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Host sharing and host manipulation by larval helminths in shore crabs: cooperation or conflict?

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Abstract

Larval helminths of different species that share the same intermediate host and are transmitted by predation to the same definitive host may cooperate in their attempts to manipulate the behaviour of the intermediate host, while at the same time having conflicts of interests over the use of host resources. A few studies have indicated that intermediate hosts harbouring larval helminths have altered concentrations of neurotransmitters in their nervous system, and thus measuring levels of neurotransmitters in host brains could serve to assess the respective and combined effect of different helminth species on host behaviour. Here, we investigate potential cooperation and conflict among three helminths in two species of crab intermediate hosts. The acanthocephalan *Profilicollis* spp., the trematode *Maritrema* sp. and an acuariid nematode, all use *Macrophthalmus hirtipes* (Ocypodidae) as intermediate host, whereas *Profilicollis* and *Maritrema* also use *Hemigrapsus crenulatus* (Grapsidae). All three helminths mature inside gulls or other shore birds. There was a significant decrease in the mean volume of *Profilicollis* cystacanths as the intensity of infection by this parasite increased in *H. crenulatus*, the only host in which this was investigated; however, there was no measurable effect of other helminth species on the size of acanthocephalans, suggesting no interspecific conflict over resource use within crabs. There was, in contrast, evidence of a positive interspecific association between the two most common helminth species: numbers of *Profilicollis* and *Maritrema* were positively correlated among crabs, independently of crab size, in *M. hirtipes* but not *H. crenulatus*. More importantly, we found that the total number of larval helminths per crab correlated significantly, and negatively, with concentrations of serotonin in crab brains, again only in *M. hirtipes*; numbers of each parasite species separately did not covary in either crab species with serotonin or dopamine, the other neurotransmitter investigated in this study. The relationship with serotonin appears due mainly to numbers of *Profilicollis* and *Maritrema* and not to nematodes. This is the first demonstration of a potentially synergistic manipulation of host behaviour by different helminth species, one that appears host-specific; our results also point toward the neurobiological mechanism underlying this phenomenon.

Keywords: Crabs; *Hemigrapsus crenulatus*; Dopamine; *Macrophthalmus hirtipes*; *Maritrema*; Neurotransmitters; *Profilicollis*; Serotonin

1. Introduction

Larval stages of parasitic helminths use intermediate hosts not only as sources of nutrients and protection against environmental hazards, but also as vehicles taking the parasites to their definitive host. Often, the same intermediate host harbours larval stages of several different helminth species that may or may not have the same definitive host (Bush et al., 1993). This creates a range of potential scenarios of cooperation or conflict among larval helminths (Lafferty et al., 2000). For instance, if all helminths in an intermediate host have the same definitive host, they have a mutual interest in the fate of the intermediate host: it serves as a vehicle taking them all to a common destination. Many helminths are capable of manipulating the behaviour or colouration of their intermediate hosts in ways that render them more susceptible to predation by the parasite's definitive host (Poulin, 1994, 2000; Moore, 2002). In situations where different helminth species share an intermediate host and also have the same definitive host, all helminths can achieve a higher transmission success to the definitive host if one of them is a manipulator (Thomas et al., 1997, 1998; Lafferty et al., 2000; Poulin and Valtonen, 2001). Instead of having a single manipulator species in these cases, natural selection may have favoured the ability to alter the phenotype of the intermediate host independently in all coexisting helminth species. If this

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were the case, we would expect a more active form of cooperation among helminths sharing an intermediate host, with an additive or even synergistic manipulative effort resulting in greater alterations in the host.

Sharing an intermediate host can also result in conflicts among helminths with respect to resource use. For example, the size of acanthocephalan cystacanth larvae inside amphipod intermediate hosts is influenced by the number of both conspecifics and cystacanths of other acanthocephalan species (Dezfuli et al., 2001). In more extreme situations, the first helminth to establish inside an intermediate host can even lower the establishment success of other species (Gordon and Whitfield, 1985; Barger and Nickol, 1999). Whether or not they can combine their efforts to manipulate the behaviour of the intermediate host, different helminth species may at the same time compete for space or nutrients within this host.

