

Numerical and functional responses of intestinal helminths in three rajid skates: evidence for competition between parasites?

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SUMMARY

Host-parasite interactions generally involve communities of parasites. Within these communities, species will co-exist and/or interact with one another in a manner either benefiting the species involved or to the detriment of one or more of the species. At the level of helminth infracommunities, evidence for intra- and inter-specific competition includes numerical responses, i.e. those regulating helminth intensity of infection, and functional responses, i.e. where the presence of competitors modifies the realised niche of infrapopulations. The objectives of this study are to assess the numerical and functional responses of helminths in infracommunities from 3 rajid skates using general linear models. Despite a lack of numerical responses, functional responses to intra- and inter-specific interactions were observed. A positive correlation between the number of individuals in an infrapopulation and its niche breadth (functional response) was observed for the tapeworms *Pseudanthobothrium* spp. and *Echeneibothrium* spp., in all their respective hosts, and for the nematode *Pseudanisakis* sp. in the little skate. Evidence for inter-specific competition includes niche shifts in *Pseudanthobothrium purtoni* (ex little skate) and *Pseudanisakis* sp. (ex thorny skate) in the presence of *Pseudanisakis* sp. and the tapeworm *Grillotia* sp., respectively. These results are consistent with other studies in providing evidence for competition between helminths of skates.

Key words: cestodes, competition, elasmobranchs, functional response, general linear model, helminths, nematodes, niche breadth, numerical response, skates.

INTRODUCTION

Competition and predation are the two most widely studied ecological interactions between species. However, the body of literature on the intra- and inter-specific interactions between parasites pales in comparison with that for free-living eukaryotes, despite parasitic organisms outnumbering the latter (May, 1992; Windsor, 1998). Host-parasite interactions generally involve communities of parasites (Lello *et al.* 2004; Graham, 2008). However, due to the aggregated nature of parasites, not all combinations of species will be encountered in each host individual (Poulin, 2001). When co-occurring, species can co-exist and/or interact to benefit both parasites or be detrimental to one of the species. For instance, in wild rabbits, the strongyloid nematode *Graphidium strigosum* is able to modulate host immunity, thus its presence could have a positive effect on the numbers of another strongyloid, *Trichostrongylus retortaeformis* (Lello *et al.* 2004). Conversely, in laboratory mice, replication of micro-parasites requiring red blood cells is reduced in the presence of helminths inducing anaemia (Graham, 2008). Evidence for intra- and inter-specific

competition in parasites includes numerical and/or functional responses (Thomson, 1980). Numerical responses are those that regulate parasite intensity of infection at the infrapopulation (conspecific parasites within each host individual) level (Poulin, 2001), such as a decrease in numbers (e.g., Dobson, 1985), size and hence fecundity (e.g., Keymer, 1982), whereas functional responses are modifications to the realised niche of infrapopulations in response to competitors (Poulin, 2001), such as interactive site segregation (e.g., Stock and Holmes, 1988).

Intra-specific interactions, numerical responses in helminth infections are generally density dependent and can prevent the exponential growth of parasite populations (Keymer, 1982; Shostak and Scott, 1993). Furthermore, the size of the realised niche of an infrapopulation is by and large correlated with intensity of infection (Bush and Holmes, 1986; Alarcos *et al.* 2006; Pie *et al.* 2006). Similarly, in inter-specific interactions, the relative intensities of infection can affect the survival, fecundity, and establishment (Silver *et al.* 1980; Holland, 1984; Dobson, 1985) of individuals in an infrapopulation, resulting in a possible reduction in recruitment and influencing transmission patterns of entire populations (Poulin, 2007). On the other hand, functional responses are generally observed in inter-specific scenarios and can be independent from numerical

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responses (Poulin, 2001). Interactive site segregation (Holmes, 1973) can be defined as the overlap in realised niches being less than that of the fundamental niche within species-pairs (Poulin, 2001). For instance, inter-specific interactions leading to niche shifts of one species have been observed in gastro-intestinal helminth communities from a wide range of hosts, such as amphibians (Hamann *et al.* 2009), birds (Bush and Holmes, 1986; Stock and Holmes, 1988), insects (Adamson and Noble, 1993), fish (Friggens and Brown, 2005; Hassanine and Al-Jahdali, 2007) and mammals (Ehman, 2001).

