Effects of the trematode *Maritrema novaezealandensis* on the behaviour of its amphipod host: adaptive or not?

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Abstract

There are many recorded cases of parasites that are capable of altering the behaviour of their host to enhance their transmission efficiency. However, not all of these cases are necessarily the results of the parasites actively manipulating host behaviour; they may rather be the 'by-products' of pathology caused by the parasite's presence. This study investigates the effect of the microphallid trematode *Maritrema novaezealandensis* on the behaviour of one of its crustacean intermediate hosts, the amphipod *Paracalliope novizealandiae*. Uninfected amphipods were experimentally infected by exposure to *M. novaezealandensis* cercariae. The activity level and vertical position of experimentally infected amphipods were compared with uninfected amphipods at 2 weeks and 6 weeks post-infection, i.e. both before and after the parasite achieved infectivity to its definitive host. Infected amphipods were found to exhibit significantly lower levels of activity and to occur significantly lower in the water column than uninfected controls during both periods. Based on the timing of the change in behaviour exhibited by infected amphipods, the results suggest that the altered behaviour exhibited by *P. novizealandiae* infected with *M. novaezealandensis* is most likely due to pathology caused by the parasite rather than a case of active, and adaptive, behavioural manipulation.

Introduction

Many metazoan parasites in natural ecosystems have complex life-cycles in which two or more host species are involved. For parasitic helminths, transmission is often achieved through the process of trophic transmission, where an intermediate host must be ingested by the next suitable host for the life cycle to be completed (Lafferty, 1999). To facilitate this process, some parasites are capable of 'adaptive' host manipulation where the parasite alters the phenotypic properties of the host to improve its transmission rates to the next host, and thus its own fitness (Poulin, 1995; Moore, 2002). Despite the interest in studying host manipulation, the problem lies in determining whether the altered behaviour displayed by the host is due to adaptive manipulation by the parasite, or merely pathology caused by the parasite's presence (Poulin, 1995). To date, few of the described cases of altered host behaviour in the literature can definitely be attributed to active manipulation on the parasite's part (Poulin, 2000).

One of the ways of assessing the adaptive value of altered host behaviour is by measuring the fitness benefit of the host behavioural change incurred by the parasite (Poulin, 1995). While this may be difficult to measure directly, if the host displays behaviour that is detrimental to both itself and the parasite, it would be more likely that the altered behaviour is pathology rather than active manipulation. One of the possible indicators of the adaptive nature of altered behaviour is the timing of the onset of altered host behaviour. In theory, an active manipulator should only alter host behaviour when it becomes infective to its next host. For instance, various species of copepods belonging to *Cyclops* serve as intermediate host for cestodes. It has been found that parasitized copepods only display altered behaviour when they harbour procercoids that have reached a developmental stage when they are potentially infective to the next host (Poulin *et al.*, 1992; Urdal *et al.*, 1995). In other host–parasite systems, if the host exhibits aberrant behaviour while the parasite is still developing and not yet infective, then it would be more likely to be due

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to pathology; altered host behaviour at that stage would not only serve no purpose for the parasite, but may actually be detrimental if it causes the host to become more susceptible to predation before the parasite is ready for transmission. For example, Shirakashi & Goater (2005) found that minnows, *Pimephales promelas*, infected with the brain-encysting trematode *Ornithodiplostomum ptychocheilus* exhibit altered behaviour at 2 and 4 weeks post-infection when the parasite is not yet infective to birds. Interestingly, this period corresponds with the time of maximum growth for the parasite within the fish's brain (Shirakashi & Goater, 2005). However, at 10 weeks post-infection, when the parasite becomes infective and ceases development, the behaviour of infected fish showed no significant difference from that of uninfected fish. This indicates that the behavioural alteration is most likely due to unavoidable pathology associated with the developing parasite.

Here, the timing of the onset of behavioural changes induced by the parasite *Maritrema novaezealandensis*, a microphallid trematode recently described from Otago Harbour, South Island, New Zealand is examined. Its life cycle is typical of most microphallids (Martorelli *et al.*, 2004). The first intermediate host is the New Zealand mud snail *Zeacumantus subcarinatus*; cercariae released from the snail can then infect a number of different intertidal crabs and amphipods that serve as second intermediate hosts, which are then eaten by the red-billed gull *Larus novaehollandiae scopulinus*, the definitive host (Martorelli *et al.*, 2004).

