

# Impact of trematodes on host survival and population density in the intertidal gastropod *Zeacumantus subcarinatus*

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**ABSTRACT:** Ecological studies have demonstrated that parasites are capable of influencing various aspects of host life history and can play an important role in the structure of animal populations. We investigated the influence of infection by castrating trematodes on the reproduction, survival and population density of the intertidal snail *Zeacumantus subcarinatus*, using both laboratory and field studies. The results demonstrate a highly significant reduction in the reproductive output in heavily infected populations compared to populations with low trematode prevalence. A long-term laboratory study showed reduced survival of infected snails compared to uninfected specimens, for snails held at 18 and 25°C. Furthermore, parasite-induced mortality in the field was inferred from a reduction in prevalence of infection among larger size classes, indicating that infected individuals disappear from the population, although the effect of parasites varied between localities. A field survey from 13 localities including 2897 snails demonstrated that prevalence of castrating trematodes had a significant negative effect on both population density and biomass of *Z. subcarinatus*. This study provides one of the first demonstrations of population-level effects of parasites on their hosts in the field. The results of this study emphasise the importance of castrating parasites as potential agents of population regulation in host species with limited dispersal ability.

**KEY WORDS:** Trematodes · Population regulation · Castration · Host fecundity · Parasites

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## INTRODUCTION

The influence of parasites on host life-history traits and behaviour has been documented in several studies (Sousa 1983, Curtis 1987, Goater & Holmes 1997, Latham & Poulin 2002, Moore 2002). The potential impact of parasites on host populations and community structure has, however, only recently been recognised by ecologists (Minchella & Scott 1991, Sousa 1991, Poulin 1999, Thomas et al. 1999, Mouritsen & Poulin 2002). A prerequisite for an impact at the population level is a parasite-induced increase in mortality and/or a decrease in fecundity of the host leading to a reduction in the mean rate of increase in the host population (Anderson & May 1981, Jaenike et al. 1995). Good examples of parasite regulation of host abundance

exist from a range of host–parasite systems (Anderson & Crombie 1984, Scott 1987, Scott & Dobson 1989, Hudson et al. 1998, Ebert et al. 2000). However, most examples come from studies on laboratory populations, and it is often not clear whether the impact of parasites is also manifested in truly natural systems.

Infections of aquatic snails with larval trematodes have been recorded from every part of the world and from most gastropod families (Pechenik et al. 2001, Poulin & Mouritsen 2003). Practically all trematodes use gastropods as first intermediate hosts; inside the snail, they multiply asexually to produce new infective stages for the next step in their life cycle. The impact of infection ranges from effects on host growth, behaviour, spatial distribution, survival and fecundity (Sousa 1983, Mouritsen & Jensen 1994, Sokolova 1995; see

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Sorensen & Minchella 2001 for review). Many trematode species castrate the snail host by replacing host reproductive tissue with their own asexual stages (rediae and sporocysts), thus greatly reducing the reproductive output of infected individuals. Furthermore, there is evidence that infected snails experience a lower tolerance to physical, thermal and/or osmotic stress compared to uninfected conspecifics (McDaniel 1969, Tallmark & Norrgren 1976, Lauckner 1980, but see also Sousa & Gleason 1989). During periods of extreme weather conditions a higher mortality of infected specimens can lead to a population crash in heavily infected snail populations (Jensen & Mouritsen 1992).

The New Zealand mud snail *Zeacumantus subcarinatus* (Batillariidae: Prosobranchia), is a widespread and in some places abundant gastropod inhabiting protected bays along the New Zealand coastline. The high density of some populations indicates that it is an important species in the grazing community on soft-bottom habitats in the intertidal zone. Size-frequency studies of this species suggest a longevity of at least 5 to 6 yr (B. L. Fredensborg unpubl. data). As commonly seen for species of Batillariidae, *Z. subcarinatus* has direct development and snails hatch from eggs into crawl-away larvae lacking a planktonic dispersal stage (B. L. Fredensborg pers. obs.).

*Zeacumantus subcarinatus* is commonly infected with larval trematodes inhabiting the gonads of the snail. Very high prevalence (i.e. proportion of hosts infected) has been found in snails at some localities within the study area. The most common trematode found is the microphallid *Maritrema novaezelandensis*. This trematode has a 3-host life cycle comprising *Z. subcarinatus* as the first intermediate host, crustaceans as second intermediate hosts and shorebirds as definitive hosts (Martorelli et al. 2004).

