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Do parasites affect burrowing depth and habitat choice of sand hoppers, *Talorchestia quoyana* (Amphipoda: Talitridae)?

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Abstract *Talorchestia quoyana* is the most abundant sand hopper (Amphipoda: Talitridae) on New Zealand's beaches. These supralittoral detritivores are host to a parasitic mermithid nematode, *Thaumamermis zealandica*. In other systems, mermithids have been found to manipulate host behaviour to facilitate the continuation of their lifecycle. The aim of the present study was to determine if the burrowing behaviour of *T. quoyana* showed evidence of manipulation by *T. zealandica*. Two studies were conducted to assess the spatial and temporal patterns in sand hopper burrowing under field and laboratory conditions. Sand hopper burrowing behaviour showed considerable variation, related to sand hopper length, sand hopper distribution, month of collection, and experimental moisture conditions. The presence of parasites was not a significant factor in determining sand hopper burrowing behaviour, a result contrary to previous laboratory findings for this system. This study illustrates the benefits of combining both field and laboratory experiments to evaluate whether or not parasites alter host behaviour.

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INTRODUCTION

Talitrid amphipods, or sand hoppers, are abundant and important members of sandy beach ecosystems worldwide (Brown & McLachlan 1990). Through their supralittoral detritivory, feeding on stranded kelp and prodigious burrowing activity, they facilitate the reincorporation of nutrients upon which sediment bacteria and meiofauna rely (Brown 2001). Sand hoppers are primarily nocturnal, burrowing beneath stranded kelp during the day to avoid predators and desiccation (Poulin & Latham 2002a). Their spatial distribution is not uniform across the shore (Inglis 1989; Marsden 1991a; Poulin & Rate 2001). However, this patchy distribution is unrelated to kelp biomass (Marsden 1991b), suggesting other factors may shape their spatial ecology.

The determinants of sand hopper burrowing depth are still not well understood. Humidity and temperature have been acknowledged as key abiotic factors (Brown & McLachlan 1990), but a potentially important biotic factor has only recently been identified. A study by Poulin & Latham (2002a) suggested that parasites might play a role in determining sand hopper burrowing depth. The talitrid amphipod, *Talorchestia quoyana* Milne-Edwards, is host to three species of organism: a digamasellid mite, a rhabditid nematode and a mermithid nematode, *Thaumamermis zealandica* Poinar, Latham & Poulin (Poulin & Rate 2001; Poinar et al. 2002). The digamasellid mite and the rhabditid nematode are external symbionts, using sand hoppers as a means of dispersal with no detectable effect on the host (Rigby 1996a,b; Pugh et al. 1997; Poulin & Rate 2001). In comparison, *T. zealandica* is a parasite found within the body cavity (Poinar et al. 2002; Poulin & Latham 2002a). The infective stage of the parasite penetrates the body wall of the sand

hopper, where it grows to lengths exceeding 20 cm. It then emerges from the host, killing the host in the process, and completes its post-parasitic maturation (Poinar et al. 2002). The adult nematode worms require a moist sand environment to emerge (Poinar et al. 2002). Despite the established physiological and evolutionary costs of host manipulation (see Poulin et al. 2005; Thomas et al. 2005), in other systems mermithid ecological requirements have resulted in the evolution of behaviour manipulation of mayflies (Vance 1996; Williams et al. 2001) and ants (Maeyama et al. 1994). Thus, any effect of *T. zealandica* on *T. quoyana* is of great ecological and evolutionary interest.

