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Parasite-induced behavioural changes to the trade-off between foraging and predator evasion in a marine snail

Tsukushi Kamiya *, Robert Poulin

Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand

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Trophic interactions in ecological communities not only affect consumer-resource densities, but also induce phenotypic responses that can ripple through a food web to influence prey resources, competitors and other predators (i.e. trait-mediated interactions). Predators affect short-term trade-off decisions in prey species through behavioural alterations, yet the role of parasites in the context of trait-mediated interactions is rarely discussed, despite our extensive knowledge of parasite-induced behavioural alterations. Adding parasites to the picture, the foraging–predation trade-off presents an interesting case where the effects of predator- and parasite-induced behavioural responses occur in conjunction. Empirical studies on the effect of parasites on trait-mediated anti-predator responses are still scarce, especially in marine systems. Our experiments used marine mud snails, *Zeacumantus subcarinatus* (Batillariidae), infected with the trematode parasites *Maritrema novaezealandensis* and *Philophthalmus* sp. to investigate the effects of trematode-induced behavioural alterations on the trade-off between food acquisition and predator avoidance in the presence of a range of predation cues. We found that the time taken to initiate anti-predator responses was overall significantly shorter in *M. novaezealandensis*-infected snails than in uninfected snails, while it was significantly longer for *Philophthalmus*-infected snails. In the simultaneous presence of odours from an injured conspecific and a crab predator, however, the reaction time between uninfected and *M. novaezealandensis*-infected snails did not differ while *Philophthalmus*-infected snails were still markedly slower than the former groups. Our results demonstrate species-specific parasite-induced behavioural changes in the presence of imminent predation risk. However, effects of the trematodes on overall feeding and fleeing times were not detected in our experiments; thus, the net effect of parasitism on the foraging–predation trade-off remains unknown.

1. Introduction

Predator–prey interactions have been traditionally discussed in terms of consumptive effects of predators that lead to changes in resource densities (Sih et al., 1985). However, empirical evidence is accumulating that predators affect the prey population not only by changing consumer-resource densities but also by inducing phenotypic responses in the prey (Schmitz et al., 1997; Werner and Peacor, 2003). Such predator-induced trait-mediated responses are known to occur at the level of morphology, life history, development and behaviour, and their effects can ripple through a food web to influence prey resources, competitors and other predators (Werner and Peacor, 2003). For example, the mere presence of predators can stimulate prey to reduce foraging activity (Peacor and Werner, 2000; Trussell et al., 2003), invest in defensive structures (Barry, 1994), or change habitat use (Turner and Mittelbach, 1990), with direct effects on the prey's overall fitness. These changes in phenotypic traits of prey species can then cascade down to facilitate reversed competitive interactions (Mowles et al., 2011), to increase growth rate in competing species (Peacor and Werner, 2000), and to alter resource availability for other predators (Eklov and VanKooten, 2001). Although the effects of these predator-induced trait-mediated indirect interactions (TMIIs) are ubiquitous in both aquatic and terrestrial systems, trait-mediated effects are generally more substantial in aquatic than terrestrial communities, perhaps because aquatic organisms are better capable of assessing chemical cues indicative of predation risk (Preisser et al., 2005).

Predation has historically been considered as a central process in community ecology (Preisser et al., 2005; Raffel et al., 2008). Consequently, trait-mediated responses have been best documented in the context of predator–prey interactions, especially through short-term responses in prey behaviour (Bolker et al., 2003; Peacor and Werner, 2000). On the contrary, trait-mediated effects of parasites have received less attention despite the fact that parasite-induced TMIIs are widespread among natural communities, and that their implications for our understanding of food webs are non-negligible (Lafferty et al., 2008; Raffel et al., 2008). It has been claimed that “parasites are just predators,” because of geometric parallels between parasite–host and predator–prey interactions (e.g. Anderson and

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* Corresponding author.
E-mail address: kamts408@student.otago.ac.nz (T. Kamiya).

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May, 1979; Earn et al., 1998). For example, predators and parasites both rely on resources from their victims to obtain energy for growth and reproduction, implying similar roles in energy and material flow in food webs (Hall et al., 2008). Although this parallel can be misleading in some contexts (Hall et al., 2008), this line of reasoning suggests that parasites can be drivers of TMIIs just as predators. Furthermore, parasite–host associations are typically characterised by longer durations than predator–prey interactions, hence the scope for longer lasting trait-mediated effects (Hatcher et al., 2006).