Whereas the potential conflicts between helminths are easily measured in terms of reduced body size or negative associations between their intensities of infection, their respective and combined effects on host behaviour are more difficult to quantify. One reason for this is that the physiological pathways used by parasites to alter host behaviour are poorly understood. In most cases, altered host behaviour appears not to be merely the outcome of energy depletion or general debilitation caused by the infection (e.g. Franz and Kurtz, 2002). Several recent lines of evidence suggest that parasites play active roles in modulating the neurochemistry of their hosts (see reviews, in Hurd and Webb, 1997; Kavaliers et al., 2000; Moore, 2002). Consider the case of the acanthocephalan *Polymorphus paradoxus*, known to alter the behaviour of its amphipod intermediate host, causing it to swim to the surface and cling to any object following a disturbance (Bethel and Holmes, 1973). Injection of the neurotransmitter serotonin in uninfected amphipods induces an almost identical behaviour, although the response does not last as long as in infected amphipods (Helluy and Holmes, 1990). In addition, the structure of serotonergic neurons in the central nervous system of amphipods infected with *P. paradoxus* differs significantly from that of uninfected amphipods (Maynard et al., 1996). Serotonin thus appears to be involved in the behavioural manipulation induced by the acanthocephalan. This biogenic amine is known to alter activity levels, aggression, escape responses, phototaxis and reproductive behaviour in crustaceans (Arnesen and Olivo, 1988; McPhee and Wilkens, 1989; Wood et al., 1995; Aggio et al., 1996; Jadhav et al., 1999; Sneddon et al., 2000; Tierney and Mangiamele, 2001) and could thus be a prime target for helminths in crustacean intermediate hosts. Altered levels of neurotransmitters in intermediate hosts have also been associated with cestode infections (Øverli et al., 2001), and are, therefore, not restricted to infections by acanthocephalans. If several kinds of helminths can somehow modify the concentrations of neurotransmitters in their intermediate host's brain, then measuring these concentrations and relating them to infection levels by different helminths may provide a quantitative assessment of the combined or cooperative influence of parasites on intermediate host behaviour.

Here, we investigate the potential cooperation and conflicts among different helminth species sharing two species of shore crabs, *Macrophthalmus hirtipes* (Ocypodidae) and *Hemigrapsus crenulatus* (Grapsidae), as intermediate hosts. First, we looked for evidence of conflict by determining whether the infection levels of the different helminth species are negatively associated among individual hosts; negative relationships could indicate strong competitive interactions leading to species exclusion, whereas positive relationships might indicate that one species associates with the other. Second, we searched for potential conflicts of a different kind, by relating the average size of acanthocephalan cystacanths (by volume, the most important parasites in the crabs) to both their numbers per crab and numbers of other helminth parasites. Third, we determined whether there were relationships between intensity of infection by all helminth species, separately or combined and concentrations of two neurotransmitters in the crabs' brains. We looked at both serotonin, known to be modulated directly or indirectly by parasitic infection in other species, and dopamine, which was not related to parasitism in other studies (Helluy and Holmes, 1990; Øverli et al., 2001). Together, these results provide an overview of the consequences of host sharing for larval helminth parasites.

2. Materials and methods

2.1. The host crabs and their parasites

The ocypodid crab *M. hirtipes* and the grapsid crab *H. crenulatus* are common inhabitants of mud flats in sheltered bays, inlets and estuaries throughout New Zealand (McLay, 1988). They are found mainly in the lower portion of the intertidal zone and in the shallow subtidal zone. Larger individuals of both species normally burrow into the sediments at low tide, whereas smaller ones often live under rocks or other objects.

The acanthocephalans *Profilicollis antarcticus* and *Profilicollis novaezelandensis* are common parasites of both crab species, in which the acanthocephalan cystacanths occur in the body cavity (Brockerhoff and Smales, 2002). The two parasites have identical life cycles and develop into adult worms after their crab intermediate host is ingested by a suitable shorebird definitive host. At our study site, these include the black-backed gull (*Larus dominicanus*) and the red-billed gull (*Larus novaehollandiae scopulinus*). Less than 1% of cystacanths found in crabs at our study site are *P. antarcticus* (Latham and Poulin, 2002a), for this reason, in the present study we have pooled these two species and refer to them as *Profilicollis* spp. There are several lines of

evidence suggesting that *Profilicollis* spp. alter the behaviour of the two crabs studied here. First, investigations on Chilean populations of *H. crenulatus* parasitised by *P. antarcticus* have shown that the parasite alters both the activity levels and carapace colouration of the crab hosts (Pulgar et al., 1995; Haye and Ojeda, 1998). Second, field data indicate that the mean number of cystacanths per crab drops in the larger size classes of both *M. hirtipes* and *H. crenulatus* (Latham and Poulin, 2002a); in the absence of density-dependent regulation of parasites or host immune reactions, this trend suggests that the heavily infected crabs are removed from the population by predation. Third, crabs found outside their burrows at low tide had more cystacanths per crab than those that were still buried, in *M. hirtipes* but not *H. crenulatus* (Latham and Poulin, 2002b).

