in moderate abundances in *E. analoga*. Host size appears to be an important factor dictating prevalence and mean intensity as larger crabs harbored more parasites. Host size is a vital factor for the parasites because it correlates with available resources, such as space and nutrients (Dezfuli *et al.*, 2001; Steinauer & Nickol, 2003). The longer the host lives and feeds, the more likely it is to acquire more parasites (Poulin, 1999). Host sex also seems to play a vital role as the majority of helminths we encountered were found in larger females. It is likely that males die off earlier than females due to their smaller size and females need to live longer to produce and maintain broods, males and females may differ in their migration rates, or females might escape predation more easily by remaining in deeper water than do males (Wenner, 1972).

The prevalence rates of *P. altmani* in *E. analoga* we obtained is lower than those reported by other investigators, as geographical location and climatological conditions play important roles
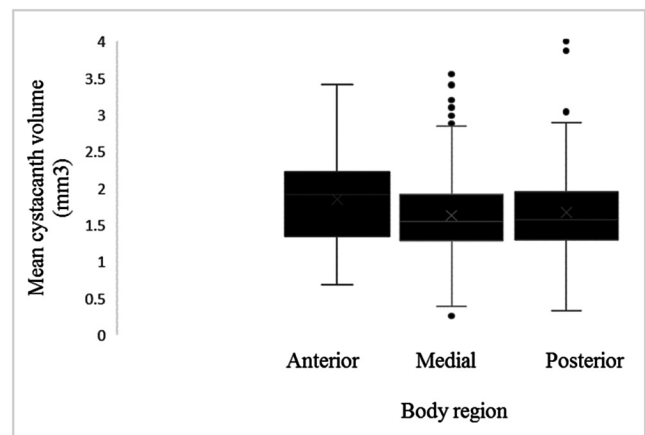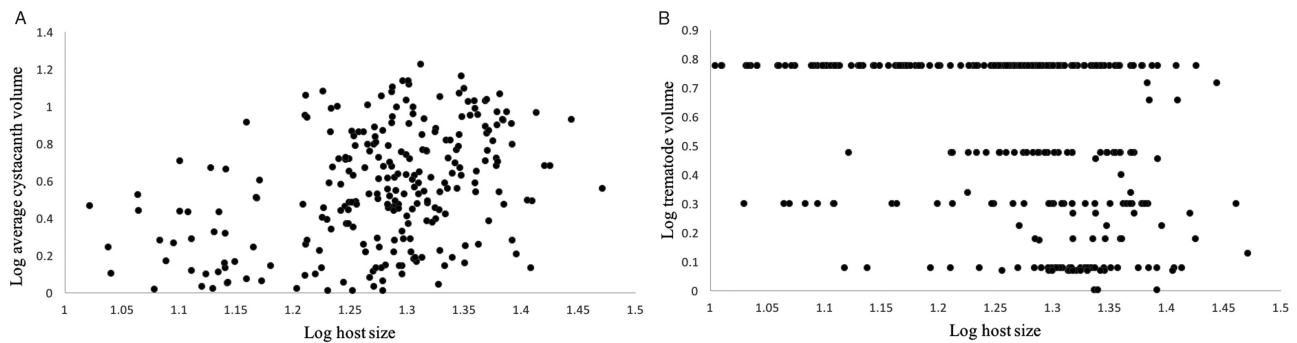

Figure 3. Mean volume of cystacanths of *Profilicollis altmani* in relation to the microhabitat within the hemocoel of its host *Emerita analoga*, showing median, interquartile ranges, and outliers.