In the field of parasitology, parasite-induced behavioural alterations that enhance parasite transmission success by facilitating predation on the current host by the next one have been the subject of extensive research (Poulin, 2010; Poulin and Thomas, 1999). Their effects have been shown to extend beyond the immediate predator–prey interactions involved in the life cycle of the parasite (Cezilly and Perrot-Minnot, 2005; Lefevre et al., 2009; Marriott et al., 1989; Mouritsen and Poulin, 2005). These parasite-induced behavioural alterations promoting increased predation have been recognised as TMIIs in the context of community ecology (Bolker et al., 2003; Werner and Peacor, 2003). However, our understanding of parasite-induced behavioural responses is seldom connected to the vast literature on predator-induced trait-mediated responses.

Any change in the prey's phenotype will be constrained by a suite of trade-offs (Bolker et al., 2003). For example, investment in predator avoidance will be at least partially offset by lost opportunities for foraging. The trade-off between food acquisition and predator avoidance is a prey property that is balanced by fitness gain from foraging and fitness loss incurred by predation risk, which can be implemented through both density- and trait-mediated effects (Schmitz et al., 2004). Adding parasites to the picture, this trade-off presents an interesting case where the effects of predator- and parasite-induced behavioural responses occur in conjunction, providing an opportunity to investigate the effect of parasite-induced behavioural alterations on other trait-mediated interactions in ecological communities. In particular, Schmitz et al. (2004) argued that the foraging–predation trade-off in mid-level species could be the ultimate mechanism driving trophic cascades in ecological communities; however, the role of parasites has not been investigated in this context despite their potential importance.

In the present study, we investigate the effects of parasite-induced behavioural alterations in mud snails, *Zeacumantus subcarinatus* (Batillariidae), on the trade-off between food acquisition and predator avoidance against purple rock crabs, *Hemigrapsus sexdentatus* (Grapsidae).

Zeacumantus subcarinatus is a relatively long-lived intertidal gastropod commonly found on the coastlines of New Zealand, which serves as first intermediate host for a number of castrating trematode parasites with complex life cycles (Martorelli et al., 2008). We focused on the effects of the most locally prevalent species: *Maritrema novaezealandensis* which accounts for 60% of infections in some populations, and *Philophthalmus* sp. which is the second most prevalent parasite of *Z. subcarinatus*. Both trematode species multiply asexually in the gonadal tissues of the snail, to produce free-swimming dispersal stages known as cercariae, which then leave the snail. In *M. novaezealandensis,* the cercariae penetrate and encyst within the body cavity of small crustaceans, to await ingestion by a definitive host, i.e. shore birds such as the oystercatchers, *Haematopus ostralegus* and the red-billed gulls, *Larus novaehollandiae* (Leung et al., 2009; Martorelli et al., 2004; Thompson et al., 2005). In contrast, following their exit from the snail, *Philophthalmus* sp. cercariae quickly encyst on hard substrate, such as the external surface of gastropod shells, where they await eventual ingestion by shorebirds (Martorelli et al., 2008; Neal and Poulin, 2012). Given that both trematodes use the snail exclusively as a long-term resource base for the asexual production of cercariae, it is in the parasites' evolutionary interest that their snail host avoids predation of any kind.

The influence of trematode parasites on food acquisition by gastropods is contentious in the literature. Previous studies show that these effects include increased feeding rate (Bernot and Lamberti, 2008), increased growth rate (Probst and Kube, 1999), reduced attraction to food (Boland and Fried, 1984), and reduced grazing (Wood et al., 2007). An attempt to compensate the resource deficit due to infection is assumed to occur when the host is observed to increase its feeding rate (e.g. Bernot and Lamberti, 2008), while pathological side-effects of infection seem to be plausible causes of feeding reduction in other cases (e.g. Wood et al., 2007).

We investigated the effects of trematode-induced TMIIs on the trade-off between food acquisition and predator avoidance in the presence of different predation cues (i.e. predator chemical cue alone, injured conspecific cue alone, and a combination of both) indicative of different risk situations. We hypothesized that the presence of predation cues would reduce foraging activity in the snails as a trade-off for predator avoidance (i.e. predator-induced trait mediation). In addition, feeding time reduction was hypothesized to be less pronounced in infected snails because of compensation for infection as well as the possibility of compromised predator detection. Predator avoidance behaviour was also expected to decrease in infected snails for the same reason.