Poulin & Latham (2002a) investigated the relationship between parasitism by *T. zealandica* and the burrowing depth of *T. quoyana* through a series of laboratory experiments. Groups of sand hoppers were placed in tubes filled with moist sand and allowed to burrow undisturbed for 24 h. After this time, sand within the tubes was partitioned by depth; the sand hoppers were recovered, measured, sexed, and dissected to determine the presence and size of parasites. Poulin & Latham (2002a) observed that intensity of infection, as measured by prevalence of infection, number of parasites per host and length of the longest parasite, all increased with host burrowing depth. These results raise an important question: are the patterns in sand hopper burrowing depth a consequence or the cause of parasite infection or are they correlated with some other unknown factor? Sand hoppers that generally burrow deeply might be more likely to be infected and may continue their trait of burrowing deeply in the laboratory. This is a common issue for studies using naturally-infected hosts (see Poulin 1995; Moore 2002). However, two factors suggest a causal relationship in this instance. First, the presence of a relationship between parasite length and host burrowing depth when controlled for the effect of host size (Poulin & Latham 2002a) implied that the smaller, infective-stage parasites penetrate sand hoppers near the surface and emerge as full grown adults from the host at depth, a scenario consistent with behaviour modification. Second, and most crucially, a recent physiological study found infected sand hoppers showed increased haemolymph osmolality, suggesting any increase in burrowing depth may be water-seeking behaviour (Williams et al. 2004). Separating the potentially entwined effects of seeking high moisture level and burrowing depth is vital to understanding the mechanism involved in any potential causal relationship. Experimentally assessing if infected sand hoppers show water-seeking behaviour when sand moisture content is unrelated to depth is a way to isolate moisture-seeking behaviour indicative of host manipulation. More importantly, the ultimate aim should be to assess if any apparent effects of parasitism are detectable under natural conditions and thus confirm the presence of host manipulation.

We tested these specific ideas through a series of field samples and a laboratory experiment on naturally infected sand hoppers. Core samples of sand containing sand hoppers were taken in the field, allowing the assessment of sand hopper burrowing depth and associated parasite load. In the laboratory, we used an apparatus with differing moisture levels to assess habitat choice and moisture-seeking behaviour separately. We predicted that prevalence of infection, the number and the length of parasites would increase with burrowing depth in the field. Further, we predicted that increased moisture levels would attract infected sand hoppers and influence their habitat choice. Validation of these predictions would provide evidence consistent with parasite manipulation of host behaviour.

MATERIALS AND METHODS

Where possible, the present methodology followed that described by Poulin & Latham (2002a) to facilitate comparison. Naturally infected sand hoppers were used throughout this investigation as previous attempts to obtain the parasite's eggs or infective stages for experimental infections have proven unsuccessful (Poulin & Latham 2002a). Infected sand hoppers are indistinguishable from their uninfected conspecifics and hence, all observations were conducted with no knowledge of infection status before dissection.

The experimental design of this investigation entailed two separate studies: an observational field study with core sampling to assess the factors associated with burrowing depth and a laboratory experiment to assess the response of burrowing behaviour to different moisture levels. Sand hoppers, identified as *T. quoyana*, were collected for both field and laboratory studies from an abundant population at Long Beach (45°45′S, 170°39′E), north of Dunedin in New Zealand's South Island. The Long Beach shoreline is sandy, with a gentle slope and exposed to moderate wave action. Sand hoppers were located in the sand beneath wrack and debris at the strandline, in mid afternoon at low tide

either through field sampling or targeted collection for laboratory experiments.

Field study

The field study was conducted over four bi-monthly sampling trips in June, August, October, and December of 2002, representing the austral winter, spring, and summer. For each bi-monthly experimental series, four core samples were taken, two core samples from each of two separate kelp patches haphazardly selected on the beach. Core samples were taken using a large cylindrical metal tube (10 cm diam., 45 cm long) with three narrow slots at 11–cm intervals and a sharpened edge at one end. The tube was pushed straight down through the sand and kelp and then the full tube was extracted quickly by digging around its edge. Once the tube was extracted, three metal depth partitions were inserted through the narrow slots to separate the sample into four depth zones: depth 1, from 0 to 11 cm; depth 2, from 12 to 22 cm; depth 3, from 23 to 33 cm; and depth 4, from 34 to 44 cm. The resulting samples of sand, kelp and associated sand hoppers were placed in containers for transport to the laboratory.