Paracalliope novizealandiae (Eusiridae: Amphipoda) is an intertidal amphipod common to the Otago Harbour. It typically lives among patches of algae and seagrasses in the intertidal zone; it has been found to be one of the crustaceans that can serve as a second intermediate host for *M. novaezealandensis* (Fredensborg *et al.*, 2004). Although the impact of the trematode on the amphipod's behaviour has not been quantified, a previous study has suggested that *M. novaezealandensis* may be capable of depressing serotonin levels in the brain of another of its second intermediate hosts, the shore crab *Macrophthalmus hirtipes* (Poulin *et al.*, 2003). This physiological effect may induce behaviour in the crab that makes it more susceptible to predation from the parasite's definitive host (Poulin *et al.*, 2003). Given this finding in crab hosts, and the well-documented effects of other microphallid trematodes on crustacean behaviour (e.g. Helluy, 1983; Kunz & Pung, 2004), we expected *M. novaezealandensis* to be capable of manipulating the behaviour of its other second intermediate hosts, such as the amphipod *P. novizealandiae*. Predation of amphipods by avian definitive hosts occurs at low tide in shallow water puddles; amphipod activity and vertical distribution are not only traits likely to determine predation rates, they are also targets of manipulation in other microphallid–crustacean systems (Helluy, 1983, 1984; Kunz & Pung, 2004). These are therefore the behavioural traits investigated here.

The aims of this study were to determine whether *M. novaezealandensis* alters the behaviour of its amphipod second intermediate host, and whether this behavioural alteration is an adaptive feature that facilitates the parasite's transmission or simply a pathological side effect caused by the parasite's presence. Since heavy infections of *M. novaezealandensis* can kill amphipods

(Fredensborg *et al.*, 2004), the side-effect scenario is plausible. To distinguish between the adaptive and side effect explanations, the behavioural alterations induced by the parasite both before and after it reached a stage where it was infective to bird definitive hosts were quantified.

Materials and methods

Experimental infection

Several hundred amphipods, *Paracalliope novizealandiae*, were collected from Hoopers Inlet on the Otago Peninsula, South Island, New Zealand (45°52′S, 170°42′E). This locality was chosen due to the local absence of the snail first intermediate host, *Zeacumantus subcarinatus*, and its complete physical isolation from areas in which the snails occur, which ensured that amphipods collected from this inlet were free of *Maritrema novaezealandensis*, as we confirmed via preliminary dissection of numerous individuals from this locality (see also Fredensborg *et al.*, 2004). Amphipods were maintained in the laboratory for a few days, in seawater with strips of sea lettuce (*Ulva lactuca*), prior to infection.

Thirty snails, *Z. subcarinatus*, were randomly collected by hand during low tide from Lower Portobello Bay and were kept in a large container half-filled with seawater and strips of sea lettuce. Snails were later placed individually in 10 ml plastic dishes and incubated at 25°C for 60 min to promote the emergence of cercariae. Amphipods were randomly selected and placed individually in 0.5 ml vials. Six *M. novaezealandensis* cercariae were transferred from a dish into a vial containing an amphipod using a 200 μl pipette and the water volume within the vial was adjusted to 0.1 ml. All vials were then incubated at 25°C for 6 h to ensure penetration of the cercariae into the amphipod. Preliminary infections indicated that exposure to six cercariae generally results in two or three establishing in an amphipod, with a prevalence approaching 100% (see also Fredensborg *et al.*, 2004). These infection intensities correspond quite well to natural infection levels, with amphipods in sheltered bays where the parasite occurs typically harbouring 1–5 metacercariae per infected individual (Fredensborg *et al.*, 2004). Amphipods belonging to the control group were also incubated under the same conditions to take into account any possible subsequent behavioural changes due to the infection procedure.

