Intra- and interspecific density-dependent effects on growth in helminth parasites of the cormorant, *Phalacrocorax carbo sinensis*

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SUMMARY

The action of intra- and interspecific competition, mediated by density-dependent effects on growth, was investigated among the 3 helminth species found in the alimentary tract of 104 cormorants, *Phalacrocorax carbo sinensis*. Intraspecific density-dependent effects on worm sizes were observed in the abundant nematode *Contracaecum rudolphii*, as shown by a negative correlation between mean worm size and intensity of infection. Higher intensities of infection by *C. rudolphii* were also associated with more variable worm sizes in the nematode *Syncuaria squamata*, suggesting a one-sided and density-dependent interspecific effect. There was also clear evidence of some form of negative interaction between the nematode *S. squamata* and the acanthocephalan *Southwellina hispida* from two fronts. First, there was a strong negative correlation between the intensities of infection of the 2 species across hosts. Second, sizes of worms of 1 species became more variable as the number of worms of the other species per host increased, and vice versa. This interspecific density-dependent effect on growth was thus apparently symmetrical. We also found evidence that worm size is a predictor of egg output in the 3 helminth species, indicating that intra- and interspecific density-dependent effects on growth can affect population dynamics in these worms. These results illustrate the complex nature of density dependence in helminth growth, and how its effects can act both within and among species.

Key words: competition, egg output, *Contracaecum rudolphii*, size dimorphism, *Southwellina hispida*, *Syncuaria squamata*, variability.

INTRODUCTION

There is much evidence for density-dependent regulation of gastrointestinal helminth populations (Keymer, 1982; Shostak & Scott, 1993). In nematodes, for instance, increases in worm burden are often associated with reductions in mean worm length or mean egg output per worm (e.g. Szalai & Dick, 1989; Irvine *et al*. 2001; Richards & Lewis, 2001), although there are exceptions (see Marcogliese, 1997). Density-dependent reductions in worm length, even if not accompanied by evidence of fecundity reduction, are likely to impact on the regulation of nematode populations because of the strong relationship between body size and egg output in practically all nematodes (e.g. Mössinger & Wenk, 1986; Szalai & Dick, 1989; Sinniah & Subramaniam, 1991; Skorping, Read & Keymer, 1991; Marcogliese, 1997; Irvine *et al*. 2001; Richards & Lewis, 2001).

Beyond their intraspecific effects, density-dependent processes can have interspecific influences, i.e. the number of worms of one species can have impacts on the growth or fecundity of worms of another species sharing the same host. Studies of interspecific interactions between parasitic helminths have often focused on functional responses, such as niche shifts, by one species when co-occurring with another species (see Poulin, 1998). The strongest evidence for competitive or other interactions, however, comes from numerical responses, i.e. changes in the numbers, growth or fecundity of one species when co-occurring with a second species (Thomson, 1980). These have also been demonstrated for pairs of helminth species in concomitant infections (e.g. Moqbel & Wakelin, 1979; Silver, Dick & Welsh, 1980; Dash, 1981; Holland, 1984). From the perspective of interspecific density dependence, however, many earlier studies failed to deliver convincing evidence for at least 3 reasons. First, they often examined how the presence of one species, at a given intensity (= density) of infection, affected a second species; there was no test of the effects of a range of intensities of infection. Second, the experimental infection procedures used in many studies result in intensities of infection much higher than those normally observed in nature. Third, most earlier studies focused on simple 2-species laboratory systems, whereas natural helminth communities usually consist of more than 2 coexisting species.

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Here, we had an opportunity to examine density-dependent effects on helminth growth in a natural system. The River Po delta (a mosaic of estuarine wetlands covering about 38000 ha) is a major area of traditional forms of aquaculture in Italy (Ardizzone, Cataudella & Rossi, 1988). The extensive fish production in the area is exploited by large numbers of cormorants *Phalacrocorax carbo sinensis* Blumenbach, 1798. The birds acquire helminths when feeding on farmed fish, which act as paratenic hosts for the nematodes *Contracaecum rudolphii* Hartwich, 1964 and *Syncuaria squamata* Linstow, 1883 (see Moravec, 1994), as well as for the acanthocephalan *Southwellina hispida* Van Cleave, 1925 (see Schmidt, 1985). Production losses incurred from bird predation have led to the implementation of control programmes aimed at reducing the cormorant population (Volponi, 1997, 1999). Birds culled as part of these control measures provided material for our study on density-dependent effects in helminth growth.

