

Recruitment rate of gymnophallid metacercariae in the New Zealand cockle *Austrovenus stutchburyi*: an experimental test of the hitch-hiking hypothesis

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Abstract The rate at which host organisms accumulate parasites is affected by a number of intrinsic and extrinsic factors. The New Zealand cockle *Austrovenus stutchburyi* is frequently parasitised by trematodes comprising of two species of echinostomes and a species of gymnophallid that use it as a second intermediate host for trophic transmission to avian definitive hosts. The echinostomes are capable of manipulating the burrowing behaviour of the cockle to enhance their transmission success, whereas the gymnophallid is not capable of host manipulation. Previous studies have found patterns of positive associations between the echinostomes and the gymnophallid. Thus, it is possible that the latter is a “hitch-hiking” parasite that preferentially infects cockles already heavily infected by echinostome metacercariae to enhance its own transmission rate. A field experiment involving cockles forced to remain either above or below the sediment surface to simulate manipulated and non-manipulated cockles was conducted to test the hitch-hiking hypothesis. The gymnophallid was not found to display any preference for either surfaced or buried cockles; therefore, it cannot be considered as a hitch-hiking parasite. Possible alternative reasons for the pattern of positive association between the gymnophallid and the echinostomes are proposed.

Introduction

The distribution of parasites within a host population tends to follow that of an over-dispersed pattern, with most of the

parasites aggregated in a few individuals (Poulin 1998). There are a number of factors that can determine why certain individuals are more susceptible to parasitic infections. These factors can be intrinsic, such as the host organism’s innate resistance to infection (Grosholz 1994; Schmid-Hempel 2003), age (Gurski and Ebbert 2003), size (Wegeberg et al. 1999; Timi and Lanfranchi 2006), mobility (Webber et al. 1989) or behaviour (Karvonen et al. 2004). There are also extrinsic factors that can influence an individual’s risk of parasitism, such as spatial distribution—over both large (Buck et al. 2005) and small scales (Poulin and Fitzgerald 1989; Grosholz 1994; Poulin and Mouritsen 2004)—or various features of its habitat (Jensen et al. 2004; Ferreira et al. 2005).

For suspension and deposit-feeding bivalves of soft-sediment intertidal communities, most of the variation in their small-scale distribution is usually expressed in the form of different patterns of burial depth. Although generally, larger individuals tend to bury deeper into the sediment (Zaklan and Ydenberg 1997), the burial depth of bivalves can be influenced by a range of factors such as food availability (Lardies et al. 2001), immersion time (de Goeij and Honkoop 2003) and predation pressure (Blundon and Kennedy 1982). Differential burial depths can also be a consequence of sub-lethal predation (de Goeij et al. 2001) or parasitism (Thomas and Poulin 1998). These variations in the pattern of vertical distribution can have different ecophysiological implications for soft-sediment intertidal bivalves—the result of which is essentially a trade-off between feeding rate and predation risk, as bivalves buried at shallower depth in the sediment have a higher feeding rate (which can lead to faster growth) but are also at greater risk of predation (Zaklan and Ydenberg 1997). However, might shallower burial depth also expose the bivalve to a higher risk of parasitism in comparison to more deeply

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buried conspecifics, especially if the pattern of burial depth itself is a consequence of parasitic infection?

The New Zealand cockle *Austrovenus stutchburyi* is commonly parasitised by metacercariae of two echinostome trematode species that encyst in its foot. At high infection intensity, they can impair the cockle's ability to bury, forcing it to remain on the sediment's surface and facilitating the parasites' transmission to the definitive host by exposing the cockle to avian predators (Thomas and Poulin 1998; Mouritsen 2002; Babirat et al. 2004). The metacercariae that encyst in the foot of *A. stutchburyi* consist of two species from the Himasthlinae subfamily, *Curtuteria australis* and *Acanthoparyphium* sp., but because of similarities in their level of abundance and the identical ecological roles they serve in the soft-sediment intertidal ecosystem, they are considered as ecological equivalents (Babirat et al. 2004) and will in this study be collectively referred to as 'the echinostomes.'

At certain localities in the Otago Harbour (South Island, New Zealand), *A. stutchburyi* are also parasitised by metacercariae of an undescribed species of gymnophallid, with infection intensity ranging from tens to hundreds of metacercariae per cockle (Poulin et al. 2000; Leung and Poulin 2007). The gymnophallid shares the same definitive host as the echinostomes, which consist of shorebirds that feed upon the surfaced cockles.