In the laboratory, the sand hoppers were recovered from the samples, measured with calipers to the nearest mm (from the anterior end of the cephalon to posterior tip of the telson), sexed, decapitated and preserved in 70% ethanol. Sex classification was based on the presence of specialised gnathopods in mature males. To prevent juvenile male misclassification, all sand hoppers <10 mm in length were classified as juveniles. Each sand hopper's infection status was ascertained by dissection using fine forceps under a dissecting microscope. The number of mermithid worms per host (if any) was recorded and the length of each straightened worm was measured to the nearest mm. This measurement permitted the calculation of total worm length (i.e., the sum of the lengths of all worms in a sand hopper), and greatest worm length (i.e., the length of the largest worm in a sand hopper).

Habitat choice experiment

For the laboratory experiment, sand hoppers were collected from the field in February 2003, late in the austral summer period. Medium and large sized sand hoppers were collected by hand from the top 30 cm of sand underneath decaying patches of kelp at the strandline. The sand hoppers were stored in containers with moist sand for return to the laboratory. Additional materials required for the experiments such as sea water and moist sand were also collected and stored in airtight containers at this time. The size-selective nature of the sand hopper collection and the seasonal timing of the experiments were chosen to maximise the chance of high parasite prevalence, especially large parasites ready to emerge from their host (Poinar et al. 2002; Poulin & Latham 2002a,b). This time is when behaviour modification, if present, would be expected to be at its peak.

The objective of the habitat choice experiment was to examine the effect of parasites on host shelter and moisture-seeking behaviour. This was assessed using an apparatus that consisted of rectangular plastic containers (30 cm long, 12 cm wide,15 cm deep) covered by lids with a central slot that bisected the longest side. The containers were filled two thirds full with moist sand, and a cellulose sponge (12 cm long, 10 cm wide, 1 cm thick) was placed at one end. The slot allowed the insertion of a metal sheet to partition the sand and sand hoppers on either the sheltered "sponge" or exposed "sand" side. Once the sand hoppers were added, the lid was put in place.

The design of this experiment involved the use of 10 containers for one experiment conducted in March 2003. Each container was supplied with 50 sand hoppers, thus 500 sand hoppers were used in total. To assess the role of moisture in habitat selection, five of the containers were supplied with sponges soaked with 200 ml of sea water, whereas the remaining five containers received sponges that were soaked in sea water and then wrung dry. The sponge moisture levels were selected to create a moisture gradient, reflecting the variation in environmental conditions below stranded kelp where sand hoppers are typically found. Once the apparatus was set up and the sand hoppers added, the containers were left undisturbed at room temperature and under a natural photoperiod for 24 h. This period was chosen as the circadian periodicity in sand hopper activity suggests 24 h is sufficient to allow them to settle at their preferred daytime depth (Poulin & Latham 2002a). After this period the containers were partitioned into two samples (sponge versus sand) using the metal sheet. The samples were sorted and the sand hoppers collected. All recovered sand hoppers were measured, sexed, decapitated, and dissected in the same manner as those obtained in the core samples.

Statistical analysis

Data from both studies were analysed with linear regression models using SPSS and GLIM. All tests were two-tailed and factors were added in a stepwise manner, termed "forward entry", to allow

those factors associated with experimental design to be incorporated first (Hill & Lewicki 2006). Sand hopper burrowing depth was analysed using ordinal regression models whereas habitat choice was analysed using logistic regression models owing to the nature of each dependent variable. In both instances, sand hopper length was treated as a covariate. Factors included in the models were parasite infection (presence or absence of mermithid parasites in each sand hopper), sand hopper sex (with juveniles treated as a third group), experimental treatment (if any), experimental unit (replicate cores or boxes), and experimental series (month of sampling). All single factors included in the analysis are reported. Two-way interactions between factors were also tested in the models but are only reported if they were significant. Owing to low parasite prevalence, separate regression analysis of greatest and total worm length per host in the infected sand hoppers was not appropriate. Thus these parameters were interpreted with descriptive statistics and product-moment correlation. To assess effect of core extraction disturbance in the field study, a paired *t* test was used to determine if there was a significant difference in the number of sand hoppers collected from the paired core samples taken from beneath the same piece of kelp.