Our objectives were to examine both intra- and interspecific density-dependent effects on worm sizes among the 3 helminth species found in cormorants. By using multivariate methods to exclude the influences of other variables, we show evidence of both intra- and interspecific competition among these helminth species. We also verify the existence of a positive relationship between size and fecundity for the 3 helminth species in our study. To our knowledge, our investigation is the first to address these questions in parasites of bird hosts.

MATERIALS AND METHODS

Cormorants were captured on several occasions during the wintering season, between January and March 2001, in the northern part of the River Po delta. Intensity of infection and percentage of gravid female worms may be higher during summer for the helminth species studied here (e.g. *C. rudolphii*, see Torres *et al*. 2000); however, sampling at other times was not permitted. Cormorants were shot under authorization as part of the programme aimed at controlling their numbers. All birds were stored in freezers at −18 °C immediately after capture. Bird age could not be determined beyond a simple categorization as immature or adult. Tarsus length was obtained for each bird, using callipers, and served as a measure of skeletal size. Fresh body mass was also determined for each bird, including dry plumage, using an electronic balance (to the nearest 2 g); these measures were later corrected for the mass of fresh fish in the oesophagus and food in the stomach. Bird sex was determined during dissection, from an internal examination of reproductive organs. The whole alimentary tract of each bird was dissected and searched for helminths, with parasites from each bird stored separately in 70 % ethanol.

Measurements of worm lengths were obtained using a stereomicroscope with an eyepiece micrometer. All acanthocephalans recovered were identified, sexed and measured (total length, mm). All individuals of the least abundant nematode species found, *S. squamata*, were also sexed and measured. Because the other nematode species, *C. rudolphii*, was very abundant, we counted all worms but only sexed and measured a random sample of 15 worms per host, unless the host harboured fewer than 15 worms, in which case all were measured. Two aspects of worm sizes were examined in analyses of the determinant of worm growth. First, we used the mean length of conspecific worms inside the same host as a measure of the average size attained by the worms in that host. Second, we used the coefficient of variation in worm length, i.e. the standard deviation expressed as a percentage of the mean length, as an estimate of the variability in worm growth among conspecific worms in the same host. For *C. rudolphii*, this estimate of variability was based only on a sample of each infrapopulation, and not on measurements of all worms as in the other two species; since the samples were taken at random, however, we feel that they provide representative estimates.

We also counted the number of uterine eggs in samples of female worms of each species, to confirm the expected positive relationship between egg production and worm size. The number of eggs *in utero* is not a measure of life-time fecundity, but it indicates the rate at which a given worm produces eggs. Five hosts infected with all 3 species of heminths were chosen at random, and 10 gravid females (or fewer if 10 were not found) of each parasite species were chosen at random from each bird. Nematodes were dissected with fine forceps, in a saline solution, under a stereomicroscope. The ovary and uterus were isolated from the rest of each nematode's body, sonicated in a saline solution, and the eggs in each female were counted, using a counting chamber, under the microscope. Acanthocephalan females were also dissected with fine forceps and the eggs within their pseudocoel were counted.

Data on intensity of infection, i.e. numbers of worms of each species per host, were log-transformed (or log $(x+1)$-transformed when data included zeros) for use in parametric statistical tests. In the main analyses of the determinants of worm sizes, multiple regressions were used to simultaneously evaluate the influences of several variables on the coefficient of variation in length and mean worm length per host, across all hosts harbouring a given helminth species. The variables examined were the number of worms of each species per host, host age, host tarsus length, host mass, and host sex; host age and host sex were coded as binary variables. Relationships between worm length and number of uterine eggs per worm were assessed on log-trans-

Table 1. Infection parameters and comparisons of the sizes of male and female worms, for each of the 3 helminth species found in 104 cormorants

Species	No. birds infected (%)	Mean intensity (range)	Mean female length (mm ± S.D.)	Mean male length (mm ± S.D.)	*P**
Nematodes					
Contracaecum rudolphii	104 (100·0)	108·0 (7–363)	27·0 ± 9·4	20·6 ± 5·3	0·0001
Syncuaria squamata	21 (20·2)	2·3 (1–10)	10·5 ± 4·7	12·2 ± 3·8	0·2818
Acanthocephalans					
Southwellina hispida	41 (39·4)	21·9 (3–49)	14·4 ± 0·8	11·5 ± 0·4	0·0001

* Two-tailed *t*-tests.