The microphallid trematode *Maritrema* sp. is also a common parasite of both crab species in our study area. After leaving their snail first intermediate host, cercariae encyst as metacercariae in the body cavity of crabs, awaiting ingestion by a bird definitive host. The red-billed gull is a confirmed definitive host of this parasite in our study area, although other shore birds are also likely to be suitable hosts. There is also a second trematode in these crabs, an unidentified microphallid; here, we counted metacercariae of this parasite with those of *Maritrema* sp. because of the likely similarities in their life cycles. Nothing is known of the effects of these parasites on crab behaviour; however, other microphallid trematodes are known to alter the behaviour of their crustacean intermediate hosts (e.g. Helluy, 1984; McCurdy et al., 2000).

Finally, juvenile acuariid nematodes (Acuariinae) also occur in the body cavity of crabs, here found only in the crab *M. hirtipes* and not in *H. crenulatus*. These nematodes also mature in birds following predation on infected crabs, most likely in the same birds as the above acanthocephalans and trematodes. Subsequent examination of the larval nematodes under a scanning electron microscope revealed that a few of them (less than $\approx$5%) are actually larvae of *Ascarophis* spp. (Cystidicolidae), which are parasitic in fish as adults. Given their relative rarity and the fact that they were only detected after pooling of all samples, we treat all juvenile nematodes together, and assume that the vast majority are acuariids maturing in birds.

2.2. Field sampling and laboratory procedures

To minimise temporal or sex-related variation in levels of neurotransmitters among crabs, we only collected adult males, and all crabs of one species were collected in the same area on the mud flat (roughly 100×50 m^2) and at the same time of year. *Macrophthalmus hirtipes* were collected over 3 consecutive days in July 2002 during low tide in Papanui inlet, on the Otago Peninsula, New Zealand. *Hemigrapsus crenulatus* were collected from Company Bay, Otago Harbour, at low tide in 1 day during August 2002. Each crab was individually wrapped in an aluminium foil and snap frozen in liquid nitrogen immediately (i.e. within 20 s) following capture. All crabs were transported back to the laboratory on dry ice and then stored at -70°C.

Twenty-four hours before dissection, crabs were moved to a refrigerator and allowed to defrost at 3°C. Each crab was placed on a bed of ice to prevent the breakdown of neurotransmitters in its nervous system, and opened under a dissecting microscope. The anterior part of the carapace was cut open, and the entire brain was removed, placed in a labelled vial and immediately frozen (-70°C).

The body cavity was then searched for parasites, and for each crab we recorded the number of acanthocephalan cystacanths, trematode metacercariae and juvenile nematodes. In addition, for all *H. crenulatus*, we measured the maximum length and width of 10 randomly chosen cystacanths per crab, or of all cystacanths if there were fewer than 10 in a crab. Cystacanths have a roughly ellipsoid shape, and their volume was determined as $(\pi LW^2)/6$, with L and W being the length and the width of the cystacanth, respectively. For each crab, we computed the mean cystacanth volume and the coefficient of variation in cystacanth volume (standard deviation $\times$ 100/mean), as an estimate of the relative amount of variation in cystacanth size per crab.

Brain samples were prepared by adding 100 μl of ice-cold perchloric acid to the frozen brains; they were then sonicated for 4 s using an ultratip, and finally centrifuged at 9,000 rpm for 20 min at 4°C. The supernatants were analysed for their concentrations of serotonin and dopamine using reverse-phase high performance liquid chromatography (HPLC) with electrochemical detection. The HPLC was run using a C18 column (4.6×100 mm^2 with 5 μm packing) and a mobile phase consisting of 0.4 g octanesulphonic acid, 13.8 g sodium dihydrogenorthophosphate, 2.65 g sodium acetate, 0.11 g ethylenediaminetetra-acetic acid (EDTA) disodium salt and 80 ml acetonitrile in 1 l of water (pH 2.5) pumped at a rate of 1 ml/min. The ESA model 5100A detector was set at 300 mV. Standard calibration curves were established every day prior to analysis of the brain samples, with known dopamine and serotonin standards in perchloric acid solution, made using 21.7 ml perchloric acid (60%), 0.25 g sodium metabisulphate and 0.5 g EDTA disodium salt in 500 ml water. The HPLC analyses were performed twice, on different days, for a sub-sample of 15 crab brains, to ensure the accuracy of the measurements; the two concentrations obtained for these crab brains were always within 10% of one another, suggesting that the HPLC results were repeatable. Finally, concentrations of either serotonin or dopamine below the limit of detection were scored as 0.