The spiral intestine in skates is relatively short. In these animals, increased surface area is achieved by the presence of intestinal mucosa of the intestine coiled along a central axis forming a descending spiral (anterior to posterior), hence its name: spiral intestine. When opened along the ventral blood vessel, the spiral opens up exposing mucosal flaps likened to the pages of a book and providing a discrete area from which attachment sites of helminths can be recorded. For a more detailed description, see Parker (1879) or McVicar (1979). The attachment sites of tapeworms within the spiral intestine have been studied in several elasmobranch species (Euzet, 1959; Williams, 1961, 1968; Rees and Williams, 1965; Carvajal and Dailey, 1975; McVicar, 1979; Borucinska and Caira, 1993; Cislo and Caira, 1993; Curran and Caira, 1995; Friggens and Brown, 2005; Alarcos *et al.* 2006; Randhawa and Burt, 2008; Twohig *et al.* 2008) and seem to be determined by: (1) adaptations of the bothridia to the mucosal topography of the spiral intestine (Williams, 1961, 1968; Rees and Williams, 1965; Carvajal and Dailey, 1975; McVicar, 1979; Borucinska and Caira, 1993; Randhawa and Burt, 2008); (2) resource availability (Williams, 1961; McVicar, 1979; Cislo and Caira, 1993; Curran and Caira, 1995); (3) physicochemical variables (McVicar, 1979); (4) scolex size (Borucinska and Caira, 1993); (5) parasite phylogenetics (Cislo and Caira, 1993; Alarcos *et al.* 2006; Randhawa and Burt, 2008); and (6) competitive interactions (Friggens and Brown, 2005). Recently, the tapeworm fauna of 4 sympatric skate species in the northwest Atlantic were described (Randhawa *et al.* 2007, 2008; Randhawa and Burt, 2008) and the attachment site of each individual worm was noted (Randhawa and Burt, 2008). Furthermore, the spatial distribution patterns for each tapeworm population was reported and their niche breadth and the overlap between each helminth species-pair quantified (Randhawa and Burt, 2008). However, specific hypotheses of intra- and inter-specific interactions within these assemblages were not tested. The objectives of this study are to assess, using general linear models (GLM), the numerical and functional responses of intra- and inter-specific interactions within each helminth infracommunity from 3 skate species: little skate *Leucoraja erinacea* (Mitchill), smooth

skate *Malacoraja senta* (Garman), and thorny skate *Amblyraja radiata* (Donovan). In addition to data presented by Randhawa and Burt (2008), I describe the distribution patterns of a nematode recovered from all 3 skates and include this nematode in my analyses and include new data on the parasites from *A. radiata*.

MATERIALS AND METHODS

Sampling

From May to August 1997 and from June 2002 to September 2004, 31 *Amblyraja radiata*, 208 *Leucoraja erinacea*, and 33 *Malacoraja senta* were collected from Passamaquoddy Bay and waters surrounding the West Isles of the Bay of Fundy, New Brunswick, Canada (see Randhawa *et al.* 2007, 2008; Randhawa and Burt, 2008 for details). Additionally, 61 *A. radiata* were collected from the North Sea in July and August 2005 by otter trawl on board the FRV Scotia. Material from the North Sea was examined fresh as described in Randhawa *et al.* (2007), the number of scoleces was used to determine the number of parasites and the site of attachment (whorl number) of each worm was noted.

Parasite distributions

Previously, tapeworm spatial distribution patterns, niche breadth and overlap between each species-pairs were assessed for each host population (Randhawa and Burt, 2008). For the purposes of this study, site of attachment and niche breadth were calculated for each infrapopulation. Site of attachment, expressed as the average for the infrapopulation, was obtained for attached worms, using the following formula: $(\sum W_i)/N$; where W_i corresponds to the attachment site (whorl number) for individual i and N corresponds to the total number of individuals in the infrapopulation. Niche breadth of each infrapopulation was calculated using Levin's niche breadth (LNB) according to the formula $1/\sum (p_i)^2$ (Simková *et al.* 2000); where p_i corresponds to the proportion of the infrapopulation exploiting whorl i . Values for LNB range from 1 to the maximum number of whorls in a species, which corresponds to niche size, with greater values representing wider niches. Unattached worms were excluded from these calculations, as their site of attachment could not be determined.

For each infracommunity the following information was collected: (1) average position of each infrapopulation; (2) niche breadth of each infrapopulation; (3) abundance of each infrapopulation; (4) abundance of all parasites; (5) host total length; (6) host sex; (7) month of collection (host); and (8) locality.

Table 1. Summary of the host distribution, prevalence, intensity of infection, average site of attachment, and niche breadth for helminths included in this study

(BF, Bay of Fundy; N, Nematoda; NS, North Sea; R, Rhinebothriidea; T, Tetracystida; Tr, Trypanorhyncha.)

Host species	Sample size	Helminth species	Prevalence	Intensity	Avg. site of attachment	Niche breadth
<i>Leucoraja erinacea</i> (little skate) 8 whorls	208 BF	^R <i>Pseudanthobothrium purtoni</i>	92.3%	13.7 ± 13.0	3.25 ± 1.00	3.38 ± 1.40
		^R <i>Echeneibothrium vernetae</i>	63.5%	11.3 ± 19.6	1.80 ± 0.92	1.73 ± 0.87
		^N <i>Pseudanisakis</i> sp.	51.0%	5.5 ± 6.7	4.22 ± 2.12	1.97 ± 1.05
<i>Malacoraja senta</i> (smooth skate) 5 whorls	33 BF	^R <i>Pseudanthobothrium hanseni</i>	48.5%	10.1 ± 17.2	1.12 ± 0.30	1.06 ± 0.17
		^T <i>Zyxibothrium kamienae</i>	48.5%	29.6 ± 60.8	1.00 ± 0.00	1.00 ± 0.00
		^N <i>Pseudanisakis</i> sp.	21.2%	1.2 ± 0.4	2.50 ± 1.76	1.00 ± 0.00
<i>Amblyraja radiata</i> (thorny skate) 9 whorls	93 (31 BF; 62 NS)	^R <i>Pseudanthobothrium hanseni</i>	90.3%	13.7 ± 14.7	3.21 ± 1.06	2.58 ± 1.22
		^R <i>Echeneibothrium dubium abyssorum</i>	16.1%	4.0 ± 5.1	3.54 ± 1.26	1.57 ± 0.88
		^N <i>Pseudanisakis</i> sp.	50.5%	4.8 ± 5.7	1.87 ± 1.47	1.42 ± 0.58
		^{Tr} <i>Grillotia</i> sp.	20.4%	3.5 ± 3.6	1.62 ± 0.87	1.21 ± 0.37