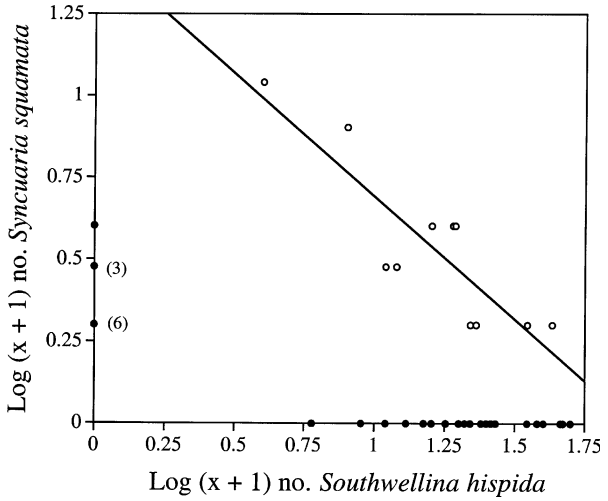

Fig. 1. Relationship between the numbers of the nematode *Syncuaria squamata* and the acanthocephalan *Southwellina hispida* across the 11 cormorants that harboured both species (○). A linear regression between the two variables is also shown ($y = -0{\cdot}751x + 1{\cdot}443$). Data for the 40 birds harbouring only 1 of the 2 species are also plotted (●); numbers in parentheses indicate stacked symbols.

formed data, using a multiple regression in which host identity (coded 1 to 5) was also included as an independent variable. This was necessary since worm numbers varied among birds and there may be direct density-dependent effects on egg production in addition to those related to worm size.

RESULTS

A total of 104 cormorants (50 males, 54 females) was obtained from the River Po delta and examined for helminths. The birds averaged 2219 g in body mass (range 1534–3064 g). Only 3 species of gastrointestinal helminths, i.e. 2 nematodes from the stomach and 1 acanthocephalan from the intestine, were found during the dissections (Table 1). No significant correlations were observed between the intensity of infection by any of these helminths and host body mass (all $P > 0{\cdot}59$) or host tarsus length (all $P > 0{\cdot}48$), and there were no differences in intensity of infection by any of the helminths

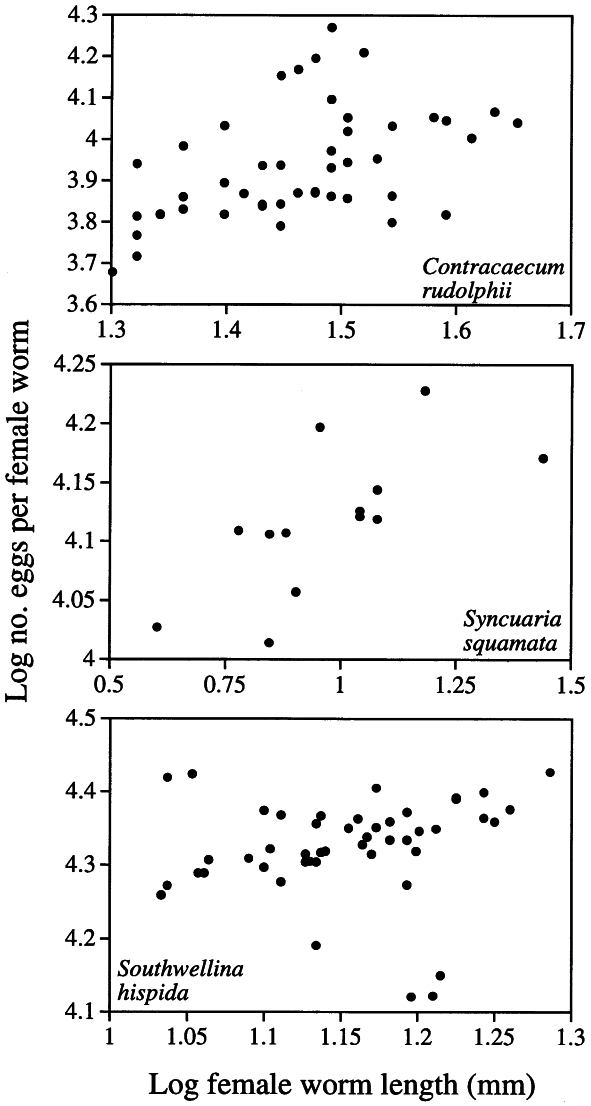

Fig. 2. Plots of numbers of eggs per female worm against worm length, for all 3 helminth species found in cormorants. Note the 3 outliers in the lower right corner of the bottom graph, which are discussed in the text.

between male and female hosts (two-tailed *t*-tests, all $P > 0{\cdot}55$) or between immature and adult hosts (all $P > 0{\cdot}18$).