Two experiments tested the following predictions. (1) Feeding time reduction as a response to perceived predation risk should be smaller in infected snails (Experiment 1). (2) The amount of food consumed and faecal pellets produced by infected snails should be on average higher than for uninfected snails, in particular under perceived predation risk (Experiment 2). (3) Predator avoidance behaviour should be more frequent in uninfected snails than in infected ones as a response to predation risk (Experiment 1). (4) The time it takes for snails to initiate predator avoidance behaviour should be significantly shorter in uninfected snails than in infected snails, and the time difference between control (no predation cue) and treatments should be greater in uninfected snails (Experiment 1).

2. Methods

2.1. Study organisms, screening and maintenance

Mud snails, *Zeacumantus subcarinatus*, and purple rock crabs, *Hemigrapsus sexdentatus*, were collected in November 2011 from a mudflat in Lower Portobello Bay, Otago Harbor, South Island, New Zealand (45°52′ S, 170°42′ E). The mudflat contains numerous boulders (approximately 20–30 cm in diameter) near the high-tide mark where both animals are typically found on and underneath the rocks in close proximity (within 1–10 cm of each other). Since the snails are often found crawling up the boulders, this provides a potential refuge from the crab predator. The snails and the crabs were maintained in separate containers (220 mm × 160 mm × 100 mm) filled with aerated filtered salt water. *Zeacumantus subcarinatus* were fed with *Ulva lactuca* and *H. sexdentatus* were fed with *Z. subcarinatus* 3 times a week. Crabs were fed with snails because in aquatic organisms, anti-predator responses mediated by chemical cues are generally enhanced when the predator has been feeding on the focal prey species (Chivers and Smith, 1998).

In order to identify infected snails, *Z. subcarinatus* were incubated individually in wells of a 12-well culture plate filled with filtered salt water at 26 °C for 20 h. This served to induce the trematode parasites, *Maritrema novaezealandensis* and *Philophthalmus* sp. to release cercariae, which were identified using a dissecting microscope. Snails were categorized into three groups based on infection status: no infection, *M. novaezealandensis* infection, or *Philophthalmus* sp. infection. Snails infected with other trematode species or doubly infected snails were excluded due to their low prevalence. Snails with different infection types were then housed in separate plastic 900 ml containers (diameter 100 mm) filled with aerated filtered salt water and fed with *Ulva*. The infection status of the snails was confirmed (as above) three times prior to the experimental procedure with 3–5 day intervals between each incubation.

2.2. Experiment 1: Behavioural assessment

This experiment aimed to investigate the trade-off between food acquisition and predator avoidance among the infection groups in the presence of different predation risks.

Each experimental container (900 ml; 100 mm diameter) contained a fresh piece of *Ulva* (30 mm×30 mm), which was pinned to the bottom of the container. The containers were thoroughly cleaned prior to each experiment, then filled with 300 ml of filtered salt water from a common source, which was kept at 20 °C. Each *Z. subcarinatus* was measured (maximum shell length), individually labelled with a marking dot (The Bee Works, Orillia, ON, Canada) glued to its shell and food deprived for 48 h; this ensured that all snails were motivated to feed in the experiment.

Crab-conditioned cue water was taken from an aquarium containing 1200 ml of salt water in which one *H. sexdentatus* had been housed for 24 h. Three crabs of similar sizes (carapace width = 44–48 mm) were interchangeably used at random to produce the predator-conditioned water. Injured conspecific cue was prepared by conditioning 100 ml filtered salt water with one crushed, uninfected *Z. subcarinatus*. The combination cue water (predator and injured conspecific) was made by mixing 50 ml of each cue waters.

Nine snails, three from each infection group (i.e. no infection, *Maritrema*-infected, *Philophthalmus*-infected), were placed in the middle of the plastic container on the piece of *Ulva*, and 100 ml of either control or treatment water (i.e. crab-conditioned, injured conspecific, or mixture) which had also been kept at 20 °C was carefully poured into the container after the snails were allowed to habituate for 20 min.

The behaviour of each snail was then recorded every 5 min for 1 h as either (1) feeding, (2) crawling at the bottom (3) crawling on the wall, (4) stationary (i.e. foot withdrawn), or (5) out of water. Crawling out of water is a typical anti-predator response well-documented in both marine and freshwater gastropods (Jacobsen and Stabell, 1999; Mowles et al., 2011). Behaviour was recorded as 'out of water' only when the anterior end of a snail was at least 5 mm above the water line, following Jacobsen and Stabell (1999). The experiments were performed within 2 h of the time of high tide at the site of collection (*H. sexdentatus* as well as most intertidal gastropods are known to be most active during high tide (McLay, 1988; Underwood, 1977), and may retain natural activity rhythms in captivity due to their internal biological clock).

