Intra-host competition between co-infecting digeneans within a bivalve second intermediate host: Dominance by priority-effect or taking advantage of others?

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ABSTRACT

We experimentally investigated the interactions between two parasites known to manipulate their host's phenotype, the trematodes *Acanthoparyphium* sp. and *Curtuteria australis*, which infect the cockle *Austrovenus stutchburyi*. The larval stages of both species encyst within the tissue of the bivalve's muscular foot, with a preference for the tip of the foot. As more individuals accumulate at that site, they impair the burrowing behaviour of cockles and increase the probability of the parasites' transmission to a bird definitive host. However, individuals at the foot tip are also vulnerable to non-host predators in the form of foot-cropping fish which selectively bite off the foot tip of exposed cockles. Parasites encysted at the foot base are safe from such predators although they do not contribute to altering host behaviour, but nevertheless benefit from host manipulation as all parasites within the cockle are transmitted if it is ingested by a bird. Experimental infection revealed that *Acanthoparyphium* sp. and *C. australis* have different encystment patterns within the host, with proportionally fewer *Acanthoparyphium* metacercariae encysting at the foot tip than *C. australis*. This indicates that *Acanthoparyphium* may benefit indirectly from *C. australis* and incur a lower risk of non-host predation. However, in co-infections, not only did *C. australis* have higher infectivity than *Acanthoparyphium*, it also severely affected the latter's infection success. The asymmetrical strategies and interactions between the two species suggest that the advantages obtained from exploiting the host manipulation efforts of another parasite might be offset by traits such as reduced competitiveness in co-infections.

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1. Introduction

For a parasite with a simple, direct life-cycle, a host may simply be a habitat and source of nutrient that it can exploit to produce offspring. However, for those with multi-host life-cycles, the role of a host is dependent on the particular developmental stage of the parasite. Such parasites often utilise intermediate hosts as vehicles to reach a definitive host to complete their life-cycle; this is usually accomplished when the definitive host ingests an infected intermediate host via trophic transmission (Lafferty, 1999). Because the likelihood of the parasitised individual being ingested by the definitive host is very low considering the number of alternative prey available, some parasites can alter the phenotype of the intermediate host to improve transmission success by making the host more susceptible to predation (reviewed by Poulin, 1995; Moore, 2002). For these host-manipulating parasites,

the altered host phenotype can be seen as a manifestation of their host exploitation strategy (Poulin, 2007).

If a host-manipulating parasite finds itself sharing an intermediate host with another manipulator that utilises the same definitive host (and thus transmission route), cooperation between the two species may occur, leading to a situation known as "co-piloting" (Lafferty et al., 2000). However, host manipulation can also be costly (Poulin, 1994; Poulin et al., 2005) and if manipulation itself is a phenotypically plastic trait that is context-dependent, one might expect that in the presence of another manipulator, a manipulative parasite may adopt a strategy more akin to "hitch-hiking": taking advantage of the other's manipulative effort without contributing to actual host manipulation (Thomas et al., 1998). Thus the outcome of co-infection between manipulators with similar life-cycles sharing the same intermediate host is not always predictable. The dynamics of interspecific associations between parasites have rarely been addressed experimentally (see Karvonen et al., 2009 for exceptions). Here we use a bivalve-trematode system, which involves two species of host-manipulating trematodes sharing a common bivalve intermediate host, to investigate the outcome of co-infection between different species of host-manipulating parasites.

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The New Zealand cockle *Austrovenus stutchburyi* is commonly infected by the encysted stage of two species of trematodes: *Curtuteria australis* and *Acanthoparyphium* sp. A (hereafter referred to as *Acanthoparyphium*) (Echinostomatidae). The cockle becomes infected when free-living larval stages of the parasites, known as cercariae, enter the cockle through its inhalant siphon. The cercariae then penetrate the cockle's foot where they form encysted stages called metacercariae – when metacercariae accumulate at the tip of the cockle's foot they impair its function (Mouritsen, 2002). Field data indicates that while the relative abundance and infection intensity of both parasites varies between different host populations, the prevalence of each species is close to 100% (Babirat et al., 2004). Thus, both trematodes almost always co-occur in the same individual host.