The main purpose of this study was to examine the effect of larval trematodes on reproduction, survival and, in turn, density and biomass of *Zeacumantus subcarinatus*. The trematode–*Z. subcarinatus* system is a good model system for studying the effects of parasites on host populations for several reasons. (1) The reproductive strategy of the snail, which lacks a planktonic larval stage and in which populations rely on local reproduction, keep snail populations relatively isolated and thus independent from each other, allowing an investigation of the direct impact of castrating trematodes on local host density. (2) Trematode prevalence in *Z. subcarinatus* populations shows a spatially heterogeneous pattern reflecting preferred feeding and roosting sites for shorebird final hosts within the study area, allowing a comparison of high- and low-prevalence populations that have otherwise similar living conditions. (3) Because *Z. subcarinatus* is a long-lived species, trematode prevalence shows only little sea-

sonal variation (B. L. Fredensborg unpubl. data), a factor that could otherwise obscure any effect that parasites may have on host population density. Laboratory experiments on egg production and survival were performed. The latter was investigated in relation to temperature-dependent release of cercariae and starvation. In addition, field measurements were conducted to determine whether the observed effects of the parasite have relevance for populations in nature.

## MATERIALS AND METHODS

**Reproduction and trematode infection.** In October 2002, 100 *Zeacumantus subcarinatus* were randomly sampled during low tide from each of 3 locations within the Otago Harbour, South Island, New Zealand. The 3 locations were selected according to prevalence of larval trematodes castrating *Z. subcarinatus*. Based on previous findings the prevalence of trematodes from the 3 localities could be characterised as low (Dowling Bay, <5%; Site 3, Fig. 1), moderate (Latham Bay, 10 to 20%; Site 9, Fig. 1) and high (Oyster Bay, >50%; Site 8, Fig. 1). For each locality, the shell height of all individuals was measured and the snails were divided into 5 replicates of 20 snails each. Each replicate was transferred to a 300 ml container supplied with seawater, a 2 mm-thick sediment layer sieved through a 500 µm sieve, and a standard amount of the sea lettuce *Ulva lactuca*. The snails were left at room temperature ( $18 \pm 2^\circ\text{C}$ ) under a 12 h light:12 h dark cycle for 40 d. During the experiment, water, sediment and sea lettuce were changed weekly. Egg-strings were recovered from the containers every second day and the numbers of eggs were recorded. At the end of the experiment shell height was measured and the snails were dissected under a stereomicroscope. Specimens of *Maritrema novaezelandensis* were identified following Martorelli et al. (2004). All other trematodes were identified to the family level according to Deblock (1980).

Because habitat quality in terms of food supply may vary among the 3 sites and because the initial nutritional status of the snails may affect the reproductive output during at least the first days of experimentation, we measured the chlorophyll *a* content in the sediment of the 3 localities (see later subsection). This provided a measure of the population of benthic microalgae as an important food source for the herbivorous snails.

To test for potential floating of juveniles, a plankton net (opening = 15 cm) was slowly dragged through the surface water of the incoming tide, parallel to the shoreline in Oyster Bay (approximately 100 m long). This procedure was carried out twice on each of

10 tides, evenly distributed throughout the reproductive period of *Zeacumantus subcarinatus* (November to March).

**Cercarial production and parasite-induced mortality—laboratory experiment.** To study the effect of temperature on cercarial production, 3 groups, each of 10 *Maritrema novaezealandensis*-infected snails of similar shell height (12 to 13 mm), were incubated individually in 10 ml dishes at 8, 18 and 25°C. After 24 h the number of released cercariae was counted.

Several hundred adult *Zeacumantus subcarinatus* were sampled from both Lower Portobello Bay and Oyster Bay (Sites 11 and 8, respectively, Fig. 1) in January 2003. The snails were screened for the presence of larval trematodes by incubating them individually in 10 ml Petri-dishes under 24 h illumination at 25°C. Normally, snails with mature *Maritrema* spp. infections will start shedding cercariae within a few hours under these conditions (Mouritsen 2002, this study). Uninfected snails were tested again after 8 d to verify their infection status. Because the majority of infections in *Zeacumantus subcarinatus* were due to *M. novaezealandensis* (61.5% of all infections, see 'Results') only snails harbouring this species were used in the experiment. The snails were separated into 2 groups (10 replicates of 20 infected snails and 10 replicates of uninfected snails), and transferred to 300 ml containers supplied with seawater and sea lettuce *Ulva lactuca* as a food source; 5 replicates of each infection status were incubated at 18 and 25°C, respectively. Water and food were changed and snail survival checked each week. After 6 wk, the amount of food in each container was limited to microalgae growing on a standard area of rocks added to the containers. These rocks were not replaced for the rest of the experiment. After 40 wk, all remaining snails were counted and dissected to confirm their original infection status.

