

Epidemiology of acanthocephalan infections in crabs from the Bahía Blanca Estuary, Argentina

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Abstract

This study was conducted in two populations of crabs, *Cyrtograpsus angulatus* and *Neohelice granulata* from the Bahía Blanca Estuary, in Argentina, to identify risk factors for infection by the acanthocephalan *Profilicollis chasmagnathi* and to assess the association between infection and mortality of these hosts. *Cyrtograpsus angulatus* and *N. granulata* crabs were sampled seasonally over the course of a year, and spring sampling included collection of dead crabs predated by Olog's gulls in a nearby breeding colony. Potential risk factors for infection were assessed and the number of cystacanth larvae per crab was counted. In *C. angulatus*, the odds of infection increased by 7% for each millimetre increase in carapace length, and were nearly 17 times greater in crabs sampled from the Olog's gull feeding area compared with those sampled from nests in the breeding colony. For every millimetre increase in carapace length in *N. granulata*, the odds of infection increased by 13% in crabs from the breeding colony, and by 32% in crabs from the feeding area. Mean intensity of infection in *N. granulata* increased by 16.5% for each additional millimetre of carapace width. The level of parasite aggregation was lowest in the largest *C. angulatus* and highest in *N. granulata* predated by Olog's gull. The results show that host size is the most important factor influencing infection prevalence in both crab species and intensity of infection in *N. granulata*, and suggest the presence of parasite-induced mortality in the populations studied.

Introduction

Parasites of the phylum Acanthocephala have an indirect life cycle in which the vertebrate definitive host becomes infected by ingesting larvae (cystacanths) contained within the haemocoel of an arthropod intermediate host (Taraschewski, 2005). Some species

are pathogenic to terrestrial and marine birds (Richardson & Nickol, 2008) and have frequently been associated with mortality in populations of aquatic birds (Sanford, 1978; Liat & Pike, 1980; Camphuysen *et al.*, 1996, 2002). Acanthocephalans are also known for their capacity to influence predator–prey interactions through behavioural, physiological or phenotypic manipulation of intermediate hosts such as crabs (Pulgar *et al.*, 1995; Haye & Ojeda, 1998; Latham & Poulin, 2001, 2002), frequently

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leading to a higher risk of predation by definitive hosts (Latham & Poulin, 2001, 2002).

The Bahía Blanca Estuary, in Argentina, is inhabited by large populations of *Cyrtograpsus angulatus* and *Neohelice granulata* crabs (Spivak, 1997), which are intermediate hosts to cystacanths of the acanthocephalan *Profilicolis chasmagnathi* (Alda *et al.*, 2011). This estuary is the most important breeding site for Olrog's gull (*Larus atlanticus*) (OG), a vulnerable species (IUCN, 2011) endemic to the Atlantic coast of Argentina, Uruguay and southern Brazil (Yorio *et al.*, 2005). During their breeding season, OG parents prey primarily on *N. granulata* (Delhey *et al.*, 2001) and *C. angulatus* (Petracci, pers. comm.), which are fed to the young from day 1 of hatching. Repeated exposure to parasites ingested from crabs has been shown to be associated with annual mortality of OG chicks in the largest known breeding colony for this species (La Sala & Martorelli, 2007).

Based on the feeding ecology of OG, the epidemiology of acanthocephalan infections in OG populations is likely dictated by the infection dynamics at the population level of the intermediate host. However, there is a gap in knowledge of the impact acanthocephalan infections have on populations of these crabs from the Bahía Blanca Estuary.

An observational, cross-sectional study was performed in populations of *C. angulatus* and *N. granulata* to identify risk factors for infection by *P. chasmagnathi* under the hypotheses that: (1) infections are associated with mortality of intermediate hosts; and (2) the risk of infection inflicted on OG varies depending on the crab prey species.

Materials and methods

Sampling procedures

Samples were collected in a feeding area of OG in the Bahía Blanca Estuary (38°44'S, 62°22'W) in south-western Buenos Aires province, Argentina, between July 2006 and July 2007. In the study site, *C. angulatus* inhabit mainly subtidal muddy sediments, and *N. granulata* are found both in the subtidal area and on mudflats of the intertidal zone. Both crab species studied here are bottom feeders in mud flats and salt marshes (Spivak, 1997). *Cyrtograpsus angulatus* was sampled from the subtidal zone using baited nets, whereas *N. granulata* was sampled systematically along transects inside a predefined area (50 × 200 m) on a large intertidal mudflat.