The experiment was replicated 16 times for each treatment, involving 48 snails of each infection type. Each individual snail was used once for each treatment, thus experiencing four different trials in total; each group of nine snails remained identical over the four trials. The order in which each treatment was given to the snails was randomized using a random number generator. In order to minimize the stress on the snails, they were given 3–4 day resting periods between experimental trials.

2.3. Experiment 2: Food consumption and excretion

Food consumption and faecal pellet production by control snails and those exposed to predation cues were compared in order to quantify the effect of perceived predation risk on changes in food acquisition and excretion among snails infected with different parasites.

The labelled *Z. subcarinatus* used in Experiment 1 were held in holding tanks with *Ulva* for at least 3 days after completion of the above experiment. The snails were then food deprived for 48 h prior to being individually placed in wells of 6-well culture plates containing 5 ml of filtered salt water and a piece of *Ulva*. The shapes of *Ulva* pieces were standardized to 20 mm diameter circles. Prior to the experiment, each *Ulva* piece was scanned and its surface area was quantified using the "Analyze Particles" function on ImageJ 1.45 s.

The mixture of crab-conditioned water and water containing injured conspecifics cues (see above) was used because it maximized the anti-predator response of uninfected snails in Experiment 1. Half of the 48 snails of each infection type were randomly assigned to the control treatment and the other half to the predation cue treatment. Wells on the same plate were allocated to the same treatment (control or predation cue) in order to avoid mixing of water types due to condensation. 0.5 ml of either fresh saltwater (control) or the predation cue mixture was pipetted into each well at the start of the experiment, and an additional 0.5 ml of either solution was added to the wells every 12 h. The plates were kept at 20 °C and the snails were allowed to graze on the *Ulva* for 48 h. The *Ulva* sheets were then re-scanned and analyzed as above, and faecal pellets produced by each snail within a well were also counted.

2.4. Statistical analysis

2.4.1. Snail behavioural data

All statistical analyses were conducted in R 2.14.1 (R Development Core Team, 2011). In experiment 1, an attempt was made to normalise the feeding and anti-predator behaviour count data (i.e. number of times out of 12 observations a snail was seen either feeding or out of the water) using the *rntransform* function from the *GenABEL* package. Since the rank transformation failed to normalise the residuals, and no other appropriate transformation was possible, the data were reduced to binary presence-absence data. Generalised linear mixed models (GLMM) with binomial error were constructed using the *lmer* function from the *lme4* package, one for each response variable (time feeding and time out of the water) (Bolker et al., 2009). In these models, predation cue (control water, crab-conditioned water, injured conspecific water, and a mixture or crab and injured conspecific water), infection (uninfected, *Maritrema*-infected, and *Philophthalmus*-infected) and snail size were incorporated as fixed effects, whereas snail identity, experimental tank, and trial were included as random effects.

In addition, the first time (out of 12 observations) that a snail was observed out of water was considered as how long it took to initiate the anti-predator crawl-out behaviour. The effects of the same set of fixed and random effects as above on this reaction time were analysed using a GLMM after the response variable was log-transformed to normalise model residuals. Tukey–Kramer pair-wise comparisons were then carried out using the *ghlt* function in the *multicomp* package to compare responses among treatments within each infection status and across infection groups within each treatment.

2.4.2. Food consumption & excretion

For experiment 2, the amount of *Ulva* consumed (Shapiro–Wilk normality test – $W = 0.983$, $p = 0.090$) and numbers of faecal pellets produced (Shapiro–Wilk normality test – $W = 0.984$, $p = 0.114$) by the snails were successfully normalised using Box–Cox transformation with the *powerTransform* function of the *car* package. Linear multiple regression models were performed separately on these two response variables, with treatment (control water, or a mixture or crab and injured conspecific water), infection and snail size as predictors.

3. Results

3.1. Snail behaviour

Overall, time spent feeding was significantly reduced in the presence of odours from either injured conspecifics alone or in combination with predators, while neither of the trematode species had an effect on snail feeding activity (Table 1). Similarly, predator avoidance activity, measured as time spent out of the water, significantly increased in the presence of each of the predation cues relative to the control; the combination cue had the largest effect, followed by the conspecifc cue and then the crab cue (Table 2). Here again, anti-predator behaviour was not affected by either trematode infections or shell size.