RESULTS

In the course of this investigation, 1003 sand hoppers were collected; 503 in field core samples and 500 selectively captured for the habitat choice laboratory experiments.

Field study

Across the field core samples, only one sand hopper was found in the deepest depth region (depth 4). It was an uninfected female, and was pooled with sand hoppers found at depth 3 in the same core for analysis. The core samples contained more juveniles than males or females and consequently showed a lower mean sand hopper length than the individuals collected for the habitat choice experiment, in which males and females were more abundant (Table 1). Overall, greatest parasite length per sand hopper and total parasite length per sand hopper were almost identical owing to the low frequency of multiple parasites in individual hosts and did not vary across experiments (Table 1).

The number and length of sand hoppers collected by core samples in the field were not normally

Table 1 Summary data for all sand hoppers *Talorchestia quoyana* and the mermithid parasites *Thaumamermis zealandica* collected from infected sand hoppers in the field study and the habitat choice experiment.

	Total no. of sand hoppers	Length (mm, mean ± SEM)	Males	Females (gravid)	Juveniles	Prevalence of infection (%)	Prevalence of multiple parasites (%)	Length of the greatest parasite per sand hopper (mm, mean ± SEM)	Total parasite length per sand hopper (mm, mean ± SEM)
Field study	503	8.8 ± 0.2	74	144 (1)	285	6.2	0.4	65.6 ± 11.2	66.1 ± 11.1
Habitat choice	448	11.7 ± 0.1	198	187 (0)	63	6.7	0.5	67.9 ± 7.0	69.3 ± 7.2
Total	951	10.2 ± 0.1	272	331 (1)	348	6.4	0.4	66.9 ± 6.6	67.7 ± 6.6

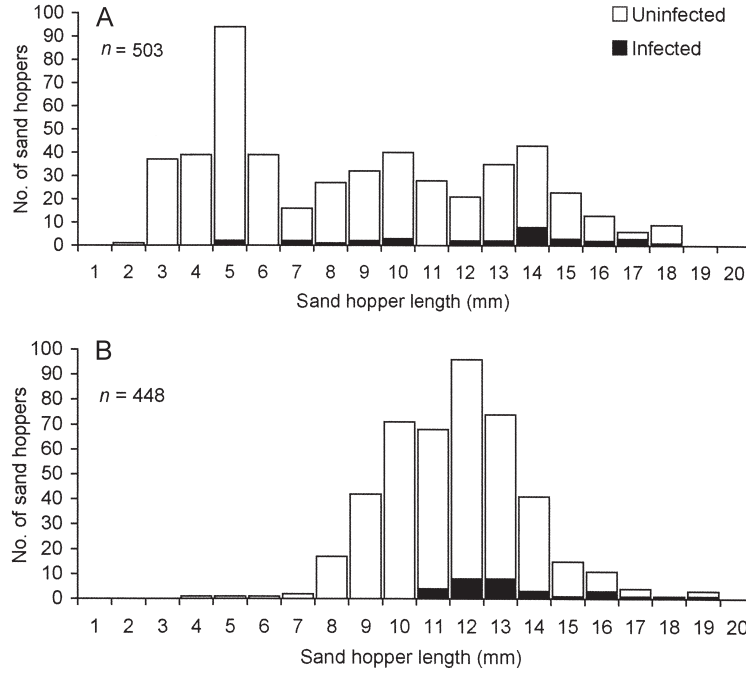

Fig. 1 Length frequency distributions of infected and uninfected sand hoppers *Talorchestia quoyana* for: **A**, the field study; and **B**, habitat choice experiment.