**Field survey of trematode prevalence in snail populations.** Quantitative and qualitative samples of *Zeacumantus subcarinatus* were collected from 10 localities in and around Otago Harbour (Sites 1, 2, 3, 5, 6, 8, 9, 11, 12 and 13, Fig. 1) in September 2002; 3 additional localities (Sites 4, 7 and 10, Fig. 1) were included in the study in September 2003. To determine the density of snails, 15 core samples (each 0.1188 m<sup>2</sup>, 5 cm deep) were collected randomly in an area of approximately 50 × 50 m parallel to the

shoreline at mid-tide level. In addition, qualitative samples were obtained by collecting the top 5 cm of sediment from patches (approximately 0.1 m<sup>2</sup>) randomly selected within the sampling area to ensure a minimum of 100 snails per locality for determination of parasite prevalence. Samples were sieved *in situ* through a 500 µm mesh sieve and the snails retained were preserved in pH-buffered 4% formaldehyde. In the laboratory, shell height was measured, and snails >6.0 mm were dissected and examined for the presence of larval trematodes (based on a preliminary study showing that no infections could be detected in snails below this size). Species of trematodes were identified according to Deblock (1980) and Martorelli et al. (2004).

Data on chlorophyll *a* content in the substrate previously collected from 10 of the 13 localities were used in the analysis. Sediment samples were obtained in May by random sampling of 5 core samples (each 0.1188 m<sup>2</sup>, 5 cm deep) from each locality. Chlorophyll *a* was extracted from the sediments in 95% ethanol and its concentration was measured using a spectrophotometer, following standard techniques.

**Data analysis.** The log-transformed mean total reproductive output as well as chlorophyll *a* data were compared among localities using a 1-way ANOVA performed in combination with post-hoc analyses (Fisher's least-significant difference, LSD test). Both trematode prevalence and mean snail size were tested against the reproductive output of replicates from all 3 populations using a Spearman rank-order correlation. Mean snail size was compared among populations

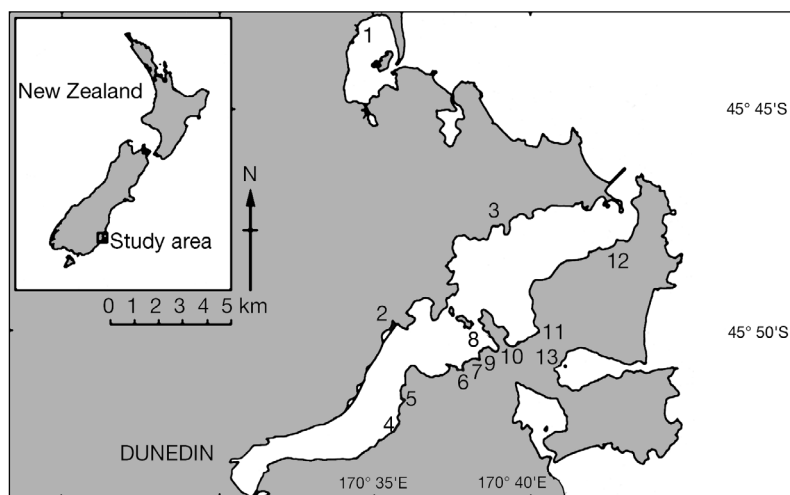


Fig. 1. Map of study area showing 13 study sites within and around the Otago Harbour, New Zealand. 1: Blueskin Bay; 2: Sawyers Bay; 3: Dowling Bay; 4: McAndrew Bay; 5: Company Bay; 6: Turnbull Bay; 7: Edwards Bay; 8: Oyster Bay; 9: Latham Bay; 10: Lamlash Bay; 11: Lower Portobello Bay; 12: Otakou; 13: Papanui Inlet

using a Kruskal-Wallis 1-way ANOVA by rank, as homogeneity of variance between populations was violated. Subsequently, post-hoc pair-wise comparisons were performed according to Siegel & Castellan (1988).

To determine the biomass of *Zeacumantus subcarinatus* in the field, the wet weight of 70 snails ranging in size from 2 to 16 mm was obtained and the relationship between snail size and weight was best fit as follows: snail wet weight = 0.0079–0.006 (shell height) + 0.0014 (shell height)<sup>2</sup> ( $R^2 = 0.98$ ). This relationship between size and weight was then applied to size-frequency data from the 13 localities to calculate mean wet weight of *Z. subcarinatus* m<sup>-2</sup> at each locality. Biomass and snail density were then included as dependent variables in 2 stepwise multiple regressions with the 3 factors Habitat quality (measured as chlorophyll a content in the sediment), Snail size and Trematode prevalence as predictor variables. Prior to analysis, density and biomass were log-transformed and trematode prevalence arcsine square-root-transformed to mitigate violations of normality and variance.