On the contrary, we found a significant effect of trematode infection on how long it took for the snails to initiate the avoidance behaviour. The time taken to exit the water was overall significantly shorter in *M. novaezealandensis*-infected snails than in uninfected snails, while it was significantly longer for *Philophthalmus*-infected snails (Table 3). A pair-wise comparison among infection groups indicated that *Philophthalmus* sp. infected snails retained their relatively slower reaction times in the presence of the combination cue, unlike either uninfected or *M. novaezealandensis*-infected snails whose reaction times did not differ from each other's (Table 4 and Fig. 1). Also, reaction times in the presence of the combination cue were faster compared to the control situation in snails that were uninfected and in those infected with *M. novaezealandensis*, but not in those infected with *Philophthalmus* sp. (Fig. 1). The reaction time to the odour of injured conspecifics alone was also significantly reduced compared to the control situation in *M. novaezealandensis*-infected snails (Fig. 1). Overall, snails infected with *Philophthalmus* sp. did not demonstrate significant reductions in their reaction times when exposed to any of the predation cues provided (Fig. 1). In general, the combination cue was consistently more effective at triggering a response than either of the predator or injured conspecific cues on their own.

3.2. Food consumption and excretion

The amount of food consumed by the snails, as measured by the change in the area of the piece of *Ulva* provided to them, did not vary between treatments or among the different infection groups; it was also unrelated to snail shell size (all $P>0.10$). However, snails infected with *M. novaezealandensis* produced a significantly larger amount of faecal pellets overall than uninfected snails during the same experiment (Fig. 2), while neither the predation cue nor shell size had a significant effect (Table 5).

4. Discussion

Our experiments demonstrated that trematode parasites play a role in influencing predator avoidance of their marine gastropod host. The two trematode parasites, *M. novaezealandensis* and *Philophthalmus* sp. studied here had distinct effects on host anti-predator response, and thus our study reveals species-specific effects of parasite infection on behavioural responses concerning the foraging–predation trade-off.

Foraging activity is a vital part of an animal's life, yet it is often associated with increased mortality from predation (Werner and Anholt, 1993). Thus prey species should adjust their foraging strategies to optimise the balance between the fitness gain of foraging and fitness loss from predation (Abrams, 1995; Schmitz et al., 2004). Accurate assessment of immediate predation risk is important as overestimation of a low risk may lead to an unnecessary reduction in foraging and subsequent reproductive output, while underestimation of a high risk would result in increased likelihood of capture (Wojdak and Trexler, 2010). Our results demonstrated a reduction in foraging activity in the presence of predation cues, regardless of parasite infection, as a result of increased time spent in predator avoidance mode. This result is consistent with previous findings in other systems (Mowles et al., 2011; Schmitz et al., 1997, 2004; Trussell et al., 2003), and supports our hypothesis that predator-induced behavioural alteration should lead to foraging reduction. However, neither feeding activity, the amount of food consumed, nor faecal pellet excretion rate was affected by parasites explicitly in the face of predation risk. We found a higher pellet excretion rate in snails infected with *M. novaezealandensis* both with and without the predation cue. Although this finding illustrates the general impact of parasites on host physiology, it does not suggest a role for parasites in interfering with the foraging–predation trade-off. Thus our a priori hypothesis that infected snails should compensate for the energy short-fall imposed by parasitism by increasing their feeding activity even in the face of predation risks was not supported by the results.

Table 2
Results of a GLMM with binomial family evaluating the effect of each cue type, two trematode infections, *M. novaezealandensis* and *Philophthalmus* sp. and shell size on the time spent out of water by the snail *Zeacumantus subcarinatus*.

	Estimate	Standard error	*Z*-value	*P*-value
(Intercept)	−1.658	0.540	−3.068	0.002
Combination cue	4.058	0.389	10.420	>0.001
Conspecific cue	2.713	0.336	8.068	>0.001
Crab cue	0.717	0.299	2.396	0.017
M. novaezealandensis	0.334	0.284	1.176	0.239
Philophthalmus sp.	0.094	0.282	0.333	0.739
Size	−0.034	0.116	−0.294	0.769

Behavioural plasticity in the context of spatially or temporally varying predation risk is a ubiquitous adaptation in many organisms, though it is not without costs (DeWitt, 1998; DeWitt et al., 1999). In the case of gastropods, crawl-out behaviour can be an effective strategy to reduce the probability of encounter with aquatic predators (Trussell et al., 2003). However, this behaviour is associated with an increased cost of locomotion (Calow, 1974), increased susceptibility to aerial predators such as birds (Morris and Boag, 1982), lost opportunities for grazing and reproduction (Covich et al., 1994; Mowles et al., 2011), and physiological stress incurred through exposure to air (Covich et al., 1994). Therefore, prey species are under strong selection to accurately assess predation risks and optimise the trade-off between food acquisition and predator avoidance (Mach and Bourdeau, 2011). In our experiment, the simultaneous presence of odours from an injured conspecific and a crab resulted in the strongest crawl-out responses in general, while odour from the crab alone elicited the weakest, though still significant, response given the concentrations used in this study. The varying responses to different predation cues were expected since chemical alarm cues released by injured prey in combination with feeding predator cues provide the best indication of predation risk, while predator-conditioned water alone is often associated with weaker responses

Table 1
Results of a GLMM with binomial family evaluating the effect of each cue type, two trematode infections, *M. novaezealandensis* and *Philophthalmus* sp. and shell size on the time spent feeding by the snail *Zeacumantus subcarinatus*.