Both of these species alter the bivalve's behaviour by inhibiting its ability to bury itself into the sediment and forcing the cockle to remain exposed on the sediment surface (Thomas and Poulin, 1998). By doing so, they increase the probability of the cockle being eaten by a bird definitive host (Thomas and Poulin, 1998). The two species are considered ecological equivalents not only because they induce the same behavioural modification in their common intermediate host, but they also coexist on multiple levels – field samples indicate that at a population level, not only are they present in the same localities, they also encyst within the same part of the cockle's body, specifically within the foot muscle, with both species sharing an apparent preference for the tip (Babirat et al., 2004).

While the metacercariae can encyst anywhere within the cockle's foot and all parasites are transmitted if the cockle is eaten by a bird, only those that encyst at the foot tip are responsible for altering host behaviour (Mouritsen, 2002). However, this manipulation comes at a cost. Those at the tip are vulnerable to foot-cropping fish which are unsuitable hosts for the parasites, whereas those at the base of the foot are safe from foot-croppers (Mouritsen and Poulin, 2003b). Thus, metacercariae at the base of the foot can benefit from enhanced transmission without incurring the associated risk. Do *Acanthoparyphium* and *C. australis* display similar or distinct selection of encystment sites in the cockle's foot? And if so, do they alter their site selection (and thus contribution to host manipulation) in the presence of metacercariae of the other species?

The coexistence of *Acanthoparyphium* and *C. australis* metacercariae within cockles indicates that competitive displacement does not occur (at least at an ecosystem-scale) and that they may act in synergy to modify host behaviour (Babirat et al., 2004). However, nothing is known about the dynamics of their co-infection within the cockle. It is unknown how interactions between these two host-manipulators are mediated by factors such as differential infectivity, if competitive exclusion or facilitation between the two species occurs within individual hosts, or whether temporal factors such as order of arrival can influence the pattern of infections. Here, we use laboratory infections to investigate the dynamics of these interactions, and shed light upon the evolutionary ecology of multi-parasite infection. Our specific aims are: (i) to experimentally determine and compare the encystment pattern of both *Acanthoparyphium* and *C. australis* within the cockle foot when they infect alone or together, and (ii) to determine if co-infection influences the infection success of both species.

2. Material and methods

2.1. Study organisms

Approximately 100 cockles, *A. stutchburyi,* were collected from a sand flat at Otakou, Otago Harbour, New Zealand's South Island. The infection intensity by trematodes is known to be relatively low at that site (Mouritsen, 2002). Prior to experimental infection, the cockles were held in plastic containers (300 mm long × 130 mm wide × 150 mm high) filled with seawater and approximately 60 mm of fine sand, and aerated with an airstone.

The free-living cercarial stages of *C. australis* and *Acanthoparyphium* are shed from their gastropod first intermediate hosts, the mud whelk *Cominella glandiformis* and the mud snail *Zeacumantus subcarinatus,* respectively. The cercariae are continuously produced through asexual multiplication by the clonal stages of the trematodes which reside within the gastropod host. Infected gastropods are induced to shed cercariae by placing them individually into a clear plastic cylindrical container (60 mm high × 40 mm wide) filled with seawater and incubated at 25 °C under constant illumination.

The collection, screening procedure and husbandry of the whelks infected with *C. australis* are detailed in Leung and Poulin (2010) and Leung et al. (2010). Approximately 800 mud snails, *Z. subcarinatus,* were collected from Lower Portobello Bay, Otago Harbour. They were screened to find individuals infected by *Acanthoparyphium* in the same manner as the whelks and kept in the same type of container under the same environmental conditions, and were provided with strips of sea lettuce (*Ulva lactuca*) ad libitum for food.

While the *Acanthoparyphium* infecting *Z. subcarinatus* has been found to consist of a species complex (Leung et al., 2009), the same study also indicated that the overwhelming majority of *Acanthoparyphium* collected from Lower Portobello Bay are sp. A, which is the species known to infect cockles. For this study, a few cercariae from each of the snails used for experimental infections were individually sequenced at the 16S region, which allows discrimination among different species of *Acanthoparyphium* (Leung et al., 2009). All resulting sequences corresponded with known sequences of *Acanthoparyphium* sp. A.