in their epidemiology. The tendency of prevalence to increase with host size seems to agree with Kolluru *et al.* (2011), who found higher prevalence rates of *P. altmani* species in larger-sized crabs. Oliva *et al.* (2007) investigated prevalence and mean intensity under El Niño and non-El Niño conditions and found a proportional increase in prevalence based on host size, with 58% in the smallest crabs and reaching 100% in the largest individuals; mean intensity also increased with host size. Smith (2007) compared prevalence rates for *P. altmani* between summer and fall and observed that rates fluctuate between seasons along the California coast. Our prevalence and infection intensity rates for the trematode *M. nicolli* are also lower than those reported by Smith (2007), who documented higher prevalence during certain seasons and sites. Violante-Gonzalez *et al.* (2015) examined the parasitic fauna of *Emerita rathbunae* Schmitt, 1935 in Mexico and found that the prevalence of *M. nicolli* to be 100% and intensity to be > 3,200 metacercariae per host, making this species the most numerically dominant helminth parasite in *E. rathbunae*. Such high prevalence is not completely unexpected as *E. rathbunae*, measuring up to 44 mm, is much larger than *E. analoga* (Ríos-Elósegui & Hendrickx, 2015). The positive correlations that we documented between host size and parasite counts and volume, respectively, are most likely attributed to host size. Furthermore, maturing individuals of *E. analoga*, which can be as high as 3 years in age, likely accrue parasites during their lives (Contreras *et al.*, 1999).

While noting the position and orientation of cystacanths in their crustacean hosts, Muzzall & Rabalais (1975) found them more frequently on the left side of the hemocoel, facing posteriorly in the

Figure 4. Relationship between the size of the host *Emerita analoga* and the volume of cystacanth of *Profilicollis altmani* (**A**) and the volume of metacercariae of *Microphallus nicolli* (**B**).
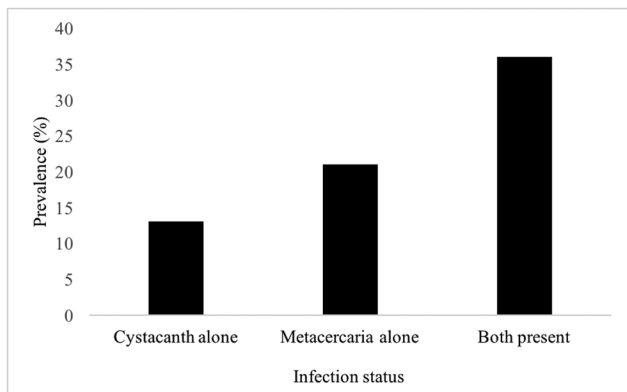
Figure 5. Prevalence of cystacanths of *Profilicollis altmani* alone, metacercariae of *Microphallus nicolli* alone, and both species together in their host *Emerita analoga*.

isopod *L. lineatus*, and Nickol & Heard (1973) also reported on cystacanths facing posteriorly in the isopod *C. scrupulosus*. In contrast, Wilson & Hubschman (1985) observed more larvae facing anteriorly, although the biological significance of such orientation remains unclear. The cystacanths we studied occupied distinct regions inside the crab's body cavity, with the vast majority located in the medial region and only a few in the anterior region. As the medial portion of the hemocoel has the greatest width, such space could accommodate large numbers of larvae, unlike the anterior portion, which is relatively narrow. The location of cystacanths affected their volume, with those located in the anterior region showing higher volume compared to those in other regions, possibly because they were fewer in numbers. Although this might constitute evidence of intraspecific interaction, the small number of cystacanths found in the anterior microhabitat precludes definite conclusions.

Many studies have focused on competition, as competitive interactions appear common among helminth parasites (Poulin, 2001). High infection intensities leading to reduction in cystacanth volume have been shown in amphipods (Cornet, 2011; Dianne *et al.*, 2012), and Saldanha *et al.* (2009) found smaller metacercarial sizes under crowded conditions in isopods. We did not find any evidence of intraspecific and interspecific competition, which corroborate the findings by other investigators. Rauque & Semenas (2011) stated lack of significant differences in the volumes of the cystacanths of *Pseudocorynosoma* sp. among single, multiple, and mixed infections (in the presence of *Acanthocephalus tumescens* von Linstow, 1896) during certain infection periods. Lack of interactions between the co-occurring acanthocephalans *Echinorhynchus truttae* Schrank, 1788 and *Polymorphus minutus* (Zeder, 1800) in *Gammarus pulex* (Linnaeus, 1758) have been noted by Awachie (1967); even in heavily infected individuals, growth and development were