Using only birds infected with at least 1 of 2 helminth species in a pair, we tested for pairwise associations among helminth species. The number of

Table 2. Effects of several variables on both the mean length and coefficient of variation in length of 3 species of helminth parasites in cormorants

(Values are partial regression coefficients from multiple regressions including infected hosts only; sample size for regressions involving coefficients of variation include fewer hosts if some hosts harboured only 1 worm.)

Variable	Mean worm length	Coefficient of variation in worm length
Contracaecum rudolphii (both $N = 104$ hosts)		
Log no. *C. rudolphii* per host	−0·424***	0·185*
% male *C. rudolphii* in host sample	−0·320***	−0·042
Log no. *S. squamata* per host	−0·077	0·134
Log no. *S. hispida* per host	0·089	−0·110
Host mass	−0·131	0·007
Host tarsus length	−0·082	−0·121
Host sex	−0·182	−0·090
Host age	0·208**	−0·077
Syncuaria squamata ($N = 21$ and 11 hosts)		
Log no. *C. rudolphii* per host	−0·390	0·636**
Log no. *S. squamata* per host	−0·313	−0·338
Log no. *S. hispida* per host	0·263	0·734**
Host mass	0·366	−0·273
Host tarsus length	−0·414	0·736**
Host sex	−0·192	−0·179
Host age	−0·322	0·521*
Southwellina hispida, females (both $N = 41$ hosts)		
Log no. *C. rudolphii* per host	0·125	0·138
Log no. *S. squamata* per host	−0·314*	0·420**
Log no. *S. hispida* per host	−0·267	−0·124
Host mass	0·078	−0·058
Host tarsus length	−0·276	−0·065
Host sex	−0·071	0·163
Host age	−0·273	−0·019
Southwellina hispida, males ($N = 41$ and 39 hosts)		
Log no. *C. rudolphii* per host	0·131	−0·248
Log no. *S. squamata* per host	0·030	0·133
Log no. *S. hispida* per host	0·097	0·278
Host mass	−0·324	0·216
Host tarsus length	0·494**	−0·025
Host sex	0·229	0·025
Host age	0·030	0·114

*$P < 0·10$; **$P < 0·05$; ***$P < 0·001$.

Contracaecum rudolphii per bird did not correlate significantly with either numbers of *Syncuaria squamata* (product-moment correlation coefficient, $r = 0·111$, $N = 104$, $P = 0·262$) or with numbers of *Southwellina hispida* ($r = -0·040$, $N = 104$, $P = 0·689$). However, there was a clear negative relationship between numbers of *S. squamata* and numbers of *S. hispida* ($r = -0·489$, $N = 51$, $P = 0·0003$); the negative correlation is also very clear when only the birds harbouring both parasite species are examined ($r = -0·869$, $N = 11$, $P = 0·0005$). As a rule, birds harbouring large numbers of *S. hispida* harboured either few or no *S. squamata* (Fig. 1).

The relationship between female worm size and number of eggs *in utero* was positive and significant for both nematode species (*C. rudolphii*: partial regression coefficient, $r = 0·458$, $N = 50$, $P = 0·0001$; *S. squamata*: $r = 0·726$, $N = 13$, $P = 0·0016$). In both species, the identity of the host from which the worms came also affected the number of eggs per worm (both $P < 0·01$). In the acanthocephalan *S. hispida*, there was no relationship between worm size and egg numbers ($r = 0·071$, $N = 47$, $P = 0·649$), and host identity also had no effect on egg production. However, the lack of a relationship between worm size and egg numbers in *S. hispida* was due to 3 clear outliers (see Fig. 2), all from the same bird; once these points are removed from the analysis, the effect of worm length becomes apparent ($r = 0·444$, $N = 44$, $P = 0·005$). Thus, egg output generally increases with female worm size in both nematode species, and most likely also in the acanthocephalan (Fig. 2).