Statistics

All continuous variables were log-transformed. No difference in average position and niche breadth were attributable to sex or month of collection (results not shown), therefore these variables were removed from the set of predictors. Numerical responses were assessed using GLM with the intensity of infection (number of parasites per infected individual host) of each infrapopulation as dependent variables and abundance (mean number of parasites in all individual hosts) of each infrapopulation(s), intensity of infection of each infracommunity, and host total length as predictor variables. Functional responses were assessed using GLM with the average position and niche breadth of each infrapopulation as dependent variables and abundance of each infrapopulation (s), intensity of infection of each infracommunity, and host total length as predictor variables. Since *A. radiata* was the only skate recovered from both sides of the Atlantic, locality was included as a predictor variable in analyses including this species. Analyses were repeated for each helminth species-pair with intensity of infection of each infrapopulation, intensity of infection of both infrapopulations and host total length as predictor variables.

All possible combinations of main effects linear regression models were computed and ranked according to their corrected Akaike information criterion values (AIC_c) obtained from the residual sum of squares for each model using the method outlined by Anderson (2008). The differences in AIC_c (Δ AIC_c) and model weights (w_i) were computed to determine the relative importance and rank of each variable (see Anderson, 2008). The latter approach provides insights into the importance of each variable, taking into account the possible multicollinearity between predictor variables (Anderson, 2008). When no single model

was overwhelmingly supported, the multi-model inference approach was used (Burnham and Anderson, 2002). Model-averaged parameter estimates were obtained by weighting parameter estimates according to model probabilities (see Anderson, 2008). The unconditional variances were obtained in order to calculate a 95% confidence interval for each predictor, taking into account the sampling variance and the variance component for model selection uncertainty (Burnham and Anderson, 2002; Anderson, 2008). This approach provides an estimate of the 'slope' for each parameter, independent from others present in the model (Anderson, 2008). *A priori*, sets of potentially biologically significant second-degree interactions between predictor variables were selected and compared to models incorporating main effects included in the interaction. The evidence ratio, between the model including the interaction term and the 'best' model from each set (based on AIC_c), was used to determine whether the inclusion of the interaction term improved the model significantly.

RESULTS

The sampling site, prevalence (proportion of infected individuals in a population), average intensity of infection, average site of attachment and average niche breadth data for each species included in these analyses are presented in Table 1 and include prevalence and intensity data reported by Randhawa and Burt (2008). Additionally, several parasite infrapopulations were excluded from this study due to their low prevalence (<10%): *Grillotia* sp. recovered from the little skate and the smooth skate, an unidentified acanthocephalan from the little skate, *Echeneibothrium canadensis* Keeling and Burt, 1996 and *Phyllobothrium piriei* Williams, 1968 from the thorny skate.

Table 2. Predictor variable relative importance weights [w₊(i)], ranks, weighted model average parameter estimates, and 95% confidence interval for Niche breadth and Average position

(Parameter estimates in bold indicate those bounded away from '0'.)