Sampling was stratified by season. Minimum sample size for each season was estimated using the Win Episcope V. 2.0 software (De Blas *et al.*, 2000) based on the following assumptions, for each species: (1) infinite population size; (2) an expected prevalence of 50%; (3) an accepted error of 10%; and (4) a confidence level of 95%. Calculations resulted in a minimum sample size of 96 crabs of each species for each season.

Examination of crabs

Crabs collected in the OG feeding area were transported live to the laboratory (Centro de Estudios Parasitológicos y de Vectores, CONICET-UNLP), humanely

killed by stunning (cooling at 4°C) followed by destruction of nerve centres (Murray, 2006), and dissected. The crabs were sexed and measured to the nearest 0.01 mm (carapace width at the level of first pair of lateral spines, and carapace length). The number of cystacanths of *P. chasmagnathi* per crab was recorded.

Statistical analysis

Prevalence of infection, 95% confidence interval (CI) and mean intensity (number of parasites per number of infected hosts) were computed and used as measures of frequency, confidence level and intensity of infection, respectively. Unpaired Student's *t*-tests were used to compare carapace length and width between sampling sites in each species. All analyses were considered statistically significant at $P \leq 0.05$.

Host size (carapace length and width, in mm) was used as a proxy for host age (McLay, 1988). To test the first hypothesis, that acanthocephalan infection is associated with mortality of intermediate hosts, methods proposed by Rousset *et al.* (1996) and Lester (1984) were implemented. For each species: (1) the relationship between infection prevalence/intensity and host size; and (2) the aggregation level of infection as a function of host size, were assessed as approximations to a causal relationship between infection and mortality. As suggested by the above-mentioned authors, a decrease of the mean parasite load, prevalence and/or aggregation level in relation to host size was interpreted as indirect evidence of parasite-induced mortality. Considering the hypothesis being tested here and the analytical approaches followed, the analyses initially included only those crabs sampled alive from the OG feeding area. Further analyses also included crabs collected from the OG breeding colony to assess the difference in prevalence, intensity and aggregation level between predated and non-predated crabs of each species.

All analyses were performed using the statistical package R (R Development Core Team, 2010). A two-stage model-fitting approach was employed to assess the role of crab size as a risk factor for infection status. First, generalized additive models (GAM) with non-parametric smoothers were fitted to explore the shape of the response (infected/not infected) to the continuous predictor variables (carapace length and width). Next, generalized linear models (GLM) with a binary, logistic response were used to reproduce the shapes identified in the GAMs by adding appropriate parametric terms in the model. For both GAMs and GLMs, binomial distributions with logit link functions were used.

GAMs were fitted using the cubic regression spline method implemented in the 'mgcv' R package (Wood, 2006), and GLMs were fitted using the R 'stats' package. GLMs were constructed using a stepwise process, starting from a saturated model which included all independent variables and two-way interactions. Terms were successively eliminated to identify the most parsimonious and explicative model based on the Akaike information criterion (AIC) (Akaike, 1974). Terms (variables or their interactions) were eliminated if variable inclusion did not reduce the AIC by more than two units (Burnham & Anderson, 2002).

Table 1. Carapace length (mm) in *C. angulatus* and *N. granulata* sampled from feeding area and breeding site of Olrog's gull.

Species and site	Mean \pm 1 SD	Range	Difference
<i>C. angulatus</i>			
Feeding area	25.43 \pm 4.84	15.77–39.49	$t = 29.24$;
Breeding colony	14.10 \pm 3.04	8.11–22.80	$P < 0.0001$
<i>N. granulata</i>			
Feeding area	12.25 \pm 5.66	2.54–31.71	$t = -8.14$;
Breeding colony	16.87 \pm 5.99	6.25–34.87	$P < 0.0001$

A set of models was constructed for each crab species using 'carapace length' (mm) and 'carapace width' (mm) as main explanatory variables, and 'sex' (male/female), 'sampling season' (spring, summer, fall, winter) and 'sampling site' (feeding area/breeding colony) as covariates. Because carapace length and width were highly correlated ($>70\%$), they were examined separately to avoid collinearity, and the variable yielding to the lowest AIC was included in the model. For continuous variables, the assumption of linearity was tested by plotting the midpoints of the quartiles of the distribution against the log-odds of the outcome for each category (Dohoo *et al.*, 2003). The association between infection status and independent variables was quantified by estimation of the odds ratio (OR). The OR for dichotomous independent variables (e.g. sampling site), which was computed as the exponential of the regression coefficient, indicates how much more likely it is for the outcome to be present in individuals of the reference group compared to those in the baseline group. For continuous independent variables, such as carapace length, the regression coefficient gives the change in the log-odds associated with an increase of one unit (mm) in the independent variable. Hence the exponential of its coefficient indicates the percentage increase in the odds for a one-unit increase in carapace length.