distributed, with peaks at 5 mm, 10 mm, and 14 mm length, possibly indicating the presence of three cohorts across the sampling period (Fig. 1). The habitat choice samples showed a more normal distribution, centred on sand hopper lengths with marginally increased parasite prevalence (Fig. 1). On average, 31 sand hoppers were collected per core sample (mean ± SE = 31.4 ± 5.2, $n = 16$). Juveniles comprised the majority of this group (mean ± SE = 17.8 ± 3.7, $n = 16$). The number of sand hoppers collected in the paired core samples of the same patch of kelp were not significantly different (paired t test: $t_7 = 0.904$, $P = 0.396$). Examining data from infected sand hoppers across both experiments combined, we observed a low prevalence of multiple parasites per host (4 individuals out of 951). Thus, the data for the greatest parasite length per host and total parasite length per host showed great similarity. As a result, only total length was used hereafter as a measure of parasite infection. Using the same pooled data, the relationship between total parasite length and sand hopper length was positive (product–moment correlation: $r_{61} = 0.290$, $P = 0.0229$) but showed considerable variation (Fig. 2).

The results of the stepwise ordinal regression to predict sand hopper burrowing depth in the field study core samples are summarised in Table 2. Through testing the significance of changes in model deviance, experimental series (month of collection), kelp patch and sand hopper length were identified as factors that offered significant improvements to the predictive power of the model, whereas sand hopper sex, parasite infection, and replicate core did not (Table 2). In general, sand hoppers were found to be most abundant in the core samples collected from depth 1 (Table 3), whereas on average the longest sand hoppers were collected from depth 2 (Table 3). Parasite prevalence and the total parasite length per sand hopper was highest in samples collected at depth 1 (Table 3). There was a high degree of variability across different kelp patches and experimental series, demonstrated in the number of sand hoppers found at each depth within each core, across kelp patches and months (Fig. 3). Two kelp patches were sampled for each series and hence variation between patches reflects heterogeneity in space and time.

Habitat choice experiment

Of the 500 sand hoppers used in the habitat choice experiment, 52 escaped and hence were excluded from further analysis. The results of the stepwise logistic regression to predict sand hopper habitat choice in the laboratory are summarised in Table 4. Testing the significance of changes in model

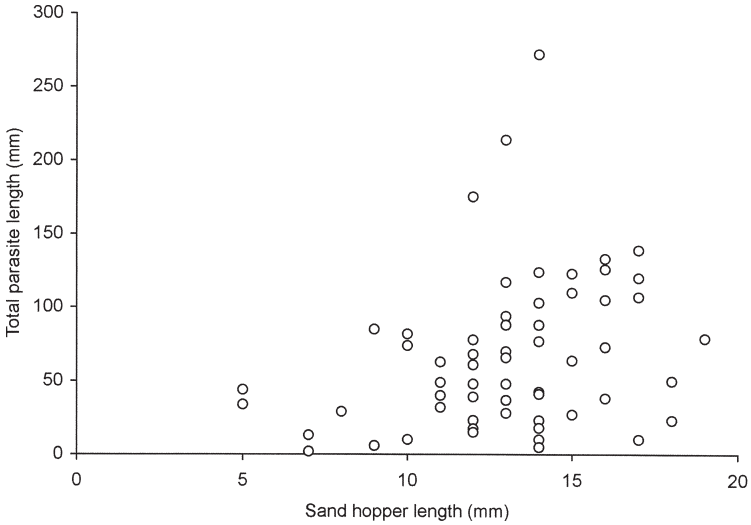

Fig. 2 Total mermithid worm *Thaumamermis zealandica* length as a function of the length of the sand hopper *Talorchestia quoyana* host (n = 61) in both the field study and the habitat choice experiment.

Table 2 Assessment of factors relevant to sand hopper *Talorchestia quoyana* burrowing depth in field study core samples by stepwise ordinal regression.

Effect	Model deviance*	Change in deviance*	d.f.	*P* value
Month of collection	89.026	89.026	3	< 0.0001
Kelp patch	101.629	12.603	4	0.0134
Replicate core	104.564	2.935	1	0.0867
Sand hopper length	122.902	18.338	1	< 0.0001
Sand hopper sex	123.919	1.017	2	0.6014
Parasite infection	124.203	0.284	1	0.5941

*Chi-square distributed.