Predictor variable	w ₊ (i)	Rank	Parameter estimate	Confidence interval
<i>Leucoraja erinacea</i> (little skate)				
Niche breadth <i>Pseudanthobothrium purtoni</i>				
Host total length	0.3851	2	-0.1475	-0.4568 to 0.1618
<i>P. purtoni</i> intensity	1.0000	1	3.0009	2.5497 to 3.4521
<i>E. vernetae</i> abundance	0.3668	3	0.0551	-0.0639 to 0.1740
<i>Pseudanisakis</i> sp. abundance	0.3181	4	0.0408	-0.0714 to 0.1531
Total intensity	0.3080	5	0.0360	-0.2195 to 0.2916
Niche breadth <i>Echeneibothrium vernetae</i>				
Host total length	0.5787	3	-0.0794	-0.3166 to 0.1578
<i>P. purtoni</i> abundance	0.8170	2	0.3594	0.0174 to 0.7015
<i>E. vernetae</i> intensity	1.0000	1	1.1290	0.7596 to 1.4984
<i>Pseudanisakis</i> sp. abundance	0.4154	4	-0.0255	-0.1179 to 0.0669
Total intensity	0.4064	5	-0.0191	-0.4485 to 0.4104
Niche breadth <i>Pseudanisakis</i> sp.				
Host total length	0.4648	5	-0.3090	-0.8568 to 0.2388
<i>P. purtoni</i> abundance	0.4763	4	0.1895	-0.1761 to 0.5552
<i>E. vernetae</i> abundance	0.6027	3	0.2279	-0.0394 to 0.4952
<i>Pseudanisakis</i> sp. intensity	1.0000	1	2.6525	2.2135 to 3.0916
Total intensity	0.6270	2	-0.5681	-1.5068 to 0.3706
Average position of <i>Pseudanthobothrium purtoni</i>				
Host total length	0.7723	2	0.4949	-0.0993 to 1.0892
<i>P. purtoni</i> intensity	0.5133	5	-0.2079	-0.6060 to 0.1903
<i>E. vernetae</i> abundance	0.7093	3	0.1738	-0.0485 to 0.3961
<i>Pseudanisakis</i> sp. abundance	1.0000	1	-0.4272	-0.7763 to -0.0781
Total intensity	0.5547	4	0.2920	-0.3200 to 0.9041
<i>Malacoraja senta</i> (smooth skate)				
Niche breadth <i>Pseudanthobothrium hanseni</i>				
Host total length	0.07571	5	0.0128	-0.0010 to 0.0356
<i>P. hanseni</i> intensity	0.99969	2	1.7596	1.4130 to 2.1062
<i>Z. kamienae</i> abundance	1.00000	1	0.9444	0.7913 to 1.0974
<i>Pseudanisakis</i> sp. abundance	0.99941	4	0.6817	0.4897 to 0.8738
Total intensity	0.99968	3	-1.7418	-2.1010 to -1.3826
<i>Amblyraja radiata</i> (thorny skate)				
Niche breadth <i>Pseudanthobothrium hanseni</i>				
Host total length	0.2700	6	0.1158	-0.3570 to 0.5886
Locality	0.2628	7	0.0156	-0.0489 to 0.0801
<i>P. hanseni</i> intensity	1.0000	1	2.4245	1.1583 to 3.6906
<i>E. dubium abyssorum</i> abund.	0.2852	4	-0.0866	-0.3693 to 0.1961
<i>Grillotia</i> sp. abundance	0.5130	3	0.3653	-0.2214 to 0.9519
<i>Pseudanisakis</i> sp. abundance	0.2799	5	0.0136	-0.1743 to 0.2016
Total intensity	0.5273	2	-0.5805	-1.6211 to 0.4602
Niche breadth <i>Echeneibothrium dubium abyssorum</i>				
Host total length	0.3393	3	0.5379	-0.7807 to 1.8565
<i>P. hanseni</i> abundance	0.0885	6	0.0005	-0.0522 to 0.0532
<i>E. dubium abyssorum</i> int.	1.0000	1	1.9350	1.1917 to 2.6783
<i>Grillotia</i> sp. abundance	0.5866	2	0.4927	-0.0292 to 1.0146
<i>Pseudanisakis</i> sp. abundance	0.0986	4	-0.0185	-0.0896 to 0.0526
Total intensity	0.0916	5	0.0046	-0.0952 to 0.1044
Average position of <i>Pseudanthobothrium hanseni</i>				
Host total length	0.6265	3	0.9512	-0.4850 to 2.3875
Locality	1.0000	1	-0.4630	-0.7101 to -0.2158
<i>P. hanseni</i> intensity	0.6258	5	0.8360	-0.6142 to 2.2862
<i>E. dubium abyssorum</i> abund.	0.3000	7	0.1177	-0.2447 to 0.4802
<i>Grillotia</i> sp. abundance	0.7011	2	0.7021	-0.1337 to 1.5378
<i>Pseudanisakis</i> sp. abundance	0.3358	6	0.0442	-0.2362 to 0.3245
Total intensity	0.6263	4	-0.9461	-2.7079 to 0.8158
Average position of <i>Echeneibothrium dubium abyssorum</i>				
Host total length	1.0000	1	4.6143	1.1127 to 8.1159

Table 2. (Cont.)

Predictor variable	$w_+(i)$	Rank	Parameter estimate	Confidence interval
<i>P. hansenii</i> abundance	0.1829	3	-0.0898	-0.3646 to 0.1850
<i>E. dubium abyssorum</i> int.	0.2482	2	-0.2153	-0.8021 to 0.3716
<i>Grillotia</i> sp. abundance	0.1362	5	0.0221	-0.2148 to 0.2589
<i>Pseudanisakis</i> sp. abundance	0.1327	6	0.0342	-0.1519 to 0.2203
Total intensity	0.1433	4	0.0108	-0.3768 to 0.3985
Average position of <i>Pseudanisakis</i> sp.				
Host total length	0.4540	6	1.0677	-1.9146 to 4.0501
Locality	0.5081	5	0.2185	-0.0597 to 0.4967
<i>P. hansenii</i> abundance	0.8701	2	1.6082	-0.1510 to 3.3674
<i>E. dubium abyssorum</i> abund.	0.8090	3	2.2448	-0.1593 to 4.6489
<i>Grillotia</i> sp. abundance	1.0000	1	3.7133	1.1839 to 6.2427
<i>Pseudanisakis</i> sp. intensity	0.3774	7	-0.2627	-0.9616 to 0.4363
Total intensity	0.7082	4	-1.5062	-4.1270 to 1.1146