2.2. Infection treatments

There was a total of five infection combinations and the number of cockles assigned to each infection varied depending on cercarial availability – *C. australis* only ($n = 24$), *Acanthoparyphium* only ($n = 20$), *C. australis* and *Acanthoparyphium* simultaneously ($n = 18$), *C. australis* followed by *Acanthoparyphium* ($n = 20$), and *Acanthoparyphium* followed by *C. australis* ($n = 17$). Each cockle was exposed to a total of 60 cercariae. In mixed-species infections, each cockle was exposed to 30 cercariae from each species. In non-simultaneous infections, a period of 1 week separated infection by the first species and the second species. The five combinations resulted in a total of three treatments for each species; (1) infecting alone or arriving first, (2) infecting after the other species, (3) infecting simultaneously with the other species.

The cercariae of both *Acanthoparyphium* and *C. australis* were obtained from their respective gastropod hosts. Snails or whelks were induced to shed in the same manner as described for screening infected individuals. A group of 20 snails and 20 whelks were selected to provide a pool of mixed genotype cercariae. The same group of snails and whelks was used throughout the entire duration of the experiment across the different treatments. For details regarding the protocol of handling and labelling the cercariae with fluorescent dye, see Leung et al. (2010). Cercariae of both species were treated identically, although the shedding period and availability of *Acanthoparyphium* cercariae from the mud snail *Z. subcarinatus* was more erratic than for *C. australis.* For infection treatments that involved both species, the cercariae of each species were labelled with a different coloured dye, alternating between a green and a red fluorescent dye to differentiate the metacercariae of the two species. The dyes used have been found not to affect

survival, infectivity or encystment site of the cercariae (Keeney et al., 2008; Leung et al., 2010).

For experimental infection, cockles were placed individually in plastic cylindrical containers (60 mm high × 40 mm wide) and completely covered with fine sand so that only the cockle's siphons would protrude above the substrate. The container was then filled with seawater to a depth of 15 mm above the sediment surface. The cockles were given at least 60 min to acclimatise to the container and start filtering the water layer, before a batch of 60 or 30 (depending on the treatment) dye-treated cercariae was added to the water with a 200 μL pipette. The cockles in mixed-species infections received a second batch of 30 dye-labelled cercariae either the following week or simultaneously, again depending on the treatment.

The container holding the cockle and the cercariae was kept in the dark for 24 h to minimise the influence of light on the fluorescence of the dye. After a 24 h exposure period, the cockles were removed from their containers and each given a unique identification number on the shell with a permanent marker. The cockles were then haphazardly assigned to a holding container. These were opaque plastic containers (240 mm long × 190 mm wide × 120 mm high) filled with seawater and 20 mm of fine sand for substrate and aerated with an airstone. Up to 10 cockles were kept in each container. The parasite's life-cycle means that cross-infection between exposed cockles held in the same container cannot occur.

Before they were dissected to locate the metacercariae, the shell length of each cockle was measured to the nearest 0.1 mm with Vernier callipers. The foot was removed by cutting along the narrow bridge between the gonad and foot basis, and placed between two glass plates that were pressed firmly together to gently flatten the foot. All encysted metacercariae were visible through the transparent foot tissue. The foot was then cut into three sections as detailed in Leung et al. (2010). The tip area matched roughly the part of the foot protruding from the shell when a cockle attempts to burrow, i.e. the part most likely to be cropped by fish, whereas the base area is never exposed to cropping; the middle section of the foot was not considered in the analyses that follow. While flattened between the glass plates, each section of the foot was examined with a fluorescence stereomicroscope. All metacercariae (red, green or non-dyed previously existing metacercariae) from each section were counted by eye.

2.3. Statistical analyses

Statistical analyses of the data were conducted with SPSS Statistics 17.0. All statistical analyses conducted were standard parametric tests. Variables that did not conform to the assumptions of normality were log-transformed. Potential confounding factors were treated as covariates. Here, we define infection success as the percentage of experimentally introduced cercariae which became established in the host tissue. Encystment patterns were calculated based on the percentage of metacercariae found at a particular part of the foot out of those which successfully established in the host tissue. P values <0.05 were considered significant.