2.3. Statistical analyses

The data were analysed using standard parametric tests. All data on intensity of infection, i.e. numbers of parasites

per host, were log-transformed (or log $(x + 1)$-transformed if there were 0s) before analysis. Concentrations of dopamine in crab brains were not normally distributed; most values were low, although few were below the detection threshold. Dopamine concentrations were log $(x + 1)$-transformed for analysis. Serotonin, in contrast, was not detectable in many crab brains. In regression analyses, we log-transformed the serotonin concentrations and used only crabs for which a serotonin value was obtained. We also performed two-group comparisons of parasite infections between crabs with measured levels of serotonin and those with levels below the detection threshold.

3. Results

All three parasites occur at high prevalence (percentage of infected hosts) and intensities (numbers of parasites per host) of infection among the 82 *M. hirtipes* and the 36 *H. crenulatus* sampled (Table 1). In addition, one *M. hirtipes* and four *H. crenulatus* harboured unidentified epicaridean isopods; excluding these few crabs from the analyses had no influence on the results, so they were included.

3.1. Parasitism in M. hirtipes

The carapace width of *M. hirtipes* in our sample ranged from 26 to 37 mm. There were positive correlations between crab size and numbers of both *Profilicollis* ($N = 82$, $r = 0.473$, $P = 0.0001$) and *Maritrema* ($r = 0.335$, $P = 0.0001$) per crab. Although there is much scatter in the data, larger crabs tend to harbour more of these two parasites (Fig. 1). There was, however, no relationship between crab size and intensity of infection by larval nematodes ($r = -0.012$, $P = 0.918$).

There was no correlation between numbers of *Profilicollis* and *Maritrema* per crab among individuals in our sample ($N = 82$, $r = 0.167$, $P = 0.1342$). Because the intensity of infection by both these parasites is influenced by crab size, their association must be evaluated while correcting for the potential influence of crab size. This was achieved by including both crab carapace width and numbers of *Profilicollis* as independent variables in a multiple regression, with numbers of *Maritrema* as the dependent variable; the resulting partial regression coefficient for numbers of *Profilicollis* was not significant ($r = 0.011$, $P = 0.9277$). There was also no correlation between numbers of either *Profilicollis* or *Maritrema* and numbers of juvenile nematodes per crab (both $P > 0.30$).

Neither host size nor intensity of infection by any of the three parasites had any significant influence on concentrations of either dopamine or serotonin in crab brains (Table 2). Because there was a hint of a negative relationship between serotonin levels and the numbers of *Profilicollis* per crab ($P = 0.085$) and also, to a lesser extent, the numbers of *Maritrema*, we repeated the multiple regression by using the total number of parasites per crab instead of the separate numbers of each species. Indepen-