With the exception of *P. hansenii* niche breadth in *M. senta*, no results yielded a well-supported model, and as such, a model-averaging approach was conducted for all other analyses (summarized in Table 2). Furthermore, in all GLM analyses, no main effects models were improved significantly by the inclusion of their respective interaction terms (results not shown), no numerical responses were detected (results not shown), and no functional responses were identified in double or triple infections (results not shown).

Leucoraja erinacea

This skate species was infected with 2 cestodes (*Pseudanthobothrium purtoni* Randhawa, Saunders, Scott and Burt, 2008 and *Echeneibothrium vernetae* Euzet, 1956) and 1 nematode (*Pseudanisakis* sp.) present in sufficient numbers to be included in subsequent analyses (Table 1). The GLM analyses included 192, 133 and 106 hosts infected with *P. purtoni*, *E. vernetae*, and *Pseudanisakis* sp., respectively (Table 1). Results indicate a niche extension of *P. purtoni*, *E. vernetae* and *Pseudanisakis* sp. in the presence of increasing numbers of conspecifics (Fig. 1a, b and Table 2). Furthermore, evidence for niche extension of *E. vernetae* in the presence of increasing numbers of *P. purtoni* was also observed (Fig. 1c), albeit a weaker predictor than the presence of conspecifics (Table 2). Lastly, the niche of *P. purtoni* in the presence of the nematode shifted anteriorly (Fig. 2 and Table 2). No evidence for niche shift was observed for other helminths infecting *L. erinacea*.

Malacoraja senta

This skate species was infected with 2 cestodes (*P. hansenii* Baer, 1956 and *Zyxibothrium kamienae* Hayden and Campbell, 1981) and 1 nematode (*Pseudanisakis* sp.) present in sufficient numbers to be included in subsequent analyses (Table 1). No GLM analyses were performed on the average site of

attachment and niche breadth for *Z. kamienae* and *Pseudanisakis* sp. since both species were confined solely to the anterior-most whorl. The GLM analyses included 15 hosts infected with *P. hansenii*. In descending order of relative effect size, the numbers of *P. hansenii* per infrapopulation, total number of helminths per infracommunity, numbers of *Z. kamienae* per infrapopulation, and numbers of *Pseudanisakis* sp. per infrapopulation had 95% confidence intervals bound away from '0' (Table 2), an indication of niche extension of *P. hansenii* in the presence of increasing numbers of conspecifics and of other helminths. Furthermore, there was no evidence that the average site of attachment for *P. hansenii* is affected by the presence of other helminths.

Amblyraja radiata

This skate species was infected with 3 cestodes (*P. hansenii*, *E. dubium abyssorum* Campbell, 1977 and *Grillotia* sp.) and 1 nematode (*Pseudanisakis* sp.) present in sufficient numbers to be included in subsequent analyses (Table 1). The GLM analyses included 84, 15, 19, and 47 hosts infected with *P. hansenii*, *E. dubium abyssorum*, *Grillotia* sp., and *Pseudanisakis* sp., respectively (Table 1). Results indicate a niche expansion only in *P. hansenii* and *E. dubium abyssorum* in the presence of increasing numbers of conspecifics (Fig. 3 and Table 2). Niche shifts were observed for *P. hansenii*, *E. dubium abyssorum*, and *Pseudanisakis* sp. and attributable to locality, host total length (Fig. 4), and abundance of *Grillotia* sp., respectively (Table 2). Therefore, *Pseudanisakis* sp. is the only species whose niche shifted in the presence of another helminth in the infrapopulation.

DISCUSSION

Evidence presented herein demonstrates that intra- and inter-specific competition is common in helminth infracommunities of the skate species included