The other outcome of interest, 'intensity of infection', was zero-inflated (Zuur *et al.*, 2009). Therefore, several types of GLMs were fitted to quantify the association between infection intensity and host size while accounting for covariates described above and handling

over-dispersion (Zeileis *et al.*, 2008). Based on the AIC criterion, zero-altered negative binomial models (ZANB), also known as hurdle models, provided a better fit to the data than Poisson, negative binomial, zero-inflated Poisson or zero-inflated negative binomial models. In ZANB models, a negative binomial GLM is used to fit the count process excluding zeros (zero-truncated count model), whereas a binomial GLM is used to fit the probability of measuring a positive count. The logistic component of the ZANB model is presented in the table but not interpreted because this was accomplished using the logistic GLMs described above. For the continuous variable in the negative binomial model, $\exp(\beta)$ indicated the percentage change in the dependent variable for a one-unit increase in carapace length.

Analyses were conducted using the 'pscl' R package (Zeileis *et al.*, 2008). The significance of each final model was tested by comparing it against a null model without predictors using likelihood ratio tests with the 'lmtest' R package (Zeileis & Hothorn, 2002).

To assess the association between parasite aggregation and host size, crabs of each species were assigned to one of four carapace length categories based on the quartiles of the distribution. The degree of parasite aggregation was calculated (regardless of the crabs' size) for each season by using the variance-to-mean ratio (Elliot, 1977). Spring level aggregation (when sampling was conducted both in the OG colony and their feeding area) was calculated for each sampling site to assess the degree of aggregation among predated crabs, because predation by OG contributes considerably to crab mortality during this season at this study site, and prey selection may be influenced by the intensity of infection in the crabs. Parasite aggregation was also estimated (regardless of sampling season) for each size-class in feeding area only crabs.

To test the second hypothesis, that the risk of infection imposed on OG varies by crab species, a subset of data for both species which included: (1) all crabs collected from nests in the colony; and (2) only those crabs from the feeding area with carapace width within the range of crabs collected from OG colony nests, was used. This was done to restrict the analysis to a dataset consisting only of hosts more likely to be predated by

Table 2. Prevalence, 95% CIs and mean intensity of infection by *P. chasmagnathi* in crabs from the Bahía Blanca Estuary are shown with number (*n*) of crabs sampled categorized by size-class.

<i>C. angulatus</i>	Small	Medium	Large	Very large
Size range (mm)	(8.11–18.81)	(18.82–23.88)	(23.89–27.78)	(27.79–39.49)
Prevalence	71.5	85.4	87.7	90.0
(95% CI)	(63.0–79.1)	(78.1–91)	(80.8–92.8)	(83.5–94.6)
Mean intensity	1.52	1.74	1.75	1.8
<i>n</i>	130	130	130	130
<i>N. granulata</i>	Small	Medium	Large	Very large
Size range (mm)	(2.54–9.00)	(9.01–12.73)	(12.74–17.07)	(17.08–34.87)
Prevalence	42.4	48.6	49.7	52.8
(95% CI)	(34.2–50.9)	(40.2–57.1)	(41.3–58.1)	(44.3–61.2)
Mean intensity	0.69	0.78	0.81	0.88
<i>n</i>	144	144	145	142

OG based on the species' prey size preference. A GLM with a binary, logistic response which included 'infection status' (infected/not infected) as the dependent variable, and 'species' (*C. angulatus*/*N. granulata*) as the primary independent variable was fitted. The model included the explanatory variables used to assess the first hypothesis and was assembled following the same procedures.

Results

The differences in carapace size (length) between sampling sites for each crab species are presented in table 1. A total of 520 *C. angulatus* and 578 *N. granulata* were collected in all four seasons, which included 98 *C. angulatus* and 147 *N. granulata* collected dead (predated) from nests in a nearby colony of OG in the spring. Prevalence with 95% CIs, mean intensity of infection, and sample sizes stratified by size-class (carapace length) are provided for each species in table 2.