similar in single as well as mixed infections. Weinersmith *et al.* (2014) reported on the lack of crowding effects for two trematodes, *Euhaplorchis californiensis* Martin, 1950 and *Renicola buchanani* (Martin & Gregory, 1951), infecting the California killifish *Fundulus parvipinnis* Girard, 1854 and attributed this to host-parasite body size ratios, i.e., as trematodes are much smaller than their hosts, they are less likely to experience resource limitation and remain unaffected even under crowded conditions. Similar conclusions can be drawn for coexisting larval species in *E. analoga* because of the small parasite-host size ratio and also because many larvae are mostly inactive and utilize few host resources (Poulin, 2001). Because the majority of mole crabs had few cystacanths, it is possible that competition for space was minimal. Larger mole crabs likely offer greater resources that help minimize crowd-induced competition, unlike those described in smaller intermediate hosts such as amphipods (Dezfuli *et al.*, 2001; Steinauer & Nickol, 2003) and isopods (Benesh & Valtonen, 2007).

We found *P. altmani* and *M. nicolli* occurring together in significantly more crabs than either species occurring alone, which implies a positive association. Species associations can result from various factors. Positive associations may result when coexisting species share similar environmental requirements, such as the same definitive hosts (e.g. *P. laevis* and *A. clavula*); conversely, negative associations (e.g. *Pseudocorynosoma* sp. and Cyclophyllidea sp.) or random associations (e.g. *P. minutus* and *P. laevis*) can occur when their definitive hosts are different (Dezfuli *et al.*, 2000; Rauque & Semenas, 2013). Poulin *et. al.* (2003) provided evidence of a positive interspecific association between the acanthocephalan *Profilicollis* sp. and the trematode *Maritrema* sp. in crabs; in this case, both helminths benefit from this association as their definitive hosts are shorebirds. Similarly, the trematodes *Stegodexamene anguillae* MacFarlane, 1951 and *Telogaster opisthorchis* MacFarlane, 1945 share fish definitive hosts and show a clear positive association (Lagrue & Poulin, 2015). In agreement with some of the above-mentioned studies, the positive association between *P. altmani* and *M. nicolli* suggests that this association is tied to their life cycles because both these parasites use surf scoters, western gulls, and other shorebirds as their definitive hosts. Smith (2007) showed positive correlations between bird abundance and prevalence of cystacanths of *P. altmani* and metacercariae of *M. nicolli* in mole crab populations along the California coast. Although shorebirds and gulls are sources of eggs for both helminth parasites, infection of crabs by *P. altmani* and *M. nicolli* are independent of each other. Acanthocephalans gain entry when their crab hosts ingest eggs during filter feeding, and trematodes infect crabs when snails release cercariae, which penetrate crabs. Nevertheless, the positive association between *P. altmani* and *M. nicolli* reported here appears to be connected to trophic relationships as evidenced by their similar definitive hosts, and that their co-occurrences are the result of adaptive infection strategies and not of coincidence.

REFERENCES

Amin, O.M., Burns, L.A. & Redlin, M.J. 1980. The ecology of *Acanthocephalus parksidei* Amin, 1975 (Acanthocephala: Echinorhynchidae) in its isopod intermediate host. *Proceedings of the Helminthological Society of Washington*, **47**: 37–46.

Awachie, J.B.E. 1967. Experimental studies on some host-parasite relationships of the Acanthocephala. Coinvasion of *Gammarus pulex* L. by *Echinorhynchus truttae* Schrank, 1788 and *Polymorphus minutus* (Goeze, 1782). *Acta Parasitologica Polonica*, **15**: 69–74.