3. Results

A total of 99 cockles were each individually exposed to cercariae; of these only two cockles did not become infected with new metacercariae. The mean shell length ± S.E. of the cockles used was 32.6 ± 0.2 mm. The total number of metacercariae already encysted in the foot of the cockles prior to the experiment ranged from 0 to 107 per cockle (mean = 18.4 ± 1.9, median = 12). Five of the cockles were free of pre-existing infection. To test for variations in size and prior infection levels of the cockles used in the

experiment, the cockles were assigned to three groups; (1) exposed only to one species, (2) exposed to two species with delay, (3) exposed to two species simultaneously. One-way ANOVAs found no significant difference in shell length of cockles in the different groups ($F_{2,96}$ = 1.23, P = 0.297), however the level of prior infection differed between the groups of cockles ($F_{2,96}$ = 4.23, P = 0.017). The pre-existing metacercariae consisted of a mixture of *Acanthoparyphium* and *C. australis* that were already well-established in the foot tissue. Cockles are known to readily regenerate their foot tissue (Mouritsen and Poulin, 2003a), and the cockle would have healed from penetration scars or other forms of host-modification from those prior infections. Therefore, their effects on experimentally introduced cercariae should be minimal. However, to correct for any potential effects of the established metacercariae, the level of prior infection was entered as a covariate in subsequent analyses.

3.1. Infection success

We found an overall difference in the infection success of *Acanthoparyphium* and *C. australis*. When infecting alone or first, the infection success of both species differed significantly (two-sample t-test, T = 2.61, d.f. = 62, P = 0.035), with *C. australis* having higher mean infection success than *Acanthoparyphium* (Fig. 1). The extent of this trend was magnified in mixed-species treatments where the focal parasite arrived after or simultaneously with the other species (two-sample t-test, T = 12.10, d.f. = 35, P < 0.001). In such situations, the infection success of *C. australis* greatly surpassed that of *Acanthoparyphium* (Fig. 1).

We then further tested how the experimental treatment (presence or absence of another species and their order of arrival) affected infection success. We analysed each of the two species separately, using an ANCOVA with the treatment as the main effect and infection success (log [x + 1]-transformed% of cercariae that become established out of the experimental dose) as the dependent variable, with shell length and log-transformed number of prior infections as covariates. The infection success of *Acanthoparyphium* was not affected by shell length of the host ($F_{1,75}$ = 1.55, P = 0.217) nor prior infections ($F_{1,75}$ = 0.13, P = 0.721),
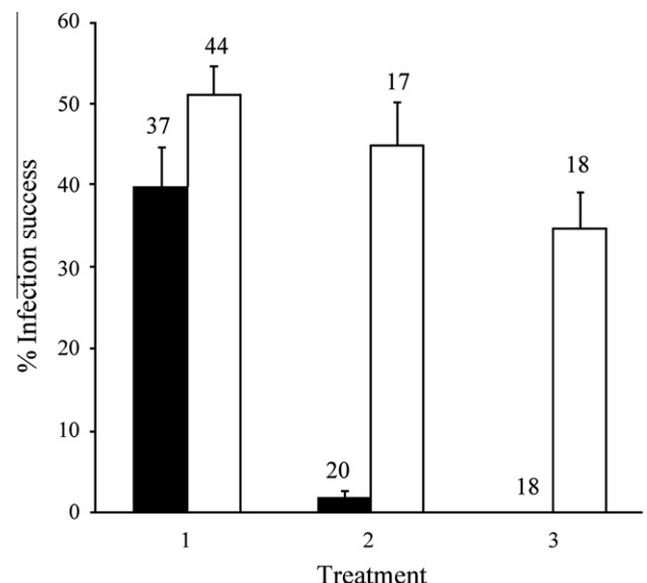

Fig. 1. Mean ± S.E. infection success of *Acanthoparyphium* (black bar) and *Curtuteria australis* (white bar) in treatments 1 (infecting alone or arriving first), 2 (infecting after the other species) and 3 (infecting simultaneously with the other species). Numbers above the bars are the number of hosts in each category.

but was significantly influenced by treatment ($F_{2,75}$ = 26.69, $P < 0.001$). The infection success of *Acanthoparyphium* dropped drastically when it arrived after *C. australis* and of the 18 cockles that were each exposed to an equal dose of 30 *Acanthoparyphium* and 30 *C. australis* cercariae simultaneously, none managed to acquire a single *Acanthoparyphium* metacercaria (Fig. 1).