	Estimate	Standard error	*Z*-value	*P*-value
(Intercept)	−0.560	0.341	−1.644	0.100
Combination cue	−0.1906	0.357	−5.339	<0.001
Conspecific cue	−1.415	0.314	−3.643	<0.001
Crab cue	−0.134	0.273	−4.512	0.624
M. novaezealandensis	0.0002	0.257	0.001	0.999
Philophthalmus sp.	−0.102	0.260	−0.393	0.694
Size	0.020	0.106	0.187	0.852

Table 3
Results of a GLMM evaluating the effect of each cue type, two trematode infections, *M. novaezealandensis* and *Philophthalmus* sp. and shell size on the time taken by the snail *Zeacumantus subcarinatus* to initiate crawl-out behaviour. Estimate and standard error values are in log scale.

	Estimate	Standard error	*T*-value	*P*-value
(Intercept)	3.031	0.123	24.582	<0.001
Combination cue	−0.565	0.120	−4.717	<0.001
Conspecific cue	−0.451	0.124	−3.643	<0.001
Crab cue	−0.167	0.135	−1.231	0.219
M. novaezealandensis	−0.189	0.086	−2.196	0.029
Philophthalmus sp.	0.174	0.088	1.985	0.048
Size	0.060	0.035	1.706	0.089

Table 4
Results of a Tukey pair-wise comparison evaluating the effect of infection status (uninfected (U), *M. novaezealandensis*-infected (M), *Philophthalmus*-infected (P)) on the reaction time of the snail *Zeacumantus subcarinatus* to initiate crawl-out behaviour in the presence of the combination cue.

	Estimate	Standard error	*Z*-value	*P*-value
U – M	0.1572	0.1106	1.421	0.155
U – P	−0.2900	0.113	−2.558	0.020036
P – M	0.4471	0.1133	3.947	<0.001

(Chivers and Smith, 1998; Cotton et al., 2004; McCarthy and Fisher, 2000; Yamada et al., 1998).

Parasite-induced host behavioural alterations are common in many systems, yet determining whether such alterations are adaptive manipulations by parasites or mere pathological side-effects of infection requires close attention to the parasites' transmission mode (see Poulin, 1995). Most importantly, an adaptive manipulation must convey fitness benefits to the manipulator, yet corroboration by empirical evidence is limited to a small proportion of host-parasite systems (Poulin, 2010). Both trematode species in this study are characterised by complex life cycles with shore birds as the definitive host (Leung et al., 2009). Both species use snails as first intermediate host, in which they asexually reproduce to release free-swimming infective stages known as cercariae. The key difference between the life cycles of *M. novaezealandensis* and *Philophthalmus* sp. is that the latter lacks a second intermediate host; instead, their cercariae quickly adhere to and encyst on hard substrates (mostly shells of gastropods, also exoskeleton of crabs; Neal and Poulin, 2012) after release from the snail host. In our study, snails infected with *M. novaezealandensis* initiated the crawl-out behaviour more readily overall than either uninfected or *Philophthalmus*-infected snails. This suggests that the assessment of predator-related trade-offs in M. novaezealandensis-infected snails may be adjusted towards overestimation of the predation risk, resulting perhaps in lost opportunities for other essential activities. In the simultaneous presence of odours from an injured conspecific and a crab, however, their reaction times did not differ from uninfected snails.