Hypothesis 1: infection is associated with mortality of intermediate hosts

The fitted GAMs showed positive linear associations between infection status (infected/not infected) and crab size (carapace length and width) for both species. Therefore, only linear variable terms were included in the final GLMs. The probability of infection in both species was better described by the length of the carapace than by its width, so only carapace length was included in the models. The crude relationship (i.e. unadjusted by other covariates) between carapace length and the probability of infection for both species is shown in fig. 1. The final models assessing the variables associated with infection in *C. angulatus* and *N. granulata* from both sites are detailed in tables 3 and 4, respectively.

In *C. angulatus*, the odds of infection increased by 7% (OR: 1.07; 95% CI: 1.03–1.12) for each millimetre increase in the size, and were ~17 times greater (OR: 16.74; 95% CI: 2.16–129.36) in crabs sampled from the OG feeding area compared to those sampled from nests in the breeding colony. In *N. granulata*, there was an interaction that suggests that the relationship between size and infection status depended on the sampling site. In crabs collected from the breeding colony and the feeding area, the odds of infection increased by 13% (OR: 1.13; 95% CI: 1.01–1.27) and 32% (OR: 1.32; 95% CI: 1.18–1.48), respectively, for each millimetre increase in host size.

In *C. angulatus*, the intensity of infection was not associated with carapace length or width when the analysis was restricted to crabs from the feeding area, nor when it included crabs from both sites and the 'sampling site' was introduced as covariate. Conversely, in *N. granulata*, the mean intensity of infection increased by 11.7% for each additional millimetre of carapace length when the analysis included only crabs from the feeding area. When crabs from both sampling sites were included in the model, the intensity of infection rose by 16.5% for every unit increase in the size (table 5).

Variability in infection level (variance-to-mean ratio) by size-class in *C. angulatus* was concave-down shaped and increased from small-sized crabs reaching a local

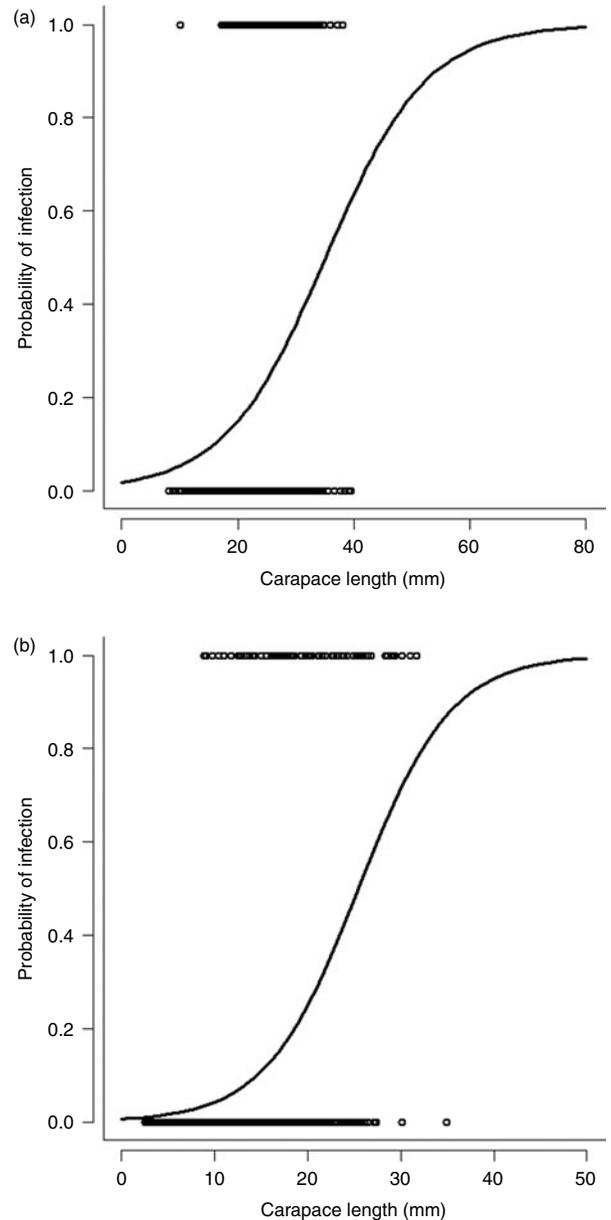


Fig. 1. Fitted probability of infection by *P. chasmagnathi* as a function of carapace length in *C. angulatus* (a) and *N. granulata* (b). Clumped black circles at 0 and 1 on the y-axis represent the observed data and show the ranges of carapace length for infected (1) and non-infected (0) crabs.

maximum in large-sized crabs, with a precipitous drop of 41.4% between large and very large crabs (fig. 2). In *N. granulata*, variability in infection level was similar between small and medium-sized crabs, but increased steadily between the latter and very large crabs, where aggregation reached its highest level (fig. 2).