For *C. australis,* the shell length of the cockle ($F_{1,79}$ = 3.47, $P = 0.066$) did not have a significant effect on infection success, however the level of prior infections ($F_{1,79}$ = 4.34, $P = 0.041$) and treatment ($F_{2,79}$ = 3.16, $P = 0.048$) had marginal influences. Unlike *Acanthoparyphium*, the infection success of *C. australis* decreased only slightly when arriving after or simultaneously with the cercariae of *Acanthoparyphium* (Fig. 1).

3.2. Encystment site

To investigate the encystment patterns of the parasites, only the experimental metacercariae that managed to successfully establish in a cockle were considered. When the encystment strategies of both species were compared, we found that the proportion of *Acanthoparyphium* encysting at the foot tip was significantly lower than that of *C. australis* (two-sample *t*-test, $T = 7.33$, d.f. = 48, $P < 0.001$; Fig. 2). The trend was reversed when considering the proportion of metacercariae encysting in the base or 'safe' part of the foot, with a higher mean proportion of *Acanthoparyphium* found at the base than *C. australis* (two-sample *t*-test, $T = 4.73$, d.f. = 50, $P < 0.001$; Fig. 2).

We investigated the effect of treatment on the encystment strategy of *Acanthoparyphium* and *C. australis,* respectively. We used an analysis of covariance (ANCOVA) with the treatment as the main effect, and the proportion of metacercariae encysting in the tip (log [$x + 1$]-transformed% of metacercariae encysted at the foot tip out of the total number established) as the dependent variable, with shell length and log-transformed number of prior infections as covariates. The analysis was also repeated for each species but with the proportion of metacercariae encysting at the base of the foot (also log [$x + 1$]-transformed) as the dependent variable.

The propensity for *Acanthoparyphium* to encyst at the foot tip was marginally influenced by host shell length ($F_{1,39}$ = 4.23,
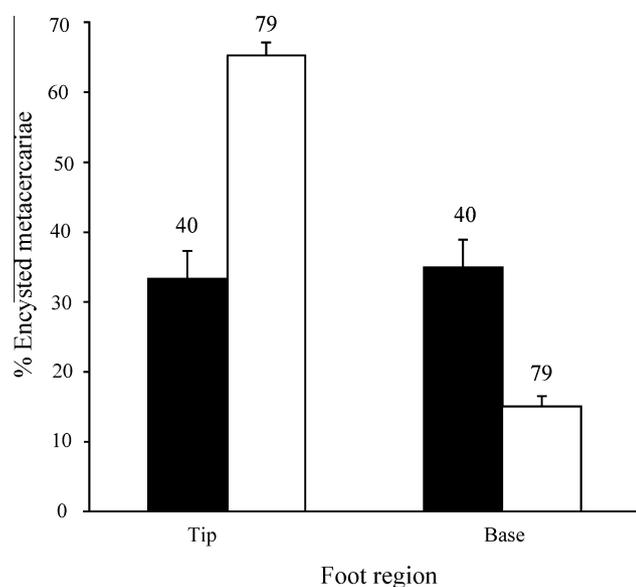
$P = 0.047$) but not affected by treatment ($F_{1,39}$ = 0.21, $P = 0.646$) or the number of pre-existing metacercariae ($F_{1,39=}$ 0.16, $P = 0.695$). The proportion of metacercariae encysting in the 'safe' part of the foot was not influenced by treatment ($F_{1,39}$ = 2.76, $P = 0.105$), host length ($F_{1,39}$ = 2.69, $P = 0.110$), nor level of prior infections ($F_{1,39}$ = 0.46, $P = 0.503$).

The proportion of *C. australis* cercariae encysting in the tip of the foot was not affected by the treatment ($F_{2,77}$ = 1.18, $P = 0.314$), host shell length ($F_{1,77}$ = 2.20, $P = 0.143$), nor the level of pre-existing metacercariae ($F_{1,77}$ = 0.14, $P = 0.709$). The mean proportion of *C. australis* cercariae that settled at the base of the foot was significantly influenced by treatment ($F_{2,77}$ = 4.28, $P = 0.018$), with fewer encysting in the base when they had arrived after *Acanthoparyphium* than when *C. australis* was by itself or the first to arrive, or if it had arrived simultaneously with *Acanthoparyphium* cercariae (Fig. 3). However, host length ($F_{1,77}$ = 1.67, $P = 0.200$) and the level of prior infections ($F_{1,77}$ = 0.21, $P = 0.648$) had no effect.