Benesh, D.P. & Valtonen, E.T. 2007. Proximate factors affecting the larval life history of *Acanthocephalus lucii* (Acanthocephala). *Journal of Parasitology*, **93**: 742–749.

Bhaduri, R.N., Valentich-Scott, P., Hilgers, M.S., Singh, R., Hickman, M.E. & Lafferty, K.D. 2017. Facultative parasitism by the bivalve *Kurtiella pedroana* in the mole crab *Emerita analoga*. *Journal of Parasitology*, **103**: 646–651.

Brown, S.P., De Lorgeril, J., Joly, C. & Thomas, F. 2003. Field evidence for density-dependent effects in the trematode *Microphallus papillorobustus* in its manipulated host, *Gammarus insensibilis*. *Journal of Parasitology*, **89**: 668–672.

Caddigan, S.C., Pfenning, A.C. & Sparkes, T.C. 2017. Competitive growth, energy allocation, and host modification in the acanthocephalan *Acanthocephalus dirus*: field data. *Parasitology Research*, **116**: 199–206.

Contreras, H., Defeo, O. & Jaramillo, E. 1999. Life history of *Emerita analoga* (Stimpson) (Anomura, Hippidae) in a sandy beach of South Central Chile. *Estuarine, Coastal and Shelf Science*, **48**: 101–112.

Cornet, S. 2011. Density-dependent effects on parasite growth and parasite-induced hostimmunodepression in the larval helminth *Pomphorhynchus laevis*. *Parasitology*, **138**: 257–265.

Cunningham, R.O. 1871. XVII. Notes on the reptiles, amphibian, fishes, Mollusca and Crustacea obtained during the voyage of the H.M.S. "Nassau" in the years 1866–69. *Transactions of the Linnean Society of London*, **27**: 465–502.

Dezfuli, B.S., Giari, L. & Poulin, R. 2000. Species associations among larval helminths in an amphipod intermediate host. *International Journal for Parasitology*, **30**: 1143–1146.

Dezfuli, B.S., Giari, L. & Poulin, R. 2001. Costs of intraspecific and interspecific host sharing in acanthocephalan cystacanths. *Parasitology*, **122**: 483–489.

Dezfuli, B.S., Rossetti, E., Rossi, R. & Fano, E.A. 1994. Occurrence of larval *Acanthocephalus anguillae* (Acanthocephala) in the *Asellus aquaticus* (Crustacea, Isopoda) from the River Brenta. *Italian Journal of Zoology*, **61**: 77–81.

Dianne, L., Bollache, L., Lagrue, C., Franceschi, N. & Rigaud T. 2012. Larval size in acanthocephalan parasites: influence of intraspecific competition and effects on intermediate host behavioural changes. *Parasites & Vectors*, **5**: 1–7.

Dugan, J.E., Jaramillo, E., Hubbard, D.M., Contreras, H. & Duarte, C. 2004. Competitive interactions in macroinfaunal animals of exposed sandy beaches. *Oecologia*, **139**: 630–640.

Fauchier, J. & Thomas, F. 2001. Interaction between *Gammarinema gammari* (Nematoda), *Microphallus papillorobustus* (Trematoda) and their common host *Gammarus insensibilis* (Amphipoda). *Journal of Parasitology*, **87**: 1479–1481.

Fredensborg, B.L. & Poulin, R. 2005. Larval helminths in intermediate hosts: does competition early in life determine the fitness of adult parasites? *International Journal for Parasitology*, **35**: 1061–1070.

Hombron, J.B. & Jacquinot, C.H. 1846. Crustacés. *Atlas d'Histoire naturelle zoologie. Voyage au Pôle Sud et dans l'Océanie sur les corvettes l'Astrolabe et la Zélée pendant les années 1837-1838-1839-1840…*, Zoologie, pls. 1–9. Gide et J. Baudry, Paris.