Seasonal patterns of parasite aggregation are presented in table 6. Aggregation levels in *N. granulata* varied (range 1.76–2.45) throughout the year in the feeding area only and the level of aggregation was 173.3% higher among

Table 3. Logistic regression model describing the association between risk factors and infection by cystacanths of *P. chasmagnathi* in *C. angulatus*.

Model = Infection ~ CARAPACE LENGTH + SITE				
$n = 520$	AIC = 505.6			
Term	Coefficient	Std Error	P	ΔAIC^{\dagger}
Intercept	-2.7724	0.602	<0.0001	-
Length	0.0704	0.023	0.0019	7.8
Site (feeding area)	2.8175	1.044	0.0069	13.7

Baseline category: breeding colony.

† AIC value increment if the single term is dropped.

crabs collected in the spring from nests in the OG colony compared to those sampled in the feeding area. Aggregation levels were more variable (range 1.78–3.51) in *C. angulatus* but, contrary to what was observed in *N. granulata*, the level of aggregation was 251% lower among crabs collected in the OG colony compared to those sampled in the feeding area.

Hypothesis 2: risk of infection varies with crab species

The odds of infection were over 2.7 times (OR: 2.74; 95% CI: 0.95–7.87) higher for *N. granulata* than for *C. angulatus*, but this difference was not statistically significant.

Discussion

This work represents the first assessment of epidemiological factors affecting acanthocephalan infection in populations of grapsid crabs in general and in *C. angulatus* and *N. granulata* in particular.

The results suggest that acanthocephalan infections are associated with mortality of intermediate hosts through predator–prey interactions. We showed that: (1) host size is the most significant risk factor for acanthocephalan infections in populations of *C. angulatus* and *N. granulata*; (2) the probability of infection does not differ between species; (3) parasite aggregation in the spring is higher in *N. granulata* crabs predated by OG compared with non-predated ones sampled in the feeding area; and

Table 4. Logistic regression model describing the association between risk factors and infection by cystacanths of *P. chasmagnathi* in *N. granulata*.

Model = Infection ~ LENGTH + SITE + LENGTH*SITE				
$n = 575$	AIC = 338.5			
Term	Coefficient	Std Error	P	ΔAIC^{\dagger}
Intercept	-4.173	0.934	<0.0001	-
Length	0.124	0.045	0.0061	-
Site (feeding area)	-1.908	1.110	0.08545	-
Length*Site (feeding area)	0.154	0.057	0.00684	4.8

Baseline category: breeding colony.

† AIC value increment if the single term is dropped.

Table 5. Zero-altered negative binomial model describing the association between carapace length and infection intensity by cystacanths of *P. chasmagnathi* in *N. granulata*.

Term	Coefficient	Std Error	P
Count model (zero-truncated negative binomial with log link)			
Intercept	-10.529	41.561	0.8000
Length	0.153	0.053	0.0039
Zero hurdle model (binomial with logit link)			
Intercept	-5.145	0.448	<0.0001
Length	0.202	0.024	<0.0001

LRT (likelihood ratio test): $\chi^2 = 102.8$; $P < 0.0001$.

(4) *C. angulatus* in the largest size-class have the lowest level of parasite aggregation.

Early works have revealed that a decrease of the mean parasite load/prevalence, or parasite aggregation level, as a function of host age, suggests the presence of parasite-induced host mortality (Lester, 1984; Rousset *et al.*, 1996). The GAM and GLM models, in this study, showed positive linear associations between crab size and the odds of infection in both species. A positive linear association between crab size and intensity for *N. granulata* was evident in the ZINB model, whereas a non-significant association was observed between size and intensity for *C. angulatus* in this analysis. Interestingly, although these models failed to reveal a causal association between *Profilicollis* infections and mortality in the populations studied, the analyses of aggregation levels suggest the opposite effect. The observation of a decreasing level of aggregation in *C. angulatus* in the largest size-class indicates that a smaller fraction of the largest and most heavily infected crabs is removed from the population. In contrast to the observation of parasite aggregation in *C. angulatus*, the aggregation pattern in *N. granulata*, which increased across size-classes, attaining its highest level in very large crabs, does not support the presence of parasite-associated mortality.