After incubation, amphipods were transferred to clear plastic containers (330 mm long × 120 mm wide × 130 mm high) half-filled with sea water, containing strips of sea lettuce and small agar weed (*Pterocladia capillacea*) also collected from Hoopers Inlet, and oxygenated with an airstone. The density of amphipods per container was kept at a maximum of 200 individuals to prevent intraspecific competition, crowding, or cannibalism (T. Leung, personal observation). Sham-infected control amphipods were also kept in the same conditions. Tanks were kept in a room that was exposed to a 12/12 h light/dark photoperiod and kept at a constant temperature of 8 ± 1°C.

At 2 weeks post-infection, behavioural studies were conducted with the infected and control amphipods to see if there were any alterations to the amphipods' behaviour prior to the parasite's infectivity to birds that might indicate pathology. No further behavioural observations

took place until 6 weeks after experimental infection to allow the metacercariae to reach full maturity. Despite Martorelli *et al.* (2004) observing that *M. novaezealandensis* metacercariae took 4–5 weeks to fully mature, subsequent observations have found that the metacercariae do not become infective in the amphipod *P. novizealandiae* until 6 weeks after the initial infection.

Behavioural study

The behavioural study was conducted using a clear plastic container (55 mm long × 25 mm wide × 73 mm high) that had been marked on its outer surface with horizontal lines at 15 mm intervals, visually dividing the water column into four layers at 15, 30, 45, and 60 mm from the bottom. The bottom of the container was darkened with opaque black material to simulate the dark sediment bottom of the amphipod's natural environment and to prevent light from being reflected from the platform supporting the containers. For the behavioural studies, the container was filled with seawater up to the 60 mm mark and five amphipods from the infected group were randomly selected and introduced into the container. A perfectly identical plastic container was also filled with the same amount of seawater and had five amphipods from the control groups introduced into it. These two containers were placed side-by-side against a white background and the amphipods were given 10 min to acclimatize before filming started. Amphipods were then video-recorded for 5 min on a Sony NP-F750 digital handycam. The zoom level was adjusted so that the entirety of the two containers was visible, while at the same time occupying the maximum possible area of the screen. The resulting video was viewed and the position of each amphipod in the water column and their activity status (swimming or not) were recorded at intervals of 10 s, for a total of 30 observations per group of amphipods. This procedure was replicated 20 times, i.e. with 20 different groups of infected and control amphipods, at 2 weeks post-infection (PI), and again at 6 weeks PI with 20 other groups of infected and control amphipods.

Two types of behaviour were recorded, the activity level and vertical position of amphipods. These are independent behaviours since amphipods can cling to any substrate, including the sides of the containers, rather than sinking when they are not swimming. For activity levels, amphipods that were actively swimming at the time when the observation was made were given a score of one, while those that were clinging and not swimming were given a score of 0. For vertical distribution, each amphipod was given a score depending on its position in the water column when the observation took place. Amphipods that were in the bottom 15 mm layer of the container were given a score of 0, those between 15 and 30 mm a score of 1, those between 30 and 45 mm a score of 2, while those at the top 45–60 mm layer were given a score of 3. Activity and vertical position scores of all five individual amphipods in a group were summed and averaged for each observation of each trial. A preliminary analysis indicated that amphipod behaviour scores did not change consistently or significantly as a function of observation time (from the beginning to the end of the 5 min period, all $P > 0.15$), so this variable was not

considered in the analyses. Instead, mean scores were averaged across all 30 observations per group, giving a total of 20 group scores for both control and infected amphipods, at both 2 and 6 weeks PI.

Data were analysed with the Minitab 14 statistical package, using two-way ANOVAs with infection status and time PI (2 or 6 weeks) as the class variables, and activity level or vertical position group scores as response variables.

Results

Parasitized amphipods were found to display significantly different behaviour from control amphipods regardless of time post-infection.

The difference in activity levels between control and parasitized amphipods was significant ($F_{1,76} = 5.327$, $P = 0.0237$). There was, however, no significant effect of time PI ($F_{1,76} = 1.628$, $P = 0.2058$) and no significant interaction between infection status and time PI ($F_{1,76} = 0.504$, $P = 0.4797$). At both 2 and 6 weeks PI, parasitized amphipods were generally less active than control ones (fig. 1). The average activity level of parasitized amphipods changed very little between 2 and 6 weeks PI (1.35 vs 1.44; see fig. 1).