Janson, S. & Vegelius, J. 1981. Measures of ecological association. *Oecologia*, **49**: 371–376.

Jaramillo, E., Dugan, J. & Contreras, H. 2000. Abundance, tidal movement, population structure and burrowing rate of *Emerita analoga* (Anomura, Hippidae) at a dissipative and a reflective sandy beach in south central Chile. *Marine Ecology*, **21**: 113–127.

Karaman, S. 1974. The genus *Echinogammarus* Stebb. (fam. Gammaridae) in Italy. *Bolletino del Museo Civico di Storia Naturale di Verona*, **1**: 71–104.

Karl, J.E. 1967. *Studies on the systematics and life history of* Polymorphus altmani *(Perry)*. Ph.D. thesis, Louisiana State University, Baton Rouge, LA, USA.

Kolluru, G.R., Green, Z.S., Vredevoe, L.K., Kuzma, M.R., Ramadan, S.N. & Zosky, M.R. 2011. Parasite infection and sand coarseness increase sand crab (*Emerita analoga*) burrowing time. *Behavioural Processes*, **88**: 184–191.

Lafferty, K.D. 1999. The evolution of trophic transmission. *Parasitology Today*, **15**: 111–115.

Lafferty, K.D., Thomas, F. & Poulin, R. 2000. Evolution of host phenotype manipulation by parasites and its consequences. *Evolutionary biology of host–parasite relationships: theory meets reality* (Poulin, R., Morand, S. & Skorping, eds.), pp. 117–127. Elsevier Science, Amsterdam.

Lagrue, C. & Poulin, R. 2008. Intra-and interspecific competition among helminth parasites: Effects on *Coitocaecum parvum* life history strategy, size and fecundity. *International Journal for Parasitology*, **38**: 1435–1444.

Lagrue, C. & Poulin, R. 2015. Spatial covariation of local abundance among different parasite species: the effect of shared hosts. *Parasitology Research*, **114**: 3637–3643.

Linnaeus, C. 1758. *Systema Naturae per Regna Tria Naturae, Secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis.* **Vol. 1**, Edn. 10. Reformata. Laurentii Salvii, Holmiae [= Stockholm].

Margolis, L., Esch, G.W., Holmes, J.C., Kuris, A.M. & Schad, G.A. 1982. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *Journal of Parasitology*, **68**: 131–133.

Muzzall, P.M. & Rabalais, F.C. 1975. Studies on *Acanthocephalus jacksoni* Bullock, 1962 (Acanthocephala: Echinorhynchidae). I. Seasonal periodicity and new host records. *Proceedings of the Helminthological Society of Washington*, **42**: 31–34.

Nickol, B.B. & Heard, R.W. 1973. Host-parasite relationships of *Fessisentis necturorum* (Acanthocephala: Fessisentidae). *Proceedings of the Helminthological Society of Washington*, **40**: 204–208.

Nickol, B.B., Heard, R.W. & Smith, N.F. 2002. Acanthocephalans from crabs in the southeastern US, with the first intermediate hosts known for *Arhythmorhynchus frassoni* and *Hexaglandula corynosoma*. *Journal of Parasitology*, **88**: 79–83.

Oliva, M.E., Barrios, I., Thatje, S. & Laudien, J. 2007. Changes in prevalence and intensity of infection of *Profilicollis altmani* (Perry, 1942) cystacanth (Acanthocephala) parasitizing the mole crab *Emerita analoga* (Stimpson, 1857): an El Niño cascade effect? *Helgoland Marine Research*, **62**: S57–S62.

Poulin, R. 1999. Body size vs abundance among parasite species: positive relationships? *Ecography*, **22**: 246–250.

Poulin, R. 2001. Interactions between species and the structure of helminth communities. *Parasitology*, **122**: S3–S11.

Poulin, R., Steeper, M.J. & Miller, A.A. 2000. Non-random patterns of host use by the different parasite species exploiting a cockle population. *Parasitology*, **121**: 289–295.