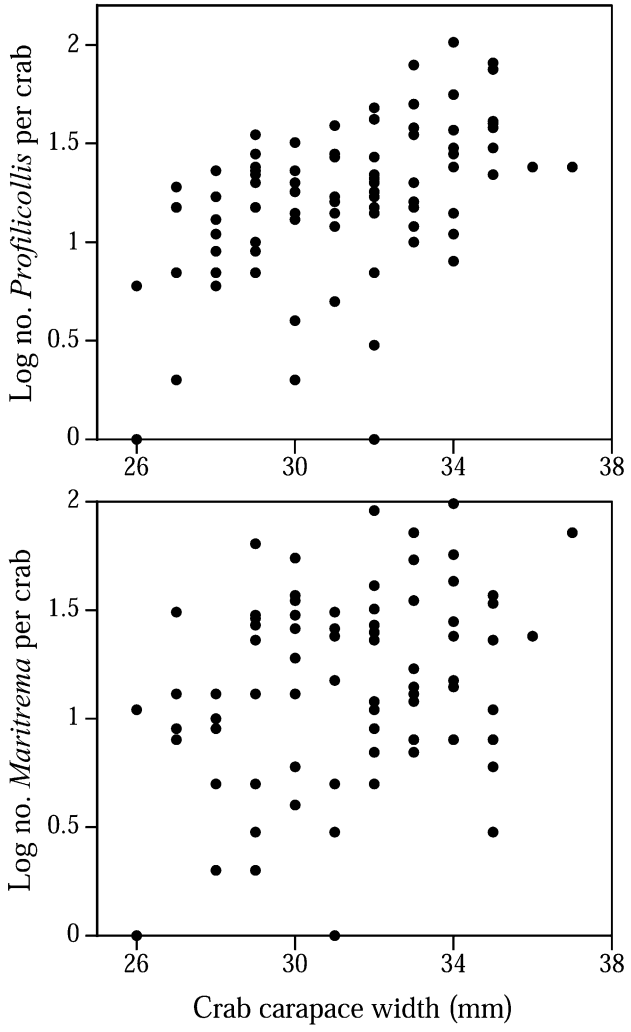

Fig. 1. Relationship between the size of crab intermediate hosts (*Macrophthalmus hirtipes*) and the numbers of two larval helminth parasites they harboured.

Table 1
Summary of the parasitological data on the two species of shore crabs investigated

Host species Parasite species	Prevalence (%)	Mean intensity[a]	Range
Macrophthalmus hirtipes ($N = 82$)			
Profilicollis spp.	100.0	23.1	1–103
Maritrema sp.	97.6	21.2	1–97
Juvenile nematodes	90.2	4.6	1–18
Hemigrapsus crenulatus ($N = 36$)			
Profilicollis spp.	86.1	31.0	1–149
Maritrema sp.	83.3	8.9	1–51

[a] Includes infected crabs only.

Table 2
Influence of host size and intensities of infection by different helminths on the concentrations of two neurotransmitters in the brain of the crab *Macrophthalmus hirtipes*

Dependent variable	Dopamine ($N = 80$ crabs)		Serotonin ($N = 52$ crabs)	
	r	P	r	P
Carapace width	0.119	0.388	0.270	0.109
Number of *Profilicollis*	−0.090	0.498	−0.271	0.085
Number of *Maritrema*	0.093	0.446	−0.230	0.130
Number of nematodes	−0.015	0.895	0.026	0.854

Partial regression coefficients from a multiple regression are shown.

dently of crab size, there was a negative relationship between total parasite load and serotonin concentrations ($r = -0.351$, $P = 0.0394$): in general, higher serotonin concentrations were observed in the least severely infected crabs (Fig. 2). Four crabs appear quite influential in this relationship (four points in the upper left corner of Fig. 2); there was nothing peculiar about these crabs with respect to any of the other variables measured. The relationship was stronger when total numbers of *Profilicollis* and *Maritrema* only were used, excluding nematodes ($r = -0.398$, $P = 0.0161$). Only data from 52 crabs could be included in these regressions, as serotonin levels in the other 30 crabs were below the window of detection by HPLC. There were no differences in either crab size or intensities of infection by any helminths between the crabs with measured concentrations of serotonin and those for which serotonin levels were undetermined (two-tailed t-tests, all $P > 0.16$).

3.2. Parasitism in H. crenulatus

Carapace width of *H. crenulatus* (range 22–38 mm) did not correlate with the numbers of *Maritrema* per crab ($N = 36$, $r = -0.112$, $P = 0.5153$), but, surprisingly, it correlated negatively with numbers of *Profilicollis* ($r = -0.419$, $P = 0.011$). Numbers of the two parasites correlated positively across crabs ($r = 0.393$, $P = 0.0177$). As earlier, to eliminate the possible influence of host size, both crab carapace width and numbers of *Profilicollis* were used as independent variables in a multiple regression, with numbers of *Maritrema* as the dependent variable. The effect of the number of *Profilicollis* remained significant (partial regression coefficient: $r = 0.420$, $P = 0.0228$), and thus there is a positive, though weak, association between the two helminths among intermediate hosts that is independent of host size (Fig. 3).

Among crabs harbouring *Profilicollis* cystacanths, a multiple regression revealed that mean cystacanth volume is negatively influenced by the number of *Profilicollis* per crab (partial regression coefficient: $N = 31$, $r = -0.628$, $P = 0.0007$) and that it almost correlates positively with host carapace width ($r = 0.273$, $P = 0.0614$). Although there were no crabs with intermediate intensities of infection in our sample, it is clear that cystacanths are larger on an average when there are few of them per crab (Fig. 4). This intensity-dependent effect applies only to conspecific parasites, however; the number of *Maritrema* metacercariae, also included in the multiple regression, did not influence the mean volume of *Profilicollis* cystacanths