Previous studies have found that among naturally infected cockles, the infection intensity of the gymnophallid is positively associated with the number of echinostome metacercariae found in the host (Poulin et al. 2000; Leung and Poulin 2007). This is not merely a product of the gradual accumulation of both types of metacercariae as cockles develop and age, because this positive association remains when the effects of cockle size is controlled for. Although gymnophallids from the Northern Hemisphere have been reported to cause various forms of pathology that would elicit or facilitate predation of their bivalve host by shorebirds (Bower et al. 1996), the local species of gymnophallid does not appear to impair its cockle host in any manner that would enhance its own transmission. Therefore, it is possible that instead of being a manipulator, it relies on associating with the echinostomes to enhance its transmission to the definitive host. For these reasons, it is suspected that the gymnophallid is a "hitch-hiker" parasite.

Hosts infected with manipulative parasites often exhibit behaviours that lead to different microhabitat preferences in comparison to non-parasitised conspecifics. This altered microhabitat of the host presents a possible mechanism through which so-called hitch-hiker parasites (Thomas et al. 1998a) can selectively infect hosts that are already harbouring manipulative parasites. Hitch-hiking parasites may accomplish this by having free-living larval stages in the same microhabitats where manipulated hosts are found.

An example of this was found by Thomas et al. (1997) regarding the microphallid trematode *Maritrema subdolum*. Another microphallid trematode, *Microphallus papillorobustus*, is known to alter the behaviour of the amphipod *Gammarus insensibilis*, inducing hyperactivity and positive phototaxis (Helluy 1983). Thomas et al. (1997) found that not only is the occurrence of *M. subdolum* in the amphipod positively associated with that of *M. papillorobustus*, but also the cercariae of *M. subdolum* tend to swim higher in the water column where *G. insensibilis* parasitised by *M. papillorobustus* are located. By doing so, *M. subdolum* can substantially improve its chances of encountering an amphipod already parasitised by the manipulative *M. papillorobustus* with which it shares the same definitive host (shore birds). Since the term "hitch-hiker parasites" has been coined by Thomas et al. (1997), the positive association between *M. papillorobustus* and *M. subdolum* in their amphipod host remains the sole existing example of the hitch-hiker hypothesis for which a mechanism behind the positive association has been identified and experimentally demonstrated.

In the case of *A. stutchburyi* infected with echinostomes, their altered microhabitat preference is on the exposed surface of the sediment (as opposed to below the sediment), as a consequence of their impaired foot muscle (Thomas and Poulin 1998; Mouritsen 2002). If the gymnophallid is indeed a hitch-hiker parasite, it should display a preference for parasitising cockles that are stranded on the sediment surface and these cockles should accumulate gymnophallid metacercariae at a significantly greater rate in comparison with buried conspecifics. In this study, this hypothesis is tested in a field experiment.

Materials and methods

Cockles were collected from a mudflat at Blue Skin Bay (north of Dunedin, New Zealand), a site where cockles are known to have low infection intensity by echinostome metacercariae. A total of 120 similarly sized cockles were collected from the site and were randomly divided into three groups. Cockles from the first group (the "baseline" group, $N=40$ cockles) were immediately processed to establish baseline morphometric and parasite infection intensity data for comparison with the experimental groups (see below).

The experimental groups consisted of the "surfaced" and "buried" cockles intended to simulate cockles with different levels of echinostome infection, with the "surfaced" cockles ($N=41$) simulating those heavily infected with echinostome metacercariae and the "buried" cockles ($N=39$) simulating those with low levels of echinostome infection. Each cockle was placed individually in a plastic cage to secure it at the

experimental site. The cages were cylinders (170 mm high, 75 mm in diameter) made of plastic mesh (mesh size: 16 × 20 mm). The sediment within the cage was cleared of local cockles and the cage was pushed 90 mm into the sediment and further secured by a metal tent peg. Each cage was equipped with a platform made from the same material as the cages. The level of the platform was adjusted to be in contact with the surface of the sediment. For the “surfaced” group, the cockle was placed in the cage above this platform, which effectively prevented the cockle from burying into the sediment below, whereas for the “buried” group, the cockle was placed below the platform to ensure the cockle remained below the sediment. Once the cage was in place, a “roof” of the same plastic mesh was placed as a lid on the top of the cage to prevent predation from birds. Identical cages had been used previously and, with success, to quantify rates of echinostome accumulation in cockles under different conditions (Mouritsen 2002; Mouritsen and Poulin 2003).