Poulin, R., Nichol, K. & Latham, A.D.M. 2003. Host sharing and host manipulation by larval helminths in shore crabs: cooperation or conflict? *International Journal for Parasitology*, **33**: 425–433.

Rauque, C.A. & Semenas, L. 2011. Parasite volume as an indicator of competition: the case of *Acanthocephalus tumescens* and *Pseudocorynosoma* sp.(Acanthocephala) in their intermediate host. *Journal of Parasitology*, **97**: 999–1002.

Rauque, C.A. & Semenas, L. 2013. Interactions among four parasite species in an amphipod population from Patagonia. *Journal of Helminthology*, **87**: 97–101.

Ríos-Elósegui, D. & Hendrickx, M.E. 2015. Abundance, relative growth and fecundity of *Emerita rathbunae* Schmitt, 1935 (Decapoda, Anomura, Hippidae) in the SE Gulf of California, Mexico. *Crustaceana*, **88**: 127–143.

Saldanha, I., Leung, T.L.F. & Poulin, R. 2009. Causes of intraspecific variation in body size among trematode metacercariae. *Journal of Helminthology*, **83**: 289–293.

Say, T. 1818. An account of the Crustacea of the United States, Part 7. *Journal of the Academy of Natural Sciences of Philadelphia*, **1**: 374–401.

Schlechte, J.A. 1969. *Seasonal variation of parasitic fauna in* Emerita analoga *and* Olivella biplicata. M.A. thesis, University of California Santa Barbara, Santa Barbara, CA, USA.

Schmitt, W.L. 1935. Crustacea Macrura and Anomura of Porto Rico and the Virgin Islands. Scientific survey of Porto Rico and the Virgin Islands, *New York Academy of Sciences*, **15**: 125–227.

Smith, N.F. 2007. Associations between shorebird abundance and parasites in the sand crab, *Emerita analoga*, along the California coast. *Journal of Parasitology*, **93**: 265–273.

Steinauer, M.L. & Nickol, B.B. 2003. Effect of cystacanth body size on adult success. *Journal of Parasitology*, **89**: 251–254.

Stimpson, W. 1857. Notices of new species of crustacea of western North America; being an abstract from a paper to be published in the journal of the society. *Proceedings of the Boston Society of Natural History*, **6**: 84–89.

Stock, J.H. 1966. A key to the species of the locusta-group of the amphipod genus *Gammarus*, with notes on their nomenclature. *Bulletin Zoölogisch Museum Amsterdam*, **1**: 1–5.

Violante-Gonzalez, J., Quiterio-Rendon, G., Monks, S., García-Ibañez, S., Pulido-Flores, G., Rojas-Herrera, A. & Larumbe-Moran, E. 2015. Parasite communities of the Pacific mole crab, *Emerita rathbunae* (Anomura: Hippidae), in sandy beaches from Guerrero and Michoacán, Mexico. *Journal of Marine Science*, **5**: 468–476.

Weinersmith, K.L., Warinner, C.B., Tan, V., Harris, D.J., Mora, A.B., Kuris, A.M., Lafferty, K.D. & Hechinger, R.F. 2014. A lack of crowding? Body size does not decrease with density for two behavior-manipulating parasites. *Integrative and Comparative Biology*, **54**: 184–192.

Wenner, A.M. 1972. Sex ratio as a function of size in marine Crustacea. *American Naturalist*, **106**: 321–350.

Williams, W. D. 1970. A revision of North American epigean species of *Asellus* (Crustacea: Isopoda). *Smithsonian Contributions to Zoology*, **49**: 1–79.

Wilson, B.A. & Hubschman, J.H. 1985. Host-parasite relationships of *Tanaorhamphus longirostris* (Acanthocephala: Neoechinorhynchidae) in the intermediate host, *Diaptomus pallidus* (Crustacea: Copepoda). *Proceedings of the Helminthological Society of Washington*, **52**: 71–75.