4. Discussion

This study found differential infection performance and encystment patterns between two species of ecologically equivalent parasites. While both species parasitise the same host and are localised in the same microhabitat within the host, our experiments have revealed subtle differences and interactions that are not observable in field samples. We found that *C. australis* is better overall at infecting cockles than *Acanthoparyphium*. While both species achieve lower infectivity if arriving after or simultaneously with infective stages of the other species, *Acanthoparyphium* was clearly more severely affected. *Curtuteria australis* was the stronger competitor overall, both in terms of infection success and performance in the presence of another species. Sequential exposure to different parasites can alter the pattern of interspecific associations from the expected outcome (Karvonen et al., 2009). Seppälä et al. (2009) found co-infection to have an asymmetrical effect on the infectivity of two species of *Diplostomum* – *Diplostomum spathaceum* and *Diplostomum gasterostei.* Co-exposure with cercariae of the other species affected the infection success of *D. spathaceum* more strongly than that of *D. gasterostei*. However, this was found to be heavily strain-dependent with certain strains of *D. spathaceum* achieving greater success while others were less infective in the presence of the other species (Seppälä et al., 2009).

In this study, the number of previously encysted metacercariae had a marginal influence on *C. australis* infection success. This is
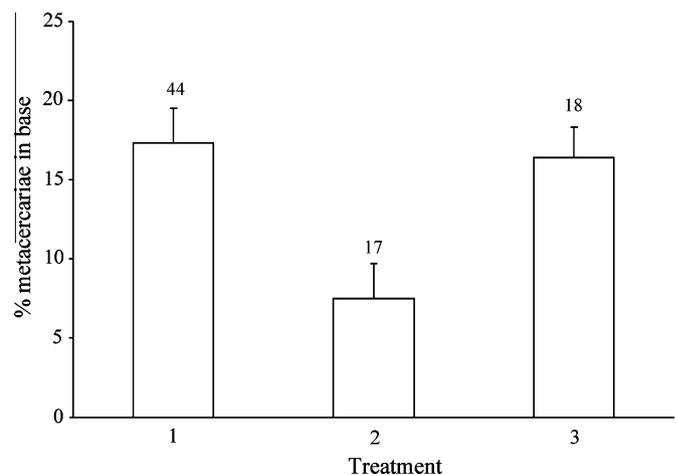
Fig. 2. Mean ± S.E. percentage of *Acanthoparyphium* (black bar) and *Curtuteria australis* (white bar) metacercariae encysting at the tip or the base of the cockle foot. Proportions of metacercariae recovered in each section of the foot were averaged across all the treatments. Numbers above the bars are the number of hosts in each category.

Fig. 3. Mean ± S.E. percentage of *Curtuteria australis* metacercariae encysting at the base of the cockle foot in treatments 1 (infecting alone or arriving first), 2 (infecting after the other species) and 3 (infecting simultaneously with the other species). Numbers above the bars are the number of hosts in each category.

similar to the findings of Leung et al. (2010), though the weaker effect found in this study may be due to dose-dependency or genotypic effects (Rauch et al., 2008; Fellous and Koella, 2009; Fox and Rauch, 2009), which have been known to alter infection outcome. Whereas each individual infection trial by Leung et al. (2010) consisted of 30 cercariae from a single clone, the exposure dose used in the present study consisted of 60 cercariae drawn from a pool of individuals consisting of different genotypes.