The difference between the vertical position of control and parasitized amphipods was also significant ($F_{1,76} = 5.137$, $P = 0.0263$). Again, there was no significant effect of time PI ($F_{1,76} = 2.755$, $P = 0.1011$) and no significant interaction between infection status and time PI ($F_{1,76} = 0.002$, $P = 0.9719$). Parasitized amphipods were generally found lower in the water column than control amphipods (fig. 2). There was also a general increase in the vertical position scores of both types of amphipods between 2 and 6 weeks PI (fig. 2).

Discussion

When interpreting behavioural and other phenotypic changes in the host organism due to parasitic infection,

Fig. 1. Average activity level (mean ± SE) for groups of control amphipods (□) and amphipods infected by the trematode *Maritrema novaezealandensis* (■), at 2 and 6 weeks post-infection. Values shown are the averages of 20 groups for each treatment. See text for method of measuring activity level.

it is necessary to be rigorous in the interpretation of the result and not attribute adaptive value to these alterations when they may simply be 'by-products' of infection (Poulin, 1995; Moore, 2002). Pathology can result in non-adaptive changes in host behaviour in a variety of ways. For instance, Franz & Kurtz (2002) found that behavioural changes in the copepod *Macrocyclops albidus* infected with the cestode *Schistocephalus solidus* were not due to energy depletion or a re-allocation of resources between muscles and lipids, but, rather, they proposed that the tapeworm directly manipulates copepod behaviour. Poulin (1995) advocated a number of criteria that must be met for a phenotypic alteration to be considered 'true' manipulation. So, how does the result of this study hold up against the scrutiny of these criteria? The change in amphipod behaviour caused by the trematode *Maritrema novaezealandensis* failed a simple test of adaptiveness; it is expressed too early in the infection to represent a parasite-induced increase in trophic transmission.

In previous studies, behavioural alteration of the host due to active manipulation was found to occur when the parasite becomes infective to its next host, but not before (Poulin *et al.*, 1992; Urdal *et al.*, 1995). In contrast, behavioural alteration due to pathology may occur while the parasite is developing and before it is infective (Shirakashi & Goater, 2005). The present results show that while parasitized amphipods displayed altered behaviour 2 weeks PI, at a stage when the parasite is not yet infective, this behavioural alteration persisted at 6 weeks PI when the parasite was infective. The only slight, and non-significant, difference in behaviour between 2 and 6 weeks PI was that all amphipods, whether infected or not, tended to occur a little higher in the water column at 6 than at 2 weeks PI; this may be a simple ontogenetic shift in microhabitat, as 4 weeks represents a significant proportion of the lifespan of these amphipods. Since altered behaviour associated with infection was expressed early, at a stage when the parasite was not
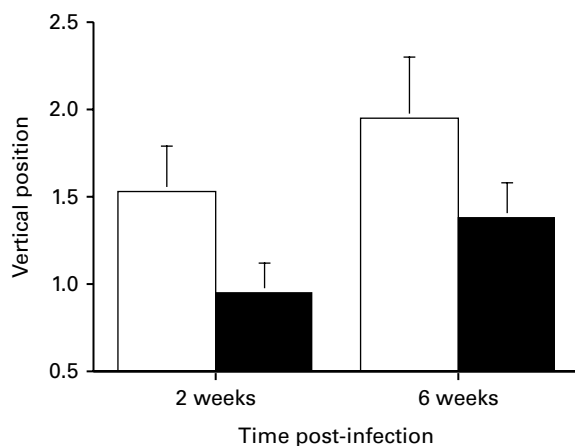
ready for transmission, the most parsimonious explanation is that it results from mere pathology. Of course, it is possible that alterations in host behaviour prior to infectivity to the definitive host could still result in net increases in transmission rates; such situations, however, would not persist long under strong natural selection.

The marked effects of *M. novaezealandensis* on the behaviour of its amphipod intermediate host are in sharp contrast to its effect on another intermediate host, the shore crab *Macrophthalmus hirtipes*. Based on a preliminary study on naturally-infected crabs, the trematode does not appear to alter crab behaviour (T. Leung, unpublished observations). This finding may be due to the masking influence of other parasite species (trematodes, acanthocephalans, and nematodes) in these crabs. It may also reflect biological differences between amphipods and crabs. For instance, compared to the small amphipods, the much larger crabs may not suffer from severe pathology following trematode infection. Fredensborg *et al.* (2004) have shown that high intensities of *M. novaezealandensis* cause severe pathology and even mortality in amphipods; the larger size of crabs may buffer them against such effects.