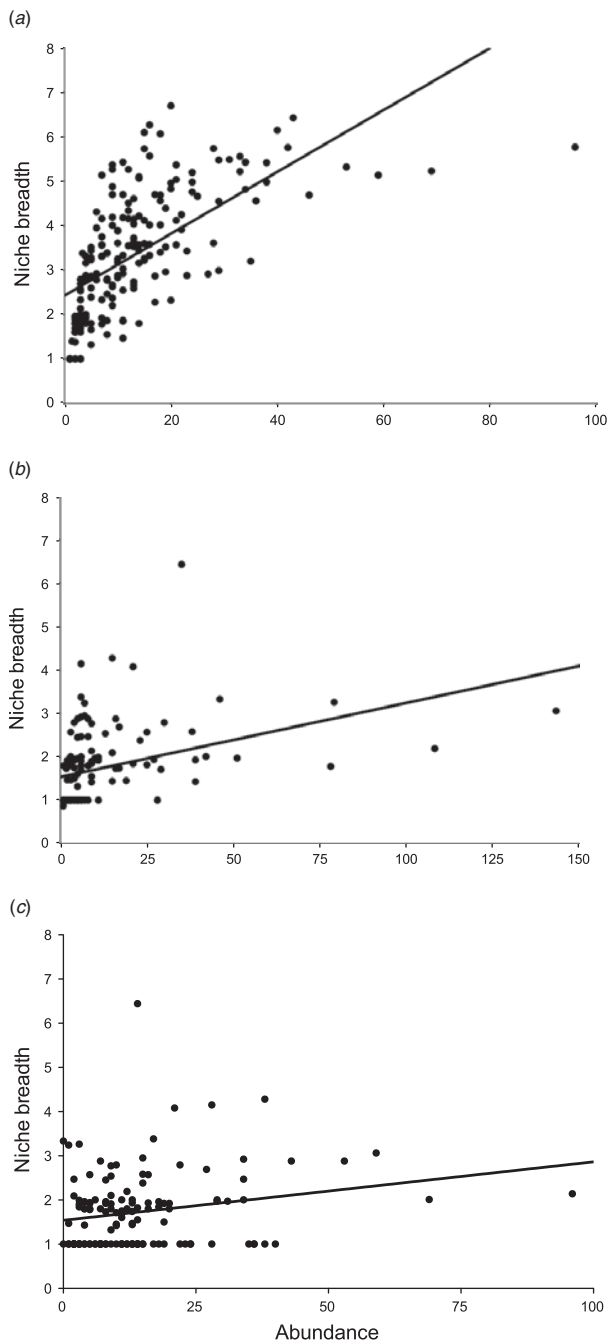


Fig. 1. Relationships between niche breadth and the abundance of helminths in the little skate (*Leucoraja erinacea*): (a) *Pseudanthobothrium purtoni*, (b) *Echeneibothrium vernetae* (intensity of infection for *E. vernetae*), and (c) *E. vernetae* (abundance of *P. purtoni*). The lines represent the best fit from simple linear regressions: (a) $r=0.6461$, $P < 0.0001$; (b) $r=0.3859$, $P < 0.0001$; and (c) $r=0.2137$, $P=0.0135$.

in this study. In fact, despite the lack of numerical responses, functional responses to intra- and inter-specific interactions were observed in helminth communities of all 3, and 2, skate species examined, respectively. No interactive site segregation was observed in the helminth community of the smooth skate and thus was not considered a universal phenomenon. Niche shifts in the tapeworm

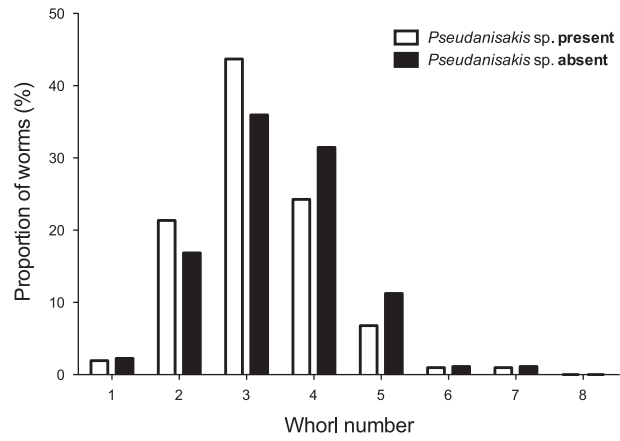


Fig. 2. Frequency distribution of the average position (whorl number) of *Pseudanthobothrium purtoni* infecting the little skate (*Leucoraja erinacea*) in the presence (open bars) or absence (full bars) of the nematode *Pseudanisakis* sp., expressed as the proportion (percentage) of the infrapopulations occupying each whorl. The preferred site of attachment of *Pseudanisakis* sp. is whorl 4.