While different strains/clones of *C. australis* were not found to differ significantly in their infectivity (Leung et al., 2010; Leung and Poulin, 2010), it is unknown whether this also applies to *Acanthoparyphium*. The performance of both *C. australis* and *Acanthoparyphium* decreased when they either arrived after or simultaneously in the host with the other species, but *Acanthoparyphium* was much more negatively affected than *C. australis*. This asymmetrical effect between species is reminiscent of the interaction between *D. spathaceum* and *D. gasterostei* (Seppälä et al., 2009). Seppälä et al. (2009) suggested that *D. spathaceum* and *D. gasterostei* interacted indirectly through modulating host defence responses, and this mechanism might explain why fewer *Acanthoparyphium* cercariae became established in cockles which had prior exposure to *C. australis*. Perhaps during sequential exposure, *C. australis* is able to evade any immune response mounted by the host, but not *Acanthoparyphium* which arrive in a host which has already been immune-primed by *C. australis*. However, we do not expect the already established metacercariae which had been naturally acquired to have the same effect, due to the time elapsed between natural and experimental infections (see Mouritsen, 2002 for rate of infection). Thus there should have been sufficient time for any immune priming effect from those prior naturally acquired infections to wear off, allowing the effects of experimentally introduced cercariae to be distinguished from those of prior infections.

A similar situation may apply during simultaneous exposure to the cercariae of both species; the cockle may mount an immune response which would result in the exclusion of one parasite but not the other. While it is possible that the infective stages themselves may physically interact, since the cercariae spend some time crawling on the surface of the cockle's foot before penetrating, there is no evidence to suggest they attempt to interfere or dislodge each other in any way (T. Leung, personal observation).

The exclusion of *Acanthoparyphium* by *C. australis* during simultaneous exposure might also be mediated by host behavioural responses to the presence of the cercariae and the behaviour of the cercariae themselves. Cockles have been observed to increase their pumping rate after cercariae enter the mantle cavity via the inhalant siphon (T. Leung, personal observation), presumably in an attempt to expel the infective stages (see Wegeberg et al., 1999). Additionally, the smaller *Acanthoparyphium* cercariae spend considerably more time crawling on the cockle's foot prior to penetration, whereas the larger *C. australis* cercariae cling within minutes to the foot and commence penetrating its epidermis (T. Leung, personal observation; see also Allison, (1979) and Martorelli et al. (2006) for dimensions of the cercariae). If the actions of *C. australis* cercariae elicit a strong behavioural response from the cockle in the form of increased pumping rate, the still crawling *Acanthoparyphium* cercariae may be more likely to be swept away by the flow than *C. australis* cercariae which are already attached.

While field samples seem to show that both *Acanthoparyphium* and *C. australis* have broadly similar encystment patterns within the cockle's foot and contribute equally to host manipulation (Babirat et al., 2004), the results of this study indicate that the actual encystment pattern of both species are masked by spatiotemporal variation in recruitment over the host's lifetime.

Although some *Acanthoparyphium* metacercariae encyst in the foot tip, the overall proportion encysted at that site is approximately half that of *C. australis* (see Fig. 2). This has major implications for the cost of host manipulation for both species. The metacercariae of *Acanthoparyphium* seem to distribute themselves more evenly throughout the foot than those of *C. australis*, with some individuals acting as manipulators by encysting in the foot tip and others not manipulating at the base of the foot where they are safe from non-host predators. Perhaps this reflects *Acanthoparyphium* adopting a less risky, bet-hedging strategy in comparison with that of *C. australis*. However, since the cercariae for this experiment came from a pool of mixed genotypes, this distribution pattern may actually result from a higher range of intraspecific variance in host manipulation strategies within *Acanthoparyphium*. The prior arrival of *Acanthoparyphium* also appears to influence the encystment site of any subsequent *C. australis* cercariae, with fewer *C. australis* metacercariae encysting in the foot base of cockles previously exposed to *Acanthoparyphium*. While Leung et al. (2010) found that the presence of metacercariae in the foot tip result in more *C. australis* encysting there, prior infections do not influence their affinity for encysting in the base. *Acanthoparyphium* exert the opposite effect in a different part of the cockle's foot. Since, as previously discussed, *Acanthoparyphium* tend to encyst at the base more so than *C. australis*, the site may become saturated for *C. australis* to settle there. Alternatively, it may even be part of the strategy of *Acanthoparyphium* as a hitch-hiker to discourage newly arriving cercariae from encysting at a site where they will not contribute to altering host behaviour.