Earlier studies have shown that manipulative parasites can have different effects on hosts of different species within the same genus, let alone organisms from different orders that vary considerably in size, life style, and microhabitat preference. For example, while the acanthocephalan *Pomphorhynchus laevis* can alter the behaviour of the amphipod *Gammarus pulex* and make it more photophilic (Bakker *et al.*, 1997), this was not found to be the case for *Gammarus roeseli* (Bauer *et al.*, 2000). Similarly, the trematode *Microphallus papillorobustus* is known to infect two species of congeneric, sympatric amphipods (*Gammarus insensibilis* and *G. aequicauda*) (Helluy, 1983). In *G. insensibilis*, the parasite induces behavioural changes such that the infected amphipod becomes photophilic, prefers to swim closer to the water surface, and becomes hyperactive when confronted with an avian predator (Helluy, 1983; Helluy & Thomas 2003). These behavioural changes have been found to increase susceptibility of *G. insensibilis* to predation by bird definitive hosts (Helluy, 1984). However, in the case of *G. aequicauda*, behavioural changes only occur if the amphipod is infected during its juvenile stage (Helluy, 1983). The consequence for the two sympatric amphipods is that they experience very different impacts from this parasite, in terms of mortality by bird predation (Thomas *et al.*, 1995). For both *Pomphorhynchus laevis* and *Microphallus papillorobustus*, several lines of evidence point toward an adaptive manipulation of host behaviour; thus, the fact that *Maritrema novaezealandensis* causes changes in behaviour in amphipod hosts but not in crabs is not in itself a sign that the alteration is simply associated with pathology.

Regardless of the mechanism behind parasite-induced host phenotype alteration, the real question is how well it contributes to the efficiency of parasite transmission. Altered behaviour, even when found to be 'true' host manipulation by the parasite, does not always guarantee or necessarily translate into increased predation (Urdal *et al.*, 1995; Webster *et al.*, 2000). The true test of the adaptive value of parasite host manipulation must be its fitness potential for the parasite (Poulin, 1995). Within

Fig. 2. Average vertical position (mean ± SE) for groups of control amphipods (□) and amphipods infected by the trematode *Maritrema novaezealandensis* (■), at 2 and 6 weeks post-infection; the higher the score, the higher the position of the amphipods in the water column. Values shown are the averages of 20 groups for each treatment. See text for method of measuring vertical position.

the ecosystem in which the parasite and its host(s) occur, there are many factors involved in determining the fitness benefit of altered host behaviour. It is important that this fitness benefit be demonstrated before one is able to reject the null hypothesis of a pathological side effect as an explanation for altered behaviour in hosts (Tompkins *et al.*, 2004). The fitness benefits potentially associated with host manipulation should be measured in terms of increased transmission efficiency. Ideally, to investigate the transmission efficiency of *M. novaezealandensis* in its crustacean hosts in the context of the intertidal ecosystem, a full set of predation trials with both 2 week PI and 6 week PI (infective) parasitized amphipods would be necessary. If possible, these predation trials should be conducted with both appropriate, e.g. gulls, and non-host, e.g. flounders, predators to better reflect the predator guild that parasitized amphipods face in the field.

In conclusion, the most parsimonious explanation for the altered behavioural pattern of *P. novizealandiae* parasitized by *M. novaezealandensis* is pathology rather than true manipulation. But this also brings up the question of which of the parasite's two crustacean hosts, the amphipod and the crab, represents the main transmission route of *M. novaezealandensis* to its definitive host, the red-billed gull? Is it the small, but plentiful amphipod *P. novizealandiae*, each harbouring at most just a few metacercariae? Or, is it the larger, but less numerous crab *M. hirtipes*, each harbouring large numbers of metacercariae? These are the questions that need to be answered before the true role and ecological importance of *M. novaezealandensis* in Otago Harbour can be revealed.

Acknowledgements

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