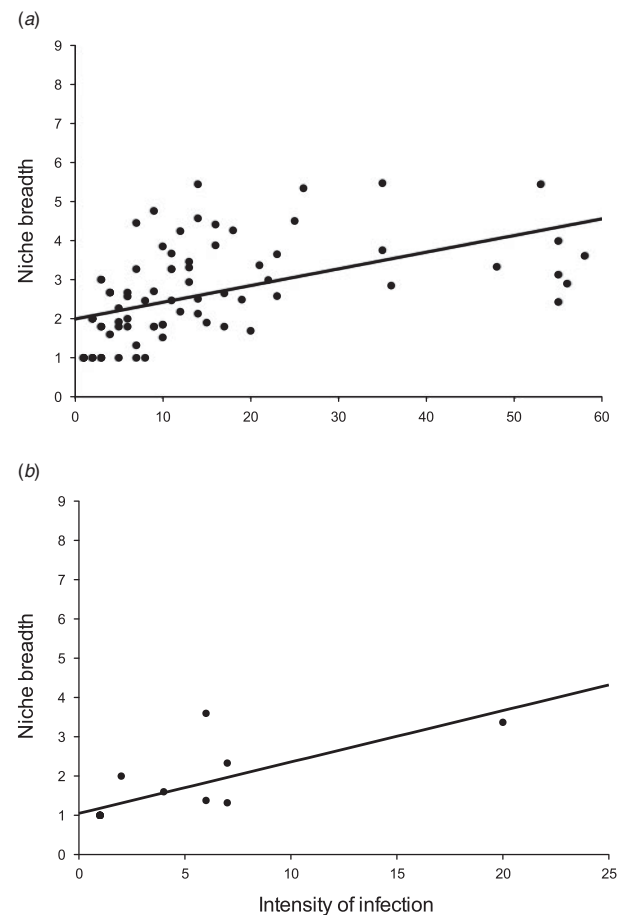


Fig. 3. Relationships between the intensity of infection of helminths and their niche breadth in the thorny skate (*Amblyraja radiata*): (a) *Pseudanthobothrium hanseni* and (b) *Echeneibothrium dubium abyssorum*. The lines represent the best fit from simple linear regressions: (a) $r=0.5148$, $P < 0.0001$ and (b) $r=0.7524$, $P=0.0012$.

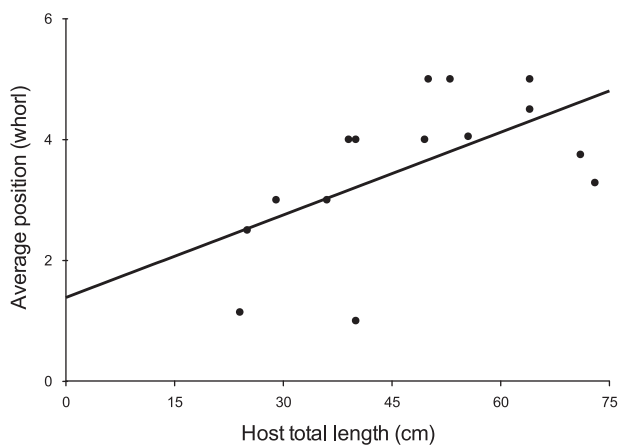


Fig. 4. Relationship between the average position (whorl number) of *Echeneibothrium dubium abyssorum* infrapopulations in the thorny skate (*Amblyraja radiata*) and host total length (cm). The line represents the best fit from a simple linear regression: $r=0.5782$, $P=0.0240$.

Pseudanthobothrium purtoni and the nematode *Pseudanisakis* sp. occurred in both the little skate and the thorny skate, respectively, in response to the presence of a competing species. However, interactions between the different helminths infecting the smooth skate provided no evidence for niche shifts. Randhawa and Burt (2008) showed that of the 5 whorls in the spiral intestine of the smooth skate, the posterior 3 were devoid of *P. hansenii* due to the incompatibility of intestinal villi and parasite bothridia. Furthermore, the compatibility between villi and bothridia in the second whorl was only possible between worms with the largest bothridia attaching to the smallest villi (Randhawa and Burt, 2008). As such, any niche shift caused by the presence of *Z. kamiense* or *Pseudanisakis* sp. may result in interactive exclusion, where inter-specific interactions leading to a shift in realised niche may cause the exclusion of a species from the infracommunity (Holmes, 1973; Poulin, 2001).

A positive correlation between the number of individuals in an infrapopulation and its niche breadth (functional response) was observed for *Pseudanthobothrium* spp. and *Echeneibothrium* spp. in all their respective hosts and for *Pseudanisakis* sp. in the little skate. This is consistent with most other studies of helminth communities (e.g., Bush and Holmes, 1986; Alarcos *et al.* 2006). However, regardless of the abundance of *P. purtoni* in the little skate, the niche breadth never exceeded 7 out of 8 whorls. This is likely due to the posterior whorl of the spiral valve generally being devoid of worms; an observation consistent with other studies on the attachment site of tapeworms in skates (Williams, 1961; Carvajal and Dailey, 1975; McVicar, 1979). Additionally, tapeworms and nematodes are trophically transmitted, thus it is expected that these will accumulate over time and that larger (older) hosts will harbour higher intensities of infection

occupying wider niches than smaller (younger) host individuals. Positive correlations between host size and parasite burden have been observed in some helminths infecting elasmobranchs (Tanzola *et al.* 1998; Sanmartin *et al.* 2000; Friggens and Brown, 2005), but not in others (Cislo and Caira, 1993; Curran and Caira, 1995; Alarcos *et al.* 2006). In this study, no evidence was found of host size affecting parasite burden (results not shown) or niche breadth. Contrary to the null model analysis approach used by Friggens and Brown (2005), the model-averaging method used in this study takes into account the possible multi-collinearity between predictors and may explain why host length seems to have negligible effects on the response variables under scrutiny.