The statistical packages SPSS for Windows (10.1), and Statistica (6.0) were used for analyses and results were considered significant at  $p < 0.05$ .

## RESULTS

### Reproduction and trematode infection

Snails from all replicates began to produce eggs within 1 wk of the start of the experiment. Egg-strings were found attached to sea lettuce and the sides of the container. The sex ratio was slightly female-biased (60%) and did not differ between the three localities ( $\chi^2 = 0.90$ ,  $p = 0.63$ ). There was a significant difference in the reproductive output between the 3 locations (1-way ANOVA,  $F_{2,12} = 50.4$ ,  $p < 0.001$ ) (Fig. 2a), all differing up to 5-fold from each other (Fisher's LSD,  $p < 0.01$  for all comparisons). In accordance, a significant difference in prevalence of larval trematodes was recorded between all 3 localities ( $\chi^2 \geq 7.95$ ,  $df = 1$ ,  $p < 0.005$  for all comparisons). Prevalence was highest in Oyster Bay ( $78.4 \pm 3.9\%$ ; mean  $\pm$  SE) and lowest in Dowling Bay ( $4.5 \pm 2.0\%$ ) (Fig. 2b). It follows that reproduction output was negatively related to trematode prevalence among the 15 replicates used in the study ( $r_s = -0.84$ ,  $n = 15$ ,  $p < 0.001$ )—presumably due to parasite-induced castration, since it was observed that larval trematodes had largely replaced host gonads in mature infections.

Mean shell height of uninfected (i.e. reproducing) snails was significantly different between the 3 localities (Kruskal-Wallis test,  $H = 29.4$ ,  $df = 2$ ,  $p < 0.001$ )

(Fig. 2c). Snails in Oyster and Dowling Bay were slightly larger than snails from Latham Bay ( $p < 0.05$  for both comparisons). However, mean snail size was unrelated to the reproductive output across all 15 replicates of snails ( $r_s = 0.01$ ,  $n = 15$ ,  $p = 0.96$ ), suggesting that the difference in size between localities did not influence the total reproductive output.

Mean chlorophyll a content was significantly different between the localities (1-way ANOVA,  $F_{2,12} = 11.0$ ,  $p = 0.002$ ) (Fig. 2d). The sediment in Dowling Bay contained significantly less chlorophyll a than both Latham and Oyster Bay (Fisher's LSD test,  $p \leq 0.002$ ), suggesting lower food abundance for the snails in Dowling Bay.

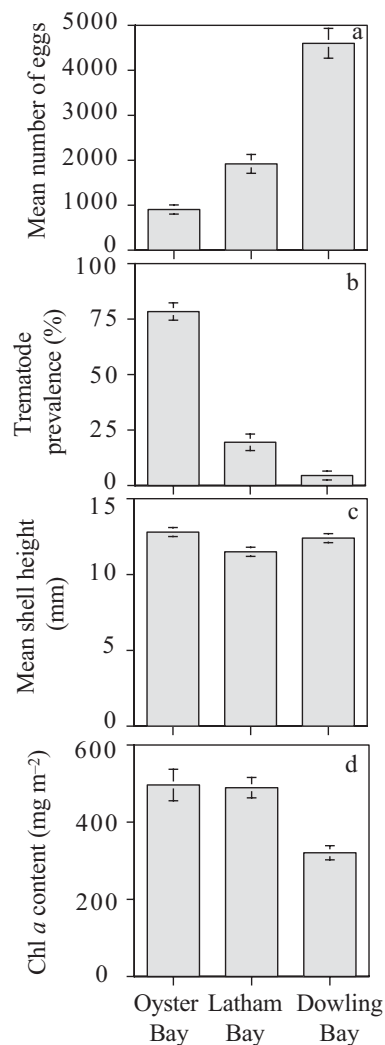


Fig. 2. *Zeacumantus subcarinatus*. Parameters monitored for each of 3 populations in recruitment experiment. Data are means  $\pm$  SE. (a) Total reproductive output over 40 d and (b) trematode prevalence ( $n = 5$  groups of 20 snails); (c) shell height of uninfected females ( $n_{\text{Oyster}} = 13$ ,  $n_{\text{Latham}} = 47$ ,  $n_{\text{Dowling}} = 49$ ); (d) chlorophyll a content of sediment ( $n = 5$  for each study site)

Not a single floating juvenile snail was caught in Oyster Bay during the entire study, suggesting that there was no demographically significant secondary settlement of juveniles from *Zeacumantus subcarinatus* populations outside the bay.