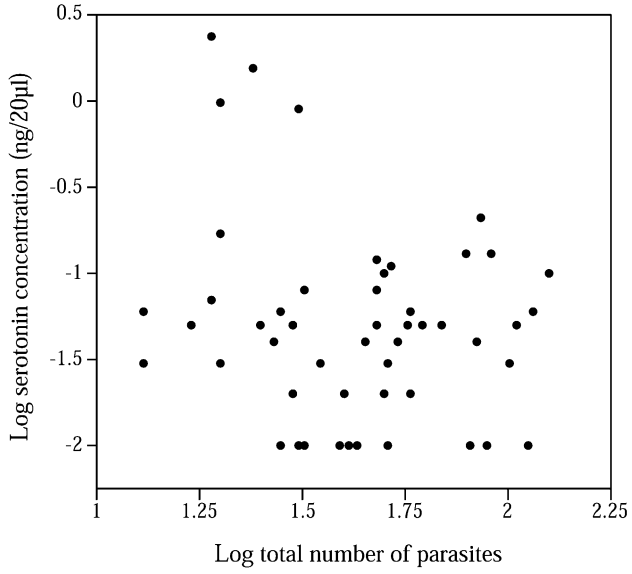

Fig. 2. Relationship between the concentration of serotonin in the brain of individual crabs, *Macrophthalmus hirtipes* and the total number of larval helminths they harboured. Numbers of *Profilicollis* cystacanths, *Maritrema* metacercariae and larval nematodes are combined to produce this total number.

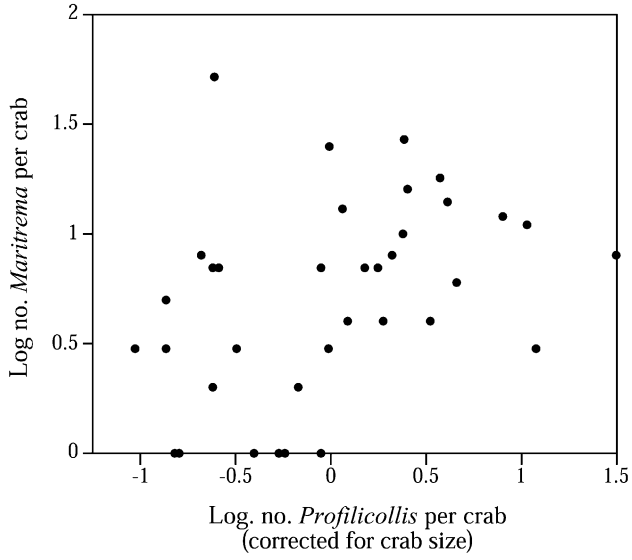

Fig. 3. Relationship between the numbers of *Maritrema* metacercariae and *Profilicollis* cystacanths among intermediate host crabs, *Hemigrapsus crenulatus*. Numbers of cystacanths are the residuals of a regression of numbers of cystacanths against crab carapace width, and are thus corrected for the influence of crab size.

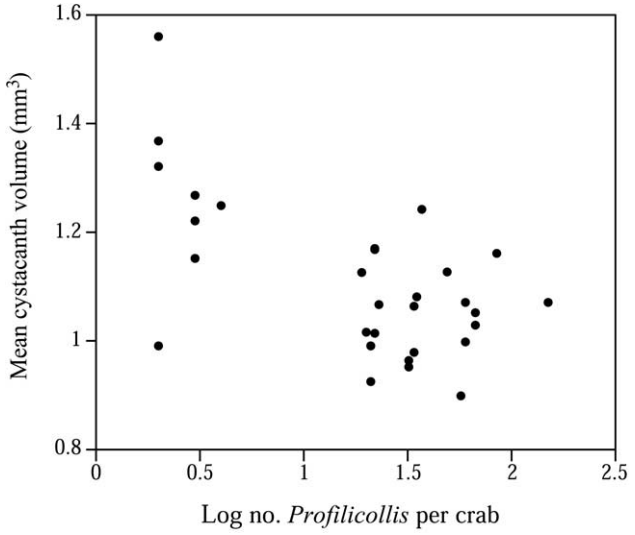

Fig. 4. Relationship between the mean volume and the number of *Profilicollis* cystacanths found in a crab, among individuals of the intermediate host crab *Hemigrapsus crenulatus*.

($r = 0.063$, $P = 0.6978$). In a similar regression analysis, the coefficient of variation in cystacanth volume was not influenced significantly by any of the three dependent variables considered (all, $P > 0.39$).