Table 3 Summary data for the sand hoppers *Talorchestia quoyana* and parasites *Thaumamermis zealandica* collected across different depths in the field study. Core samples were from 0 to 11 cm, 12 to 22 cm, and 23 to 33 cm below the sand surface for depth 1, depth 2, and depth 3, respectively.

Depth of core sample	No. of sand hoppers (mean ± SEM, *n*)	Sand hopper length (mm, mean ± SEM, *n*)	Prevalence of infection (%)	Total parasite length per sand hopper (mm, mean ± SEM, *n*)
Depth 1	19.9 ± 3.8 (16)	7.6 ± 0.21 (318)	7.4	80.6 ± 13.8 (22)
Depth 2	8.3 ± 3.6 (16)	12.3 ± 0.28 (132)	5.6	32.0 ± 16.0 (7)
Depth 3	3.3 ± 1.3 (16)	7.4 ± 0.51 (53)	3.9	26.0 ± 8.0 (2)

deviance revealed that the interaction of moisture treatment and replicate box (associated with the experimental design) and sand hopper length offered significant improvements to the predictive power of the model, whereas sand hopper sex, parasite infection, and all other interactions did not (Table 4). Across the habitat choice experiment, sand hoppers were found to be most abundant in the sheltered habitat beneath the sponge within each container (Table 5). This distribution did not vary with moisture treatment (Table 5). Sand hopper length, prevalence of infection, and total parasite length per sand hopper showed little variation across habitat or moisture treatment (Table 5).

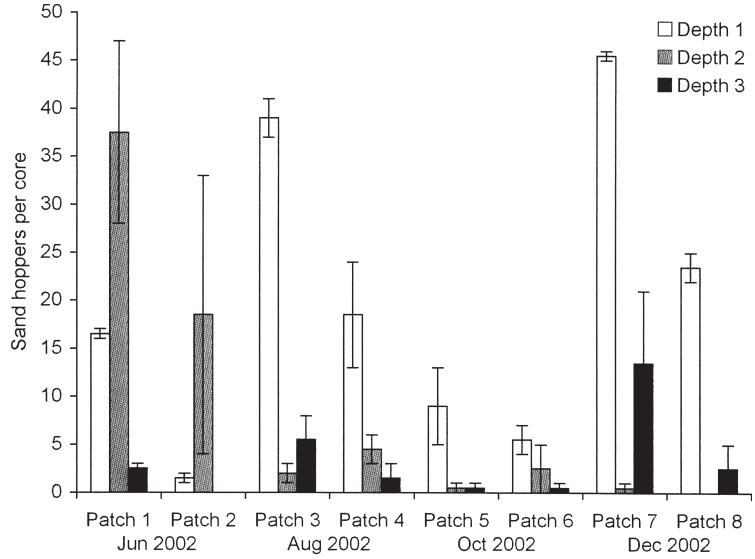

Fig. 3 Number (mean ± SE) of sand hoppers *Talorchestia quoyana* collected in core samples (n = 2) as a function of the depth zone, kelp patch and month.

Table 4 Assessment of factors relevant to sand hopper *Talorchestia quoyana* distribution in the laboratory habitat choice experiments by stepwise logistic regression.

Effect	Model deviance*	Change in deviance*	d.f.	P value
Error	532.34			
Moisture	530.25	2.09	1	0.1483
Moisture treatment × Replicate box	331.72	198.53	8	< 0.0001
Sand hopper length	327.80	3.92	1	0.0477
Sand hopper sex	327.25	0.55	2	0.7596
Parasite infection	322.61	3.41	1	0.0648

*Chi-square distributed.

Table 5 Summary data for the sand hoppers *Talorchestia quoyana* and parasites *Thaumamermis zealandica* collected in both habitats in the habitat choice experiment.