Size of Contracaecum rudolphii

The nematode *C. rudolphii* was the most abundant helminth (Table 1); a total of 11 219 worms of this species were recovered. Female *C. rudolphii* were significantly longer than males (Table 1), and thus

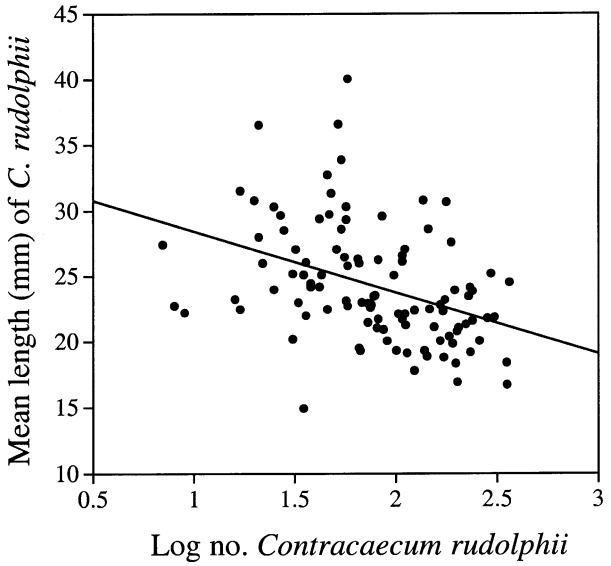

Fig. 3. Relationship between the mean length of the nematode *Contracaecum rudolphii* per host and its intensity of infection, across 104 infected cormorants. A linear regression between the two variables is also shown ($y = -4{\cdot}678x + 33{\cdot}121$).

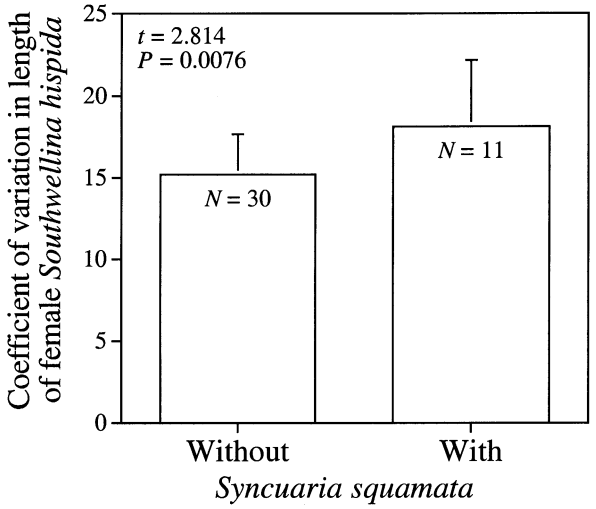

Fig. 4. Mean (±S.D.) value of the coefficient of variation in the length of females of the acanthocephalan *Southwellina hispida*, as a function of whether or not the host also harboured the nematode *Syncuaria squamata*. Sample sizes and the result of a two-tailed *t*-test are also shown.

the proportion of male worms in each sample taken from each host for body length measurements had to be included in analyses of the determinants of worm sizes. In addition to this variable, the intensity of infection by *C. rudolphii* and host age were the only variables significantly related with mean length of *C. rudolphii* per host (Table 2). Worm length was on average slightly greater in adult than in immature birds; also, as intensity of infection increased, the mean length of worms decreased (Fig. 3), suggesting a density-dependent effect. There was a hint that numbers of *C. rudolphii* per host also tended to be associated with an increase in the variation in worm length, but this trend was not quite significant (Table 2). Numbers of worms of the other species and sex, tarsus length or mass of the host had no apparent effect on either of the two measures of length in *C. rudolphii*.

Size of Syncuaria squamata

The nematode *S. squamata* was the least abundant helminth in the sample of cormorants (Table 1), with only 49 specimens found in the birds. There was no difference between the mean length of male and female worms (Table 1). None of the variables investigated showed any correlation with the mean length of *S. squamata* per host (Table 2). The coefficient of variation in *S. squamata* length could only be computed for worms in 11 hosts, because other infected hosts each harboured a single worm. Despite low sample size limiting the power of the regression analysis, several variables appeared to influence the variation in worm length per host: high infection intensities by both *C. rudolphii* and *S. hispida*, and longer host tarsus length were associated with higher variation in the sizes of *S. squamata* (Table 2).