Neither host size nor intensity of infection by either of the two parasites had any significant influence on concentrations of either dopamine or serotonin in crab brains (Table 3). Combining numbers of both parasite species in a separate regression analysis did not change these results. Only data from 17 crabs could be included in these regressions, as serotonin levels in the other 19 crabs were not measurable. Although crabs for which serotonin could be measured were slightly larger than those for which serotonin levels were undetermined (two-tailed *t*-test, $t = 2.054$, df = 34, $P = 0.0477$). There were no differences in intensities of infection by either helminth between the two groups of crabs (all $P > 0.95$).

4. Discussion

Interactions among helminth species in their vertebrate definitive hosts have received considerable attention over the past two decades (see Esch et al., 1990; Sousa, 1994). Potential interactions among larval helminths in intermediate hosts have not generated as much interest, possibly because of the apparent 'dormant' nature of many larval helminths. Given the importance of the larval stages inside intermediate hosts for trophically transmitted parasites, however, it is unlikely that helminths simply accumulate randomly and rest passively in these hosts. When several helminths of the same or different species share an intermediate host, there are either potential conflicts of interests among them, or opportunities for active 'cooperation' with benefits for all (see Thomas et al., 1998; Lafferty et al., 2000; Poulin and Valtonen, 2001; Outreman et al., 2002). Here, we have found several lines of evidence, albeit each from only one of the two crab species investigated, suggesting that larval helminth species sharing the same intermediate host: (1) are not randomly distributed among hosts with respect to one another, (2) show intensity-dependent growth and (3) can join forces to alter the physiology and behaviour of their hosts.

Table 3
Influence of host size and intensities of infection by different helminths on the concentrations of two neurotransmitters in the brain of the crab *Hemigrapsus crenulatus*

Dependent variable	Dopamine ($N = 34$ crabs)		Serotonin ($N = 17$ crabs)	
	r	*P*	*r*	*P*
Carapace width	0.211	0.298	−0.339	0.287
Number of *Profilicollis*	0.258	0.243	−0.182	0.574
Number of *Maritrema*	−0.160	0.419	−0.025	0.929

Partial regression coefficients from a multiple regression are shown.

First, in one of the two crab species investigated, we found a positive association between numbers of the acanthocephalan *Profilicollis* and the trematode *Maritrema* among crab intermediate hosts. This was not merely the result of passive accumulation inside hosts, as the crabs grow older, because the relationship remained after a correction for host size. It was, however, observed only in *H. crenulatus* and not *M. hirtipes*; this may be due to a range of factors, including the fact that the two crab species were collected in different areas. Although the correlation between numbers of *Profilicollis* and *Maritrema* was only found in one crab species, it remains intriguing because of the way the two parasites infect crabs. Crabs acquire acanthocephalans when they accidentally ingest eggs deposited in bird faeces, and they become infected with trematodes when cercariae released by snails penetrate their bodies at joints in their exoskeleton. These two infection processes appear completely independent of one another. The most parsimonious explanation for the association is that, although via different infection routes, the two parasites originally come from the same source, i.e. bird faeces. Small-scale spatial co-variation in the density of infective stages could result in exposure to *Profilicollis* being correlated with exposure to *Maritrema*. A second explanation is that one parasite preferentially infects crabs already harbouring the other parasite. Acanthocephalans could alter the behaviour of crabs in a way that renders them more susceptible to infection by cercariae of *Maritrema*. If acanthocephalan-infected crabs are more likely to be captured by bird hosts (Latham and Poulin, 2002b), the trematode could thus benefit. Although plausible (see Thomas et al., 1997, 1998; Dezfuli et al., 2000; Poulin and Valtonen, 2001), this scenario is not as parsimonious as the previous one. In any event, our results suggest that these

parasites show patterns of associations that are not by-products of a common infection process.

Second, we observed a decrease in mean cystacanth volume in crabs harbouring large numbers of *Profilicollis* cystacanths in *H. crenulatus*, the only host species for which this was investigated. The decrease was independent of host size, and cystacanth volume was not influenced by the presence of large numbers of *Maritrema* metacercariae. However, using the coefficient of variation as a measure of variability independent of the mean, we did not find that cystacanth volumes became more variable as the intensity of infection increased. There have been previous reports of intensity-dependent growth in acanthocephalan cystacanths (e.g. Dezfuli et al., 2001). Cystacanth size may be an important component of fitness in acanthocephalans. Activation of the cystacanth and initial establishment in the host gut following ingestion by a definitive host are energetically costly processes, requiring all the stored glycogen available to the cystacanth (Lackie, 1974; Taraschewski, 2000). The reduced size achieved at high intensities in the intermediate host can only have negative consequences later in life. Thus, sharing an intermediate host with many conspecifics (but not heterospecifics) can impair an acanthocephalan's chances of establishment in the definitive host, although it might make it easier to reach the definitive host (see below).