Habitat and moisture treatment	No. of sand hoppers (mean ± SEM, n)	Sand hopper length (mm, mean ± SEM, n)	Prevalence of infection (%)	Total parasite length per sand hopper (mm, mean ± SEM, n)
Sponge low moisture	31.4 ± 7.6 (5)	11.4 ± 0.1 (157)	6.1	66.3 ± 14.8 (9)
Sponge high moisture	33.0 ± 4.6 (5)	11.6 ± 0.2 (165)	7.8	71.1 ± 10.9 (12)
Sand low moisture	14.2 ± 8.5 (5)	11.8 ± 0.3 (71)	6.0	62.0 ± 27.3 (4)
Sand high moisture	11.0 ± 3.6 (5)	12.6 ± 0.4 (55)	10.0	76.2 ± 14.9 (5)

DISCUSSION

Parasite manipulation of host behaviour has been identified in many parasite taxa (Poulin 1995, 1998; Moore 2002) including mermithid nematodes (Maeyama et al. 1994; Vance 1996; Williams et al. 2001). In mermithid systems, parasites requiring a wet habitat for survival and reproduction have evolved host manipulation strategies (Maeyama et al. 1994; Vance 1996). The physiological requirement of moist sand for the mermithid nematode *T. zealandica* to emerge from its sand hopper host is consistent with other mermithid examples where behaviour manipulation has been identified. In this physiological and phylogenetic context, the patterns

in host burrowing depth and infection status detected by Poulin & Latham (2002a) were justifiably interpreted as evidence for parasite manipulation. This position was further supported by the findings of Williams et al. (2004), suggesting a physiological mechanism for parasite manipulation through host thirst response. However, the results of the present study do not reflect the patterns found by Poulin & Latham (2002a) and do not show evidence of an effect of moisture as predicted by the findings of Williams et al. (2004). In the study by Poulin & Latham (2002a), four parameters associated with parasite manipulation were tested: (1) parasite prevalence; (2) the number of parasites per host; (3) the length of the longest parasite per host; and (4) the total parasite length per host. These parameters were all expected to be elevated in sand hoppers found in deep, moist sand. Poulin & Latham (2002a) found three of the four parameters (1, 2, and 3) were elevated in sand hoppers found deeper in the sand. In contrast, none of the parameters were elevated in the present study. Given the differences in experimental approaches, this finding may indicate that parasites in this system can be observed to affect behaviour but only under certain conditions, possibly under predominantly unnatural conditions. To interpret this possibility further, it is necessary to consider the conditions found to influence burrowing depth in the present study and address the differences between studies that may account for variability in the effect of parasites.

In the field study, samples contained mostly juvenile sand hoppers, which were concentrated in the upper levels of the sand. The presence of juveniles close to the surface may account for the importance of sand hopper length in predicting burrowing depth. Seasonal differences may account for the presence of a gravid female in core samples. The number of sand hoppers collected in the paired core samples taken from the same patch of kelp was not significantly different, confirming the minimal disturbance effect of core extraction.

The most striking pattern in the field study was the variation in sand hopper abundance and parasite prevalence across different depths, kelp patches, and months. This variation across patches and months was identified as an important factor in determining sand hopper burrowing depth. Heterogeneity in host abundance between kelp patches and its associated effect on parasite prevalence has been established in a previous study (Poulin & Rate 2001), but variation in host burrowing depth between patches was heretofore unknown. One could suggest that this variability may have masked any effects of parasite presence or parasite length on host behaviour. However, this interpretation is not supported by reduced parasite prevalence and reductions in total parasite length in sand hoppers found deeper in the sand. These results are contrary to predictions of parasite manipulation, where the larger, mature worms would be expected to occur within these deep-burrowing sand hoppers. The present results are also the opposite of the experimental results found by Poulin & Latham (2002a).

The results of the habitat choice experiment examined the effect of moisture in a horizontal gradient, thereby removing the effect of depth. The dominant pattern observed in this experiment was that sand hoppers preferred the side of the container sheltered by the sponge. Further analysis identified the length of the sand hoppers, the moisture treatment, and individual differences across replicates, as other significant factors in habitat selection. Parasite infection was not a significant factor in determining sand hopper burrowing behaviour. However, given the considerable variability in the data, the most parsimonious conclusion was that the role of parasites remains unclear for both experiments. Future experiments using sponges on both sides, one wet and one dry, may help to clarify the role of moisture and separate this from the shelter-seeking behaviour observed.