Size of Southwellina hispida

In the acanthocephalan *S. hispida*, female worms were significantly larger than males (Table 1). Females also occurred in higher numbers per host than males (paired, two-tailed *t*-test, $t = 3{\cdot}014$, D.F. $= 40$, $P = 0{\cdot}0045$). Because all worms (897 in total) of this species were sexed and measured, we treated males and females separately. We found that intensity of infection by the nematode *S. squamata* correlated negatively, though not quite significantly, with the mean length of female *S. hispida* (Table 2). This suggests the potential action of interspecific density dependence. In addition, the only factor associated with within-host variation in the length of female *S. hispida* was the intensity of infection by *S. squamata* (Table 2): as the intensity of infection with *S. squamata* increases, the variability in sizes of female *S. hispida* also increases. In fact, the mere presence of the nematode *S. squamata* in a bird host is associated with higher variability in the length of female acanthocephalans, with the coefficient of variation in length of female *S. hispida* being higher in birds also harbouring the nematode *S. squamata* (Fig. 4). These effects were not observed for male *S. hispida*, although average male sizes increased with host tarsus length (Table 2).

Other intraspecific density-dependent effects are also apparent in infrapopulations of the acanthocephalan *S. hispida*. The female-to-male sex ratio of these worms correlated negatively with the number of *S. hispida* per host ($r = -0{\cdot}320$, $N = 41$, $P = 0{\cdot}0414$) i.e. as intensity of infection increases, the

number of males increases relative to the number of females in a host. This does not appear to simply reflect the age of infections, since there was no difference in worm sex ratio between immature and adult hosts (two-tailed t-test, $t = 0{\cdot}700$, D.F. $= 39$, $P = 0{\cdot}488$). At the same time, the female-to-male sex ratio covaries with sexual size dimorphism: as the sex ratio becomes more female-biased, the female-to-male body length ratio increases ($r = 0{\cdot}335$, $N = 41$, $P = 0{\cdot}0321$). Therefore, high intensities of infection with the acanthocephalan *S. hispida* are generally characterized by relatively more and relatively larger male worms than found in bird hosts infected with few worms.

DISCUSSION

Density-dependent reductions in growth or fecundity in gastrointestinal helminths have been reported in several studies (e.g. Shostak & Scott, 1993; Irvine *et al*. 2001; Richards & Lewis, 2001). Here, the sizes of all 3 helminth species studied proved good predictors of their egg output, as female worm size correlated positively with the number of eggs *in utero* in these species. We observed intraspecific density dependence in the nematode *C. rudolphii*, as measured by worm size. More importantly, we found relatively strong evidence of competitive (or at least negative) interactions between the nematode *S. squamata* and the acanthocephalan *S. hispida*, and to a lesser extent between the nematodes *C. rudolphii* and *S. squamata*. The former interspecific interaction is the most evident. First, there was a strong negative correlation between the intensities of infection of *S. squamata* and *S. hispida* across hosts, indicating that when one species occurs in high numbers the other is unlikely to do so. Second, the growth of one species was negatively affected by concurrent infections with the other, in a density-dependent way. Female but not male acanthocephalans achieved increasingly variable sizes as the numbers of nematodes per host increased. This means that variance in acanthocephalan egg production is increased by interspecific competition with *S. squamata*. There was no evidence that this interspecific effect was limited to only one sex in *S. squamata*. Interestingly, the growth of worms of one species was more influenced by the numbers of worms of the other species in the host than by numbers of their own species.