Third, in one of the two crab species investigated, we found that the total number of larval helminths harboured by a crab correlated negatively with the concentration of the neurotransmitter serotonin in its brain. Again, this result was independent of any influence of crab body size. Interestingly, this relationship was found only in the crab *M. hirtipes* and not in *H. crenulatus*. In an earlier study, we had found that high numbers of *Profilicollis* cystacanths per crab influenced the burrowing behaviour of *M. hirtipes* but not that of *H. crenulatus*, making the former species more likely to be exposed at low tide but not affecting the latter species (Latham and Poulin, 2002b). Thus there may be differences between the two crab species in their susceptibility to manipulation by parasites. Although belonging to different families, the two crab species are closely related (Kitaura et al., 2002). Such differences in effects of parasitism between closely related host species have rarely been reported. One example involves the trematode *Microphallus papillorobustus* and two related amphipod, *Gammarus* spp. hosts. The parasite alters the behaviour of one host species only (Helluy, 1983), and induces mortality in that same host species but not in the other (Thomas et al., 1995). In this example as well as in our study system, parasitism may influence the respective abundance of related host species and affect host community structure, via different host-specific levels of host manipulation and associated mortality.

The fact that a correlation with serotonin levels in the brain appears only when numbers of parasites of different species are combined suggests that the synergistic effect of the different parasites is greater than the influence of either of them taken separately. This appears to support a scenario in which different helminth species, with a common definitive host, cooperate to manipulate the behaviour of their intermediate host. Alternatively, lower serotonin levels may simply be a general host response to the stress induced by severe parasite infections. However, is it justifiable to pool the numbers of parasite of different species in the analysis? Because they belong to different phyla, their impacts on host physiology are unlikely to be comparable. From an epidemiological perspective, on the other hand, they all have the same crab-to-bird transmission route, and can derive identical benefits from host manipulation. Although this does not make them equivalent to each other, it suggests that the total number of these different helminth species is a rough approximation of the cumulative pressure exerted on crabs. It is true that a small fraction of the larval nematodes do not use birds as definitive hosts: a few belong to the cystidicolid genus *Ascarophis*, which uses fish as definitive hosts. This is not a major problem, however, for at least two reasons. First, the only relevant study of cystidicolid nematodes has failed to find even the slightest hint that they might be capable of altering the behaviour of their crustacean intermediate hosts (Knudsen et al., 2001). Second, the relationship with serotonin concentration is stronger when nematodes are excluded, and only *Profilicollis* and *Maritrema* are included in the total number of parasites per crab.

Based on studies in other crab species, reduced concentrations of serotonin can be associated with a range of behavioural responses, from altered locomotor activity (Wood et al., 1995) to changed levels of aggression (Sneddon et al., 2000; Tierney and Mangiamele, 2001). Infection by *Profilicollis* spp. in *M. hirtipes* has been shown to influence burrowing behaviour and the outcome of agonistic encounters between males (Latham and Poulin, 2001, 2002b). In *H. crenulatus*, these parasites have no apparent effect on burrowing behaviour (Latham and Poulin, 2002b) but can modify activity levels and carapace colouration, at least in the related Chilean system (Pulgar et al., 1995; Haye and Ojeda, 1998). Most importantly, there is evidence that, at our study sites, *Profilicollis* spp. can increase the mortality of the two crab species (as well as a third one, *Hemigrapsus edwardsii*), through what appears to be bird predation (Latham and Poulin, 2002a). It is, therefore, anti-predator responses that may be affected by the parasites, via lowered levels of serotonin. Interestingly, Aggio et al. (1996) have found that raised concentrations of serotonin in the crab *Chasmagnathus granulatus*, which is a grapsid like *H. crenulatus*, enhances the escape response to an overhead passing shadow, a visual stimulus associated with predatory birds. Perhaps *Profilicollis* and *Maritrema* jointly depress serotonin levels in their shared host, causing it to be less responsive to approaching danger. Our data only provide a snapshot of neurotransmitter levels in wild crabs, and we need to learn more about the roles they play in host

behavioural manipulation. Although the neurobiological details remain to be sorted out, our results still bring a new perspective of host manipulation by parasites. They are not only the first to suggest a possible joint manipulation of host behaviour by more than one parasite species, but also they point toward a mechanism by which this is achieved. The links between the different parasite species, serotonin and host behaviour will need to be further explored experimentally before these suggestions are confirmed.

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