The experiment commenced on the 25th of October 2005 at Company Bay, Otago Harbour. The cockles from this site are known to have high infection intensity of echinostome and gymnophallid metacercariae. The cages were placed at the low tidal line along a transect parallel to the waterline. A total of 80 cages were placed along the transect at 1-m intervals, alternating “surfaced” and “buried” cockles to prevent clustering of the treatments. The cages were collected 205 days later on the 17th of May 2006. During this period, the experimental site was regularly visited to ensure that the cockles and cages were in place, that the “buried” cockles actually remained buried and to remove any macroalgal growth on the cages.

All cockles, including the “baseline” cockles, were processed as follows. The length of each cockle’s shell was measured with digital callipers to the closest 0.1 mm. After the measurements were taken, the cockles were dissected and inspected for parasites under a dissecting microscope. The gymnophallid metacercariae were easily visible and were usually found in clumps on the outer mantle epithelium of the cockle, most of them accumulating near the hinge. The mantle cavity of the cockle was extensively examined for the presence of other symbionts or parasites known to be present in *A. stutchburyi* of Otago Harbour, such as the sporocysts of *Cercaria pectinata*, the mycolid copepod *Pseudomyicola spinosus* and pinnotherid crabs. All the metacercariae of both echinostome and gymnophallid found in each cockle were counted. The echinostome metacercariae were encysted within the foot of the cockle; therefore, to obtain an accurate count, each cockle’s foot was removed and placed individually in a tube of pepsin digestion solution (6 g pepsin and 7 ml 36–38% HCl in 1,000 ml water) and incubated at 40°C for 20–30 h. The process partially dissolves most of the host tissue

while leaving the metacercariae intact. After the incubation period, all the metacercariae in the tube were recovered and counted under a dissecting microscope.

Statistical analyses were conducted using the SPSS 11 statistical package. When the data deviated from normality, non-parametric tests were used for analyses. Otherwise, the data were analysed using parametric methods. All tests were two-tailed.

Results

After a storm that occurred 2 weeks before the cages were collected, 12 cockles from the buried group died, presumably because of anoxic conditions in the sediment layer they were located, caused by the increased level of sediment. A further two cages disappeared.

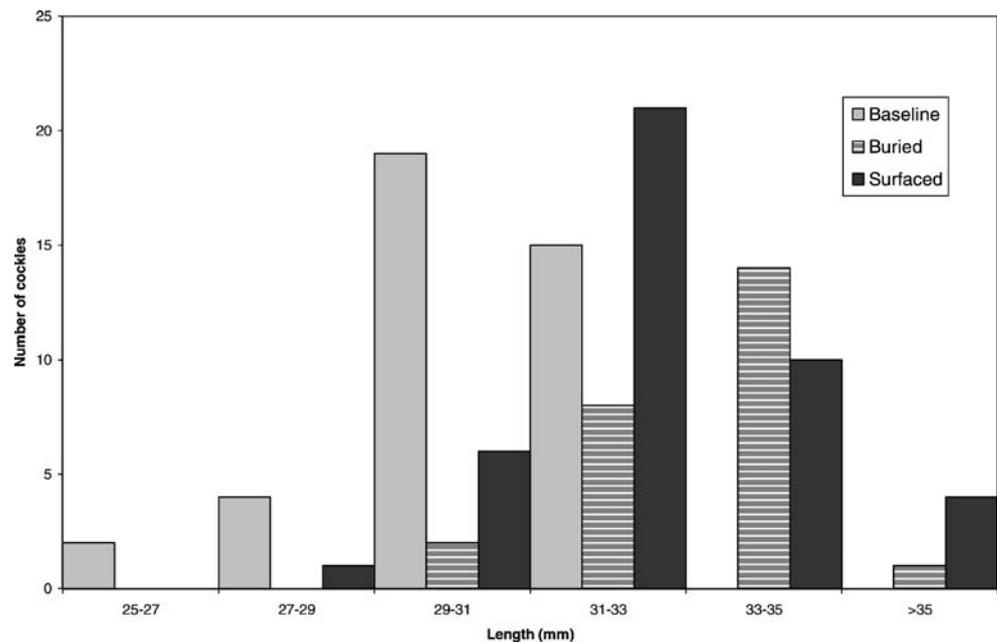
A total of 66 cockles from the experimental groups were recovered from Company Bay, consisting of 25 individuals from the buried group and 41 individuals from the surfaced group.