In contrast, *Philophthalmus* sp. infected snails showed slower responses to the combined predator-injured conspecific cue, implying increased exposure to crab predators. Potential fitness benefits to *Philophthalmus* sp. obtained through this behavioural change of their snail host may include increased time available to release cercariae in water, reduced physiological stress on the host, or increased energy supply from host foraging (though this last possibility was not supported by our results). However, these benefits would apply equally well to *M. novaezealandensis*, and any adaptive interpretation of this host behavioural change contradicts previous findings that some trematode infected gastropods spend more time out of water in the absence of predation cues (Bernot, 2003). With no fitness benefit to the parasites assumed a priori, adaptive arguments fail to explain the difference between the effects of the two parasite species on host anti-predator response. We therefore argue here that the parasite-induced change in the trait-mediated anti-predator response of *Z. subcarinatus* observed in our study is more likely to be a pathological side-effect of infection. Perhaps *Philophthalmus* sp. coincidentally damages the chemosensory apparatus of the snail and thus its ability to detect predation cues, whereas *M. novaezealandensis* does not. Earlier studies have highlighted other differences between the effects of these two trematodes that suggest they cause different

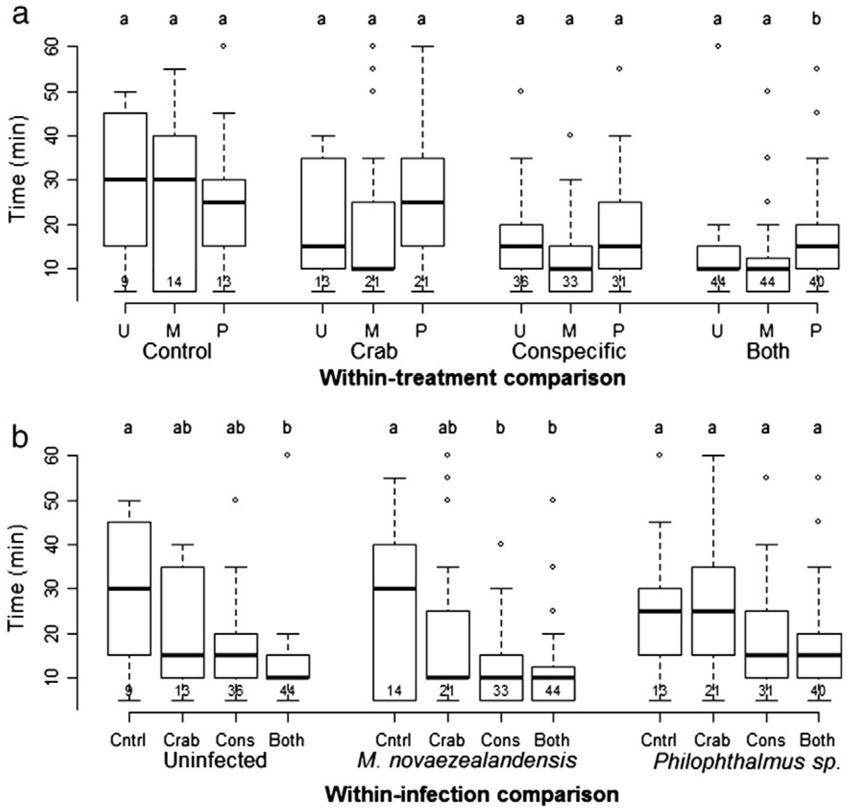

Fig. 1. Within-treatment comparison of infection status (a) and within-infection comparison of treatment (b) of time to initiate the crawl-out behaviour by the snail *Zeacumantus subcarinatus* (see Tables 3 and 4 for detailed statistical information). Letters (a and b) on the figure indicate statistically significant differences ($\alpha = 0.05$) within each treatment and infection status. Sample minimum, lower quartile, median, upper quartile, sample maximum, and outliers are shown respectively for each box. The number at the base of each box indicates the sample size. The variation in sample sizes arose because there were snails that did not leave the water during the entire course of the experiment. Abbreviations are as follows: U = Uninfected, M = *M. novaezealandensis*-infected, P = *Philophthalmus*-infected, Cntrl = Control, Crab = Crab-conditioned treatment, Cons = Injured conspecific treatment, Both = Combination treatment of crab and injured conspecific cues.