There is other evidence for intra-specific competition, which cannot be assessed with the data in hand. For instance, crowding effect, a numerical response, is described as a negative correlation between intensity of infection and the size of the worms (Read, 1951). Although the exact mechanisms remain enigmatic, causes may be exploitative competition, interference competition, and/or host immune response (Andreassen *et al.* 1999; Roberts, 2000; Bush and Lotz, 2000). A reduction in size, influenced by density-dependent mechanisms such as crowding, has also been associated with decreased fecundity (Szalai and Dick, 1989; Irvine *et al.* 2001; Richards and Lewis, 2001), but so has competition (Moqbel and Wakelin, 1979; Silver *et al.* 1980; Holland, 1984). The outcome of competition can also be affected by the order of establishment (Poulin, 2007), although this can only be assessed in experimental infections. Although numerical responses were not observed on the scale examined, it is possible that a relationship exists between worm burden, worm size and individual fecundity at the level of infra-populations, but these data are not available. Biomass may have been a more appropriate measure than intensity of infection to uncover underlying patterns of competition. Parasite volume, a good correlate of biomass, has been used as an indicator of competition (Poulin *et al.* 2003; Rauque and Semenas, 2011) and may be a more representative measurement of biomass than worm length. However, in tapeworms, width generally increases posteriorly (immature proglottides are narrower than mature or gravid ones) and thickness measurements are rarely reported (Randhawa and Poulin, 2009), thus parasite volume would have been difficult to estimate accurately. Nonetheless, in this study, the range in lengths for all species excepting *Grillotia* sp., overlapped and their sizes were similar (Randhawa, 2000); therefore it is plausible that *Grillotia* sp. can outcompete other helminths in skates due to the size differential. This trypanorhynch caused the nematode *Pseudanisakis* sp. to shift distributions in the thorny skate, yet the nematode influenced the tapeworm *P. purtoni* to shift niches in

the little skate, albeit the effect of size is relatively small in the latter. Although further sampling is necessary, it may be that trypanorhynch are the strongest competitors in rajid skates, followed by nematodes and other cestodes, respectively.

Differences in functional responses, or average site of attachment, in *P. hansenii* and *E. dubium abyssorum* are attributable to locality and host total length, respectively. In the case of *P. hansenii*, differences in average site of attachment of infrapopulations may be attributable to differences in helminth communities. *Amblyraja radiata* from the Bay of Fundy is infected with *E. dubium abyssorum*, a tapeworm absent from populations in the North Sea. Conversely, *P. piriei* is a tapeworm only recovered from the North Sea population. Both populations are also infected with *E. canadensis*, although this tapeworm is more prevalent in the North Sea population. The latter 2 parasite populations were not abundant enough to include in these analyses, but *E. canadensis* is a tapeworm whose preferred site of attachment is the anterior half of the spiral intestine (Keeling and Burt, 1996; observations herein), whereas *P. piriei* attaches preferentially in the posterior half of the spiral intestine (Williams, 1968; observations herein). It is possible that the presence of *E. canadensis* may cause a posterior niche shift in *P. hansenii*. A larger sample size of individuals infected with this species may allow us to address this question using the approach described herein. *Echeneibothrium dubium abyssorum* is generally found in larger individuals. Although no elasmobranch tapeworm life cycle has been described to date, we can assume that *E. dubium abyssorum* is acquired following an ontogenetic shift in diet, possibly occurring at approximately 20–25 cm in total length. Furthermore, the average position of this tapeworm is positively correlated with size of its host. From these data, it can be inferred that *P. hansenii* may establish first and that with increasing numbers of *P. hansenii* over time (age and/or size) being correlated with niche breadth, it may out-compete *E. dubium abyssorum* and push it further back.

Putting these results in the context of helminth interactions in elasmobranch fishes, an interesting pattern emerges: evidence for competition is only apparent in batoids, not in sharks. Three earlier studies have examined the attachment sites of helminths in sharks and found no evidence of interaction or competition between species (Cislo and Cairns, 1993; Curran and Cairns, 1995; Alarcos *et al.* 2006), whereas 2 previous studies of helminths in batoids have (McVicar, 1979; Friggens and Brown, 2005). Twohig *et al.* (2008) also suggested competition as an explanation for differences in attachment sites for 2 helminth species in the whipray *Himantura walga* (Müller and Henle), but did so cautiously in the absence of data on other helminth species comprising the infracommunity. Sharks are

generally larger than batoids, but correcting for host body size, there are no differences in tapeworm size between those infecting sharks and batoids, respectively (Randhawa and Poulin, 2009). Although not enough data are available on the relationship between elasmobranch length or weight and size of their spiral intestine, it is likely to be strongly positive (Randhawa *personal observations*). However, assuming that the length and width of the spiral intestine are proportional to body size, spiral valve volume would have a greater scaling exponent than either length or width. Therefore, sharks likely provide much larger habitats to helminths than batoids and this may explain why no competitive interactions have been observed between helminths of sharks. However, this hypothesis needs to be tested by including both linear and 3-dimensional size measurements for spiral intestines, relative to fish length, in future analyses when and if these data become available.

In summary, this study suggests that the spatial distribution of helminths in skates is not random and is determined by the functional responses stemming from both the intra- and inter-specific interactions between parasites. Further work is needed to understand the role played by these interactions in the evolutionary processes shaping the adaptations of helminths to specific niches.

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