The majority of cockles from the baseline group were in the 30–31-mm size class (Fig. 1), and most had 20–30 echinostome metacercariae (Fig. 2) with a geometric mean of 27.7 metacercariae per cockle. No gymnophallid metacercariae were found in cockles from the baseline group.

The majority of cockles collected from the buried group were in the 33–34-mm size class (Fig. 1) with a mean difference of 2.8 mm when compared with the cockles of the baseline group. The majority of cockles from the surfaced group were in the 32–34-mm size class (Fig. 1) with a mean difference of 1.8 mm when compared with the cockles of the baseline group. An analysis of covariance conducted using the cockles groups as the main factor and the number of echinostome metacercariae as the covariate found significant differences in the size of the cockles based on groups ($F_{2, 102}=16.823, P<0.001$). Pairwise comparisons found the size of the cockles from both experimental groups to be significantly different from the baseline group (both $P<0.001$), whereas there was only marginal differences between the surfaced and buried groups ($P=0.057$), with the buried group experiencing slightly greater growth. Parasite load was not found to significantly increase with the size of the cockles ($F_{1, 102}=1.903, P=0.171$).

Most of the cockles from the buried group had between 40 and 100 echinostome metacercariae (Fig. 2), with a geometric mean of 70.8 metacercariae per cockle. The buried cockles had a mean of 38.8 more echinostome metacercariae than the baseline cockles. Most of the cockles from the surfaced group had between 50 and 120 echinostome metacercariae (Fig. 2), with a geometric mean

Fig. 1 The frequency distribution of the shell length of the cockles from the baseline group compared with the shell length of the cockles from the buried and surfaced treatment groups after the experiment



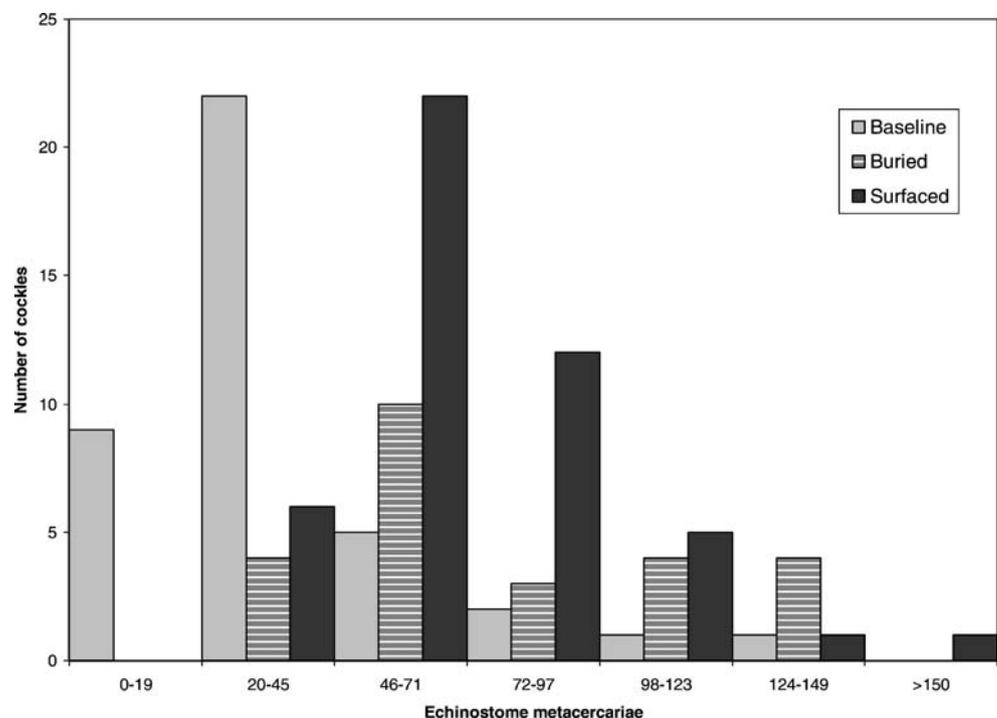
of 70.2 metacercariae per cockle. The buried cockles had a mean of 43.1 more metacercariae than the baseline cockles, whereas the surfaced cockles had a mean of 42.5 more echinostome metacercariae than the baseline cockles. A non-parametric two-tailed Mann–Whitney *U* test found that the echinostome metacercariae load of the cockles from both of the experimental groups were significantly different from that of the baseline group (both $P < 0.001$).

The difference in the number of echinostome metacercariae between cockles from the baseline and experimental groups corresponds to a mean accumulation rate of 0.2

metacercariae per cockle per day for both the buried and surfaced cockles.