Mouritsen (2002) suggested that most metacercariae encyst at the foot tip because it is the most prominent feature within the mantle cavity, thus it may be where incoming cercariae are most likely to accumulate. However, the encystment pattern of *Acanthoparyphium* shows that this is not necessarily the case. Clearly, choice of encystment site within the cockle's foot is not a passive process as different clonal lineages of *C. australis* vary in their affinity for settling at the foot-tip (Leung et al., 2010).

The encystment pattern of *Acanthoparyphium* makes it an "incomplete hitch-hiker" (or an "incomplete manipulator"). In previously reported cases of hitch-hikers or potential hitch-hikers (Thomas et al., 1997; Leung and Poulin, 2007), the hitch-hiker makes no contribution to altering host phenotype. However, the life-history strategies of trophically transmitted parasites exist along a continuum, with hitch-hiking at one end and host manipulation at the other. Even within known "manipulator" species there is intraspecific variation in manipulation investment and virulence (e.g., Franceschi et al., 2008; Cornet et al., 2009; Leung et al., 2010). Therefore, it is not altogether surprising that *Acanthoparyphium* and *C. australis* differ in their respective host exploitation strategies. While *C. australis* invests more in host manipulation, and pays a higher potential cost of non-host predation, it also out-competes *Acanthoparyphium* in terms of infectivity and establishment success.

Such trade-offs between two infection characteristics are also seen within *Diplostomum* spp., where different genotypes seem to exhibit a trade-off between their ability to penetrate the fish second intermediate host and their ability to migrate to their encystment site within the eye lens (Voutilainen et al., 2010). The interaction between *Acanthoparyphium* and *C. australis* may shed light upon why costly host manipulation persists even in the presence of hitch-hikers that exploit manipulators without sharing the potential costs. The manipulators may exhibit other traits that allow them to outperform hitch-hikers in other aspects of fitness (in this case infectivity and coping with competition).

Given the apparent overwhelming competitive advantage of *C. australis* over *Acanthoparyphium* in infecting cockles, how is it that the latter species still co-exists in sympatry with a superior competitor? There are a number of potential factors that could maintain *Acanthoparyphium* in the presence of *C. australis*.

Firstly, the relative abundance of the first intermediate host in the environment can play a role in compensating for the lower

infectivity of *Acanthoparyphium*. While it has not been quantified, at many sites the abundance and density of the algal-grazing mud snail *Z. subcarinatus*, *Acanthoparyphium's* first host, is noticeably higher than that of the first host of *C. australis*, the carnivorous mud whelk *C. glandiformis* (T. Leung, personal observation). Thus, *Acanthoparyphium's* lower infection success and competitive ability may be compensated by its larger pool of cercariae in the environment. This highlights the potential role of upstream host abundance in determining the community of parasite larvae being recruited into the downstream host.

Secondly, the encystment strategy of *Acanthoparyphium*, whereby proportionally fewer metacercariae are found in the cockle's foot tip, means that fewer metacercariae are lost to foot-cropping by non-host predators. Because *C. australis* has a much stronger preference for encysting at the foot tip, they could also suffer a correspondingly higher cost. In fact, Mouritsen and Poulin (2003b) calculated that approximately 17% of all encysted metacercariae are lost to foot-croppers. Given the findings of this study, it is possible that even though *Acanthoparyphium* and *C. australis* co-occur in cockles (Babirat et al., 2004), loss to non-host predators is likely to be incurred disproportionately by *C. australis* metacercariae.

Finally, nothing is known about the relative infectivity, life-span, reproduction capacity and competitive interactions between *Acanthoparyphium* and *C. australis* within their bird definitive host. When an oystercatcher swallows a cockle, hundreds of metacercariae of both species are recruited into its gastrointestinal tract, so they would most definitely co-occur. Further ecological interactions such as facilitation, displacement or site segregation can also be expected to occur given what is known about other gastrointestinal parasites (e.g.: Friggens and Brown, 2005; Behnke et al., 2009; Hamann et al., 2009). Studies on potential interactions at this stage would require the development of some kind of in vitro systems which allow direct observations of the adult worms.

There is a need to view patterns of parasite aggregation and interaction within a wider ecological context, especially when considering parasites with multi-host life-cycles. The patterns seen in field samples may have come about as a result of differential interspecific interactions that can occur at each stage of the life-cycle. The nature of such interactions can only be revealed through experimental infections.

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