Three features of these results are worthy of mention. The first is that the outcome of interspecific negative interactions is asymmetrical in one case, and symmetrical in the other. Whereas the acanthocephalan *S. hispida* and the nematode *S. squamata* have reciprocal effects on one another, the nematode *C. rudolphii* affects *S. squamata* but not vice versa. There are many possible reasons for this. Perhaps the power of the analyses examining the effects of *S. squamata* were limited by the small number of cormorants infected with this parasite, or the range of intensities of infection was not high enough to have measurable impacts on the growth of *C. rudolphii*. It is also possible that the interaction between these two helminths is truly asymmetrical, as is the case in many other systems where one species seems to be dominant (see Poulin, 1998). This leads to a second interesting aspect of our results: a negative interaction takes place between two different helminth species although they inhabit different microhabitats in the host (*S. squamata* occurs in the stomach, and *S. hispida* in the intestine). Nematode and acanthocephalan species are known to interact when occurring in the same microhabitat, such as the intestine (e.g. Holland, 1984). The situation in cormorants actually makes the establishment of symmetrical competition more difficult. Given that *S. squamata* occurs in the stomach of the birds and *S. hispida* is found in the intestine, the effects of the 'upstream' species on the 'downstream' one may be unidirectional: *S. squamata* may change the conditions in which *S. hispida* lives, for instance by feeding on nutrients before they enter the intestine, but not vice versa. Thus the nematode *S. squamata* should experience no negative effects from the presence of the acanthocephalan; the apparent reciprocal effects of the two parasites on one another remains unexplained. This effect need not be competitive in the strict sense: it may also be the result of host immune responses that are enhanced in concomitant infections. It is also possible that the strong negative correlation between numbers of *S. squamata* and *S. hispida* has been transferred from their shared paratenic hosts: there is evidence that associations between helminth species in definitive hosts may not be the result of competition in that host, but a reflection of associations in earlier hosts (see Poulin & Valtonen, 2001).

A third noteworthy feature of our results is that they apply only to female *S. hispida* i.e. the growth of male acanthocephalans does not appear to be influenced by infection intensities of *S. squamata*. Female acanthocephalans are larger than males, and the greater nutrient requirements associated with their size and egg production could make them more sensitive to the presence of competing species. Although the nematode *S. squamata* does not affect the growth of male acanthocephalans, it still has a negative impact on their numbers: the negative correlation between numbers of nematodes and numbers of acanthocephalans applies equally well to only male ($r = -0{\cdot}804$, $N = 11$, $P = 0{\cdot}0029$) and only female ($r = -0{\cdot}817$, $N = 11$, $P = 0{\cdot}0021$) acanthocephalans. The influence of the nematode *S. squamata* on numbers and female sizes of *S. hispida* can also be viewed in the light of data on acanthocephalan sex ratio and sexual size dimorphism. In dioecious helminths such as acanthocephalans, both

theory and comparative data suggest that as the intensity of infection increases, the sex ratio should become less female-biased to increase the probability of mating, and at the same time the size of males relative to females should increase in response to stronger male–male competition for access to females (May & Woolhouse, 1993; Poulin, 1997*a*, *b*). These expected patterns were indeed noted in this study, with relatively more and larger male acanthocephalans being found in birds with higher intensities of infection. It is easy to see how a competing species, such as *S. squamata*, can weaken these patterns via its influence on numbers of *S. hispida* and sizes of its females.

The most abundant helminth species found in the cormorants, the nematode *C. rudolphii*, despite its high numbers and relatively large size, did not influence intensities or growth of the acanthocephalan. Its one-sided effect on variability in sizes of *S. squamata* may be the result of straightforward competition for resources, as both nematodes live in the host's stomach. The growth of *C. rudolphii* was only affected by its own intensity, in a typical density-dependent manner, and not by the other helminth species. In some cases, the growth of 1 or more of the 3 helminth species was associated with host size or age. Not surprisingly, older or larger birds harboured either longer worms, or more variable worm sizes, as one would expect in hosts that have acquired parasites over a longer period. Host sex had no influence on infection levels or worm sizes, although this host feature is often associated with various parameters of infection. For instance, in mammals and birds, host sex is commonly a determinant of the intensity of infection as well as a determinant of parasite growth (Poulin, 1996*a*, *b*; McCurdy *et al*. 1998). The cormorants examined here were collected during winter; sex differences in infection among birds, if any, may appear during and after the breeding season only, when sex hormones may have influenced the susceptibility of hosts to infection.

In summary, our study demonstrates the occurrence of both intra- and interspecific density-dependent effects on helminth sizes in a natural system. Growth of the most abundant parasite in our system, the nematode *C. rudolphii*, showed signs of intraspecific density dependence. This helminth also had interspecific density-dependent effects on the growth of another nematode, *S. squamata*. Furthermore, growth of the latter species was also markedly affected by the intensities of infection of the acanthocephalan *S. hispida*, in a fully reciprocal, interspecific, density-dependent relationship. These results highlight the complex nature of density dependence and competitive interactions, and the need to consider natural systems in which many helminth species co-exist in the same hosts, in addition to simpler 1- or 2-host laboratory models.

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