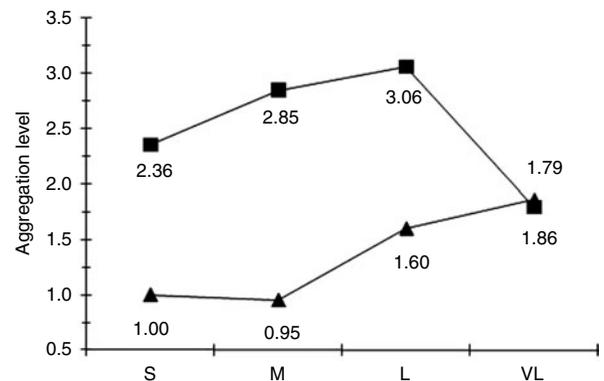


Fig. 2. Changes in parasite aggregation (variance-to-mean ratio) with host size-class in *C. angulatus* (■) and *N. granulata* (▲) sampled from the feeding area of OG. Host size (carapace length) is expressed as four categories (S, small; M, medium; L, large; VL, very large).

Table 6. Parasite aggregation (variance-to-mean ratio) by sampling season. The spring sample collection for both species was stratified by sampling site.

Species by site	Spring	Summer	Autumn	Winter
<i>C. angulatus</i>				
Colony	1.00	*	*	*
Feeding area	3.51	2.04	2.90	1.78
<i>N. granulata</i>				
Colony	5.33	*	*	*
Feeding area	1.95	1.76	2.45	2.22

*Not applicable because samples were not collected.

As mentioned in the introduction, the phylum Acanthocephala is widely known for its ability to mediate predator–prey interactions through various mechanisms (Pulgar *et al.*, 1995; Haye & Ojeda, 1998; Latham & Poulin, 2001, 2002). OG numbers in the study area peak during the species' breeding season in the austral spring and, as described above, gull parents feed chicks and themselves almost exclusively on *C. angulatus* and *N. granulata* during this period. This strong trophic specialization, combined with the capacity of acanthocephalans to manipulate their intermediate hosts, initially suggested that parasite-related mortality in *C. angulatus* was associated with predation by OG. However, predation by OG could be ruled out as the main source of mortality because *C. angulatus* predated by OG and collected in the colony: (1) were considerably smaller than those sampled from the feeding area; (2) had a negligible (0.01%) prevalence of infection; and (3) showed no parasite aggregation as a consequence of (2). Alternatively, other species known for preying on these crab species, such as sharks (Cortés, 1999) and fish which occur in the study site, might be responsible for the suspected *C. angulatus* mortality.

Despite the apparent lack of association initially observed between infection and mortality in *N. granulata*, there was a remarkably higher level of parasite aggregation (regardless of size-class) among crabs collected in nests from the colony during the spring. This may indicate that *N. granulata* with the highest infection intensity are being removed from the population via predation by OG. Given the host manipulation capacity of acanthocephalans, it is plausible that this biological trait somehow leads to an increased likelihood of predation in heavily infected *N. granulata*, which eventually would be reflected by the higher parasite aggregation observed among predated individuals.

The odds of infection were higher for *N. granulata* than for *C. angulatus*, although this difference was not significant. This lack of significance could be explained by the life history of crab species, such as habitat use and feeding ecology, and by characteristics of the parasite's life cycle. Adult females of *P. chasmagnathi* infecting vertebrate final hosts shed their eggs through faeces. These relatively small eggs are picked up inadvertently over time by *C. angulatus* and *N. granulata* through feeding, and eventually reach their cystacanth stage in these intermediate hosts. The bottom-feeding foraging habit of these crabs makes infection a random process which is expected to follow a density-independent infection pattern, as suggested by Latham & Poulin

(2002) for other species of shore crabs. Therefore, infection patterns in both species should have similar parasite infection transmission rates, explaining the similar rates of infection observed.

In conclusion, our results suggest that mortality of the largest and most heavily infected *C. angulatus* is associated with predators other than OG, whereas mortality among the most heavily infected *N. granulata* does seem to be associated with a higher risk of predation by OG. The latter findings underline the importance of incorporating, whenever possible, data from both live and dead hosts when studying the epidemiology of helminth infections in intermediate hosts within systems where their predators can be identified and predated hosts can be sampled.

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