The difference in parasite prevalence across experiments may help to account for the variation in results. It is possible that sample size prevented the detection of an effect. The design of the experiment entailed the use of naturally infected hosts that are indistinguishable from their conspecifics. Thus the present study was designed to obtain adequate samples based on previous infection levels that had been considerably higher (31.3%, Poulin & Latham 2002a) than those found in the present study (Table 1). Parasite prevalence was low in the field study, and the use of selective collection of larger sand hoppers to increase parasite prevalence was unsuccessful in the habitat choice experiment, possibly owing to the different month in which samples were collected. Despite this possible shortcoming, significant factors in burrowing depth and habitat choice were identified. Sand hopper length was a significant factor in both the field study and the habitat choice experiment. Additionally, the month of collection and kelp patch were significant factors for the field study, whereas the interaction of moisture treatment and replicate box was significant for the habitat choice experiment. Furthermore,

reduced sample size of infected sand hoppers does not account for the increased size and prevalence of larger, mature worms closer to the surface in the burrowing depth experiment. A further possibility worth considering is the role that external factors may play in triggering host behavioural change. The plasticity of mermithid size at maturity (Poinar et al. 2002; Poulin & Latham 2002a,b) could indicate that size was important, but may not be the only factor governing parasite emergence. Possibilities worthy of further investigation include a seasonal trigger of behaviour manipulation, based on changes in photoperiod or temperature, or seasonal and inter-annual cycles in behaviour modification, based on synchronous timing of events across both parasite and host life-cycles. Interactions between hosts could be a factor. Host density was found to be considerably lower (3947 per m^2) in the field study than used by Poulin & Latham (2002a) (6366 per m^2). In comparison, the habitat choice experiment used a lower host density (1389 per m^2) owing to the shallowness of the containers and to encourage movement of the animals along the horizontal moisture gradient.

We need to also consider the assumptions of our experimental design further in seeking to explain the observed patterns in our results. Burrowing depth of sand hoppers may be related to moisture levels, but equally, in the field, other factors such as oxygen concentration, distance from food sources, and probability of predation may also be involved, perhaps resulting in tradeoffs not observed in the laboratory. Additionally, although a sand moisture gradient produced an effect in previous laboratory studies, there may be a threshold above which increased moisture is of no further benefit. Despite our efforts to ensure consistency in sampling, inherent variability in the field study may have resulted in optimal moisture levels at intermediate depths. In essence, the behavioural response to a moisture gradient in the laboratory, as observed by Poulin & Latham (2002a), might not have been observed in the field as moisture levels may have been more variable and other factors may have been of greater importance. Future experiments quantifying both sand moisture and oxygen concentration as well as experimentation with food sources and the presence of predators may clarify these relationships.

The present study illustrates the difficulty in interpreting behaviour variation as the product of parasite manipulation in an inherently variable system. This problem was compounded by the nature of the behavioural change. Poulin (1995) stated that manipulations of host behaviour can only be considered adaptive if they satisfy certain conditions: (1) they must be complex; (2) they must show signs of a purposive design; (3) they are more likely to be adaptations if they have arisen independently in several lineages of parasites; and (4) they must be shown to increase the fitness of the parasite. In his review, Poulin (1995) concluded that although some host behavioural changes are very complex and extremely well-fitted to their presumed function, most are simple increases or decreases in an activity already performed before infection. The present system was an example of the latter and hence the adaptiveness of any change in host behaviour is ultimately difficult to gauge.

In summary, there remains considerable ambiguity concerning the role of the parasite *T. zealandica* and its effect on the burrowing depth and habitat choice of its host *T. quoyana*. The present study sheds light on the complexity underlying subtle variations in an ecologically significant behaviour. This complexity is an important factor in shaping the ecology of the New Zealand sandy shore community.

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