The buried group accumulated a geometric mean of 0.17 (range=0–2) gymnophallid metacercariae per cockle, whereas cockles from the surfaced group accumulated a geometric mean of 0.27 (range=0–7) gymnophallid metacercariae per cockle. A non-parametric two-tailed Mann–Whitney *U* test did not find any significant difference between the number of gymnophallid metacercariae ($P = 0.301$) or echinostome metacercariae ($P = 0.786$) accumulated by the buried and surfaced cockles.

Fig. 2 The frequency distribution of numbers of echinostome metacercariae in the cockles from the baseline group compared with those in the cockles from the buried and surfaced treatment groups after the experiment



Two of the cockles from the surfaced group were found to have each acquired a single *P. spinosus* copepod during the course of the experiment; both individuals were adult females with egg sacs.

Discussion

Our experiments did not demonstrate that surfaced cockles accumulate gymnophallid metacercariae at a higher rate than buried cockles.

Company Bay was selected as the experimental site to test the hitch-hiking hypothesis because of the high prevalence (close to 100%) and infection intensity by gymnophallid metacercariae in the cockles of the area. The field experiment took place during the summer months, as it was the period of highest cercariae production because of the higher ambient temperature. The resulting low gymnophallid accumulation rate over a period of more than 6 months has interesting implications for the transmission dynamics of the gymnophallid and the ecological relationship between the parasite and its potential first intermediate host, the identity of which at present remains unconfirmed but is most likely to be the tellinid bivalve *Macomona liliana*. Although the accumulation rate found in the present experiment is compatible with the number of metacercariae found in cockles with moderate infection intensity measured in tens, it does not explain the individuals with higher infection intensities that can harbour hundreds of metacercariae (Poulin et al. 2000; Leung and Poulin 2007). It is possible that *M. liliana* infected with the sporocyst stage of the gymnophallid is sparsely distributed and that the prevalence is relatively low. High variation in infection intensities in the cockle may be the result of infection occurring in “bursts,” as clouds of cercariae are released from the first intermediate host in irregular bouts instead of being gradually released at a relatively constant rate. The high density of cockles may also serve to further “dilute” the infection intensity by spreading out the number of cercariae in the environment among more potential hosts. Combined with variation in the spatial distribution of *M. liliana* that possibly serve as first intermediate hosts, this can result in the uneven pattern of gymnophallid infection seen in the field-collected cockles while also explaining the low level of metacercariae recruitment in the present study.

It is interesting to briefly note that two of the cockles from the surfaced group became infected by the parasitic copepod *P. spinosus* during the experimental period. This shows similarities to earlier findings that *P. spinosus* occurs slightly more commonly among surfaced cockles (Leung and Poulin 2007). The more exposed position of these surfaced cockles may make them more accessible to the

infective copepodid of *P. spinosus* than its buried conspecifics. Tentative hints to how and why this might occur may rest with another parasite in the local system that utilises the cockle as a host. It has been found that surfaced cockles heavily infected with echinostome metacercariae were more likely to be infected by the sporocysts of *C. pectinata*, possibly because of the swimming behaviour of the miracidia of *C. pectinata* and the altered siphon position of the surfaced cockles (Poulin et al. 1998). It is possible that *P. spinosus* may be involved in a parallel situation.

Regarding the growth of the cockles during the course of the experiment, the literature reports overall greater growth for shallower buried individuals in both suspension and deposit-feeding bivalves resulting from greater feeding opportunities (Zaklan and Ydenberg 1997; de Goeij and Luttkhuizen 1998). Zaklan and Ydenberg (1997) reported greater food intake rates at shallower burial depth for the suspension-feeding bivalve *Mya arenaria*. However, although surfaced cockles may have better access to food particles suspended in the water column, as with other endobenthic bivalves that are exposed on the sediment surface, they are also subjected to environmental stressors such as desiccation and higher temperature build-up because of greater exposure to solar irradiance (Kurihara 2003). In addition, for the local system, surfaced cockles will also be the targets of predation attempts by groups of the local mud whelk *Cominella glandiformis* (Ansell 2001). An exposed cockle would be forced to close its valve in response to these sources of stress, thus periodically interrupting food intake and resulting in adverse effects on its growth rate. Although this study only found marginal differential growth between cockles of the buried and surfaced group, if the experiment had continued for longer, it is possible that this marginal difference would become more pronounced.