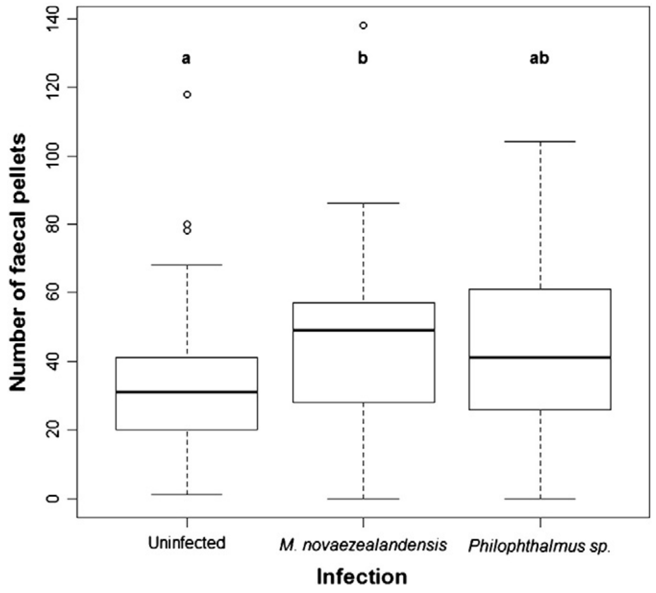

Fig. 2. Number of faecal pellets produced by uninfected ($n=48$), *M. novaezealandensis*-infected ($n=48$) and *Philophthalmus*-infected snails ($n=48$) over 48 h. Letters (a and b) on the figure indicate statistically significant differences ($\alpha=0.05$) among the infection groups. Sample minimum, lower quartile, median, upper quartile, sample maximum, and outliers are shown respectively for each box.

collateral pathology in the snail. For instance, snail thermal preferences are altered in completely different ways by *Philophthalmus* sp. and by *M. novaezealandensis* infection (Bates et al., 2011).

Empirical studies on the effect of parasites on trait-mediated anti-predator responses are still scarce, with no marine example to the authors' knowledge. A species of New Zealand freshwater snail infected with a trematode has been shown to demonstrate time-specific behavioural responses which coincide with increased encounter with the host predator and reduced encounter by non-host predators (Levri, 1998, 1999; Levri and Lively, 1996). Here an adaptive explanation for parasite manipulation was supported since the trematode benefits from the reduced probability of ingestion by unsuitable hosts, thereby increasing the probability of successful transmission (Levri, 1998, 1999). In contrast, changes in anti-predator responses may be due to pathological side-effects of infection in other cases. Bernot (2003) found complex context-dependent responses in trematode infected snails, reporting decreased use of refuge habitat in infected snails in the presence of crayfish, increased refuge use in the presence of fish, and increased crawl-out behaviour in the presence of fish but not in the presence of crayfish. However, crawl-out behaviour seems to be an implausible adaptation for the trematodes in this case because the larval stages are released in the water before reaching their definitive host (Bernot, 2003).

It has been previously reported that approximately 8% of *Z. subcarinatus* are singly infected with *Philophthalmus* sp. and a further 12% suffer double infections from both *Philophthalmus* sp. and *M. novaezealandensis* (Keeney et al., 2008). Although the combined effect of the two parasites on the predator-induced trait-mediated response is unknown, our findings raise the possibility that up to 20% of the entire local snail population could be more susceptible to crab predation due to reduced anti-predator response caused by *Philophthalmus* sp.. On the contrary, the availability of snails to aerial/terrestrial predators could be reduced by a similar extent. This suggests a large shift in resource availability to the local aquatic and terrestrial food webs driven indirectly by parasite-induced behavioural alteration.

Table 5
Results of a linear multiple regression model evaluating the effect of the combination cue, two trematode infections, *M. novaezealandensis* and *Philophthalmus* sp., and shell size on the number of faecal pellets produced by the snail *Zeacumantus subcarinatus*.

	Estimate	Standard error	*Z*-value	*P*-value
Intercept	18.906	5.555	3.403	<0.001
Combination cue	−0.224	0.710	−0.315	0.753
M. novaezealandensis	1.851	0.871	2.126	0.035
Philophthalmus sp.	1.570	0.866	1.813	0.072
Size	−0.698	0.390	−1.792	0.075

The extent of these indirect effects on the intertidal community remains unclear on larger temporal and spatial scales. For example, seasonal variability in prevalence is a common characteristic of many trematode parasites (Kube et al., 2002; Sithithaworn et al., 1997). It is likely that temporal and spatial variation in food availability, prevalence of parasites, abundance, activity and identity of predators all have roles to play in determining the direction and strength of the trade-off between food acquisition and predator avoidance of mid-level species.

Empirical evidence has been accumulating in recent years in support of key roles for parasites in affecting food web properties (Lafferty et al., 2008). Based on numerous reports of parasite-induced behavioural alteration in the parasitology literature (reviewed in Poulin, 2010), we predict that a substantial proportion of such alterations inevitably result in behaviourally mediated indirect effects (Lefevre et al., 2009; Mouritsen and Poulin, 2005). Our current study showed parasite-induced behavioural changes in the presence of imminent predation risk. However, no effects of the two trematodes on the overall feeding and fleeing times were detected in our experiments; thus, the net effect on the foraging–predation trade-off remains unknown. Given the importance of trait-mediated indirect interactions in predator–prey interactions, parasite-induced indirect effects also demand equal attention from researchers in community ecology.

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