With regards to the recruitment rate of echinostome metacercariae, the estimated rate of accumulation of 0.2 metacercariae per cockle per day may at first seem surprisingly low in comparison with the findings of Mouritsen (2002), who estimated an accumulation rate of 0.5 metacercariae per cockle per day. This is especially striking considering the higher overall prevalence and infection intensity of metacercariae at Company Bay in comparison with Lower Portobello Bay where Mouritsen (2002) conducted his experiment. However, this can be explained by the higher cockle density at Company Bay compared with Lower Portobello Bay. As discussed earlier, in regards to the gymnophallid cercariae, the high density of cockles could mean that the finite number of echinostome cercariae released into the environment will be divided between more potential second intermediate hosts.

This long-term field experiment found no evidence that gymnophallid cercariae actively seek out cockles manipu-

lated by echinostomes. The recruitment of gymnophallid metacercariae into the experimental cockles does not indicate that they discriminate between surfaced and buried cockles, as would be expected if it was a hitch-hiking parasite. So, if the gymnophallid is not a hitch-hiker parasite, what could explain the positive association between its metacercariae and those of the echinostomes? This positive association cannot have just arisen through mere passive simultaneous accumulation of both the echinostomes and the gymnophallid from the environment because previous studies have found that the association remained even after correcting for cockle size (Poulin et al. 2000; Leung and Poulin 2007).

The factor(s) involved could be extrinsic to the cockle itself and be attributable to the properties of the environment or the first intermediate host. The pattern of positive association could have arisen because of close spatial proximity of the first intermediate hosts of the echinostomes and gymnophallid, as suggested by El-Darsh and Whitfield (1999) to explain the pattern of positive association between certain metacercariae that parasitise flounder, *Platichthys flesus*, from the tidal Thames. However, the reason could also be intrinsic and be attributable to something that is innate to heavily infected cockles (apart from impaired burying ability). It is possible that echinostome metacercariae may still predispose their host to future infection by gymnophallids in some way, even if the latter is not a hitch-hiker that actively seeks out surfaced cockles.

Although generally, under normal environmental conditions, echinostome metacercariae are considered relatively harmless to their bivalve second intermediate host (Wegeberg and Jensen 2003), they have been found to cause tissue damage during the process of cercarial penetration (Jensen et al. 1999), as well as making their host more vulnerable to hypoxic conditions (Wegeberg and Jensen 1999). Overall, infection by echinostomes has been implicated in causing stresses that can result in immunodepressive effects on their host (Desclaux et al. 2004), which make the host more vulnerable to future parasitic infections.

The gymnophallid may just be a “lucky passenger” (Thomas et al. 1998b) that shares the same second intermediate and definitive host as the echinostomes. It can still benefit from the echinostome’s host manipulation, even if it does not actively or preferentially parasitise manipulated host. In our system, because of the high prevalence and infection intensity of echinostomes in the cockles of Company Bay, it may be unnecessary for a non-manipulative parasite like the gymnophallid to evolve hitch-hiking life history traits to enhance transmission rate. In any case, despite the striking impact of the echinostomes on their host’s burying ability, their actual transmission rate is quite low because of the dietary preference of the local avifauna and the presence of non-host predators (Tompkins

et al. 2004). This may mean that the actual net enhancement in transmission rate gained from associating with manipulated cockles may be quite low; thus, selection pressure may not be strong enough to allow for hitch-hiking to evolve.

In terms of trophic transmission, hitch-hiking may not be a common life history strategy because of the potential costs involved in discriminating between parasitised and non-parasitised individuals. Hitch-hiking parasites may in fact be quite rare unless under extreme circumstances, such as the *M. papillorobustus* and *G. insensibilis* system, where the population is segregated into two very discrete units of parasitised and non-parasitised individuals by the effects of the manipulative parasite (Ponton et al. 2005). Despite the potential for an array of adaptations to have evolved among parasites co-occurring within a common intermediate host—described by Lafferty (1999) with the host-as-vehicle analogy—perhaps because of the actual fitness cost and benefits of such adaptations, the evolution of traits such as “hitch-hiking,” “hijacking” and “sabotage” among trophically transmitted parasites may be left unrealised in actual ecosystems.

In conclusion, the local species of gymnophallid appears to display no preference towards infected manipulated hosts and cannot be considered as a hitch-hiker parasite. Ultimately, as Poulin (2001) remarked, non-random patterns of association between parasites are only circumstantial evidence for interactions that must always be evaluated by experimental results, and alternative explanations are usually plausible without invoking direct interactions.

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