### Cercarial production and parasite-induced mortality — laboratory experiment

Cercarial production was highly temperature-dependent (Fig. 3). No cercariae were released from snails incubated at 8°C, whereas the mean number of cercariae released from a snail was significantly higher at 25 than at 18°C (Mann-Whitney *U*-test,  $Z = -2.123$ ,  $p = 0.034$ ).

Small differences in the survival of snails were observed between replicates within treatments, but as they were all distinct from other treatments, data from replicates were pooled before between-treatment analysis. Snail survival was influenced by temperature (Fig. 4). The survival of snails incubated at 25°C was significantly reduced compared to that of snails incubated at 18°C, regardless of infection status (Kolmogorov-Smirnov 2-sample test,  $p < 0.05$  for all pair-wise comparisons). Within both temperature treatments, the survival curves for infected (inf) and uninfected (uninf) individuals were significantly different (Kolmogorov-Smirnov test; 18°C:  $n_{\text{inf}} = 40$ ,  $n_{\text{uninf}} = 23$ ,  $Z = 1.728$ ,  $p = 0.005$ ; 25°C:  $n_{\text{inf}} = 98$ ,  $n_{\text{uninf}} = 74$ ,  $Z = 4.662$ ,  $p < 0.001$ ). For snails kept at 18°C the difference in survival between the 2 infection groups was mainly due to an increased mortality of infected snails after Week 23, whereas infected snails at 25°C showed a higher mortality rate than uninfected specimens from Weeks 10 to 22 (Fig. 4).

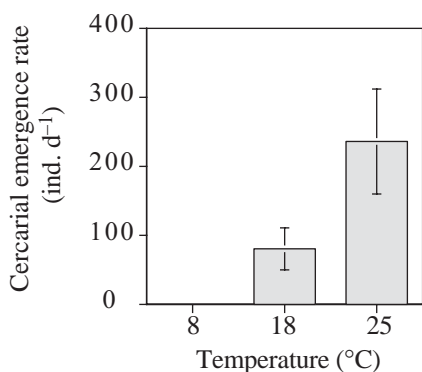


Fig. 3. *Maritrema novaehelandensis*. Effect of temperature on emergence of cercariae from *Zeacumantus subcarinatus* (mean  $\pm$  SE ind. snail<sup>-1</sup> d<sup>-1</sup>;  $n = 10$  per temperature treatment)

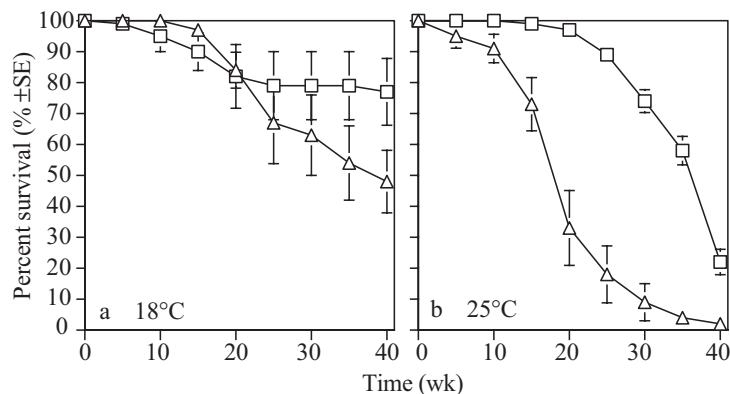


Fig. 4. *Zeacumantus subcarinatus*. Mean ( $\pm$  SE) percent survival of uninfected ( $\square$ ) and infected ( $\Delta$ ) snails, incubated at: (a) 18 and (b) 25°C. For clarity, only survival at 5 wk intervals is shown. Analysis was, however, performed on all available data ( $n = 5$  groups of 20 snails for each treatment at start of experiment)

### Field survey of trematode prevalence in snail populations

A total of 2897 snails from 13 localities were examined for larval trematodes. We recorded 5 species of trematodes, all of which were found to occupy the gonads of the snails. The variation in trematode prevalence between localities was large, ranging from 4.4 % (Dowling Bay, Site 3, Fig. 1) to 86.8 % (Lower Portobello, Site 11, Fig. 1). *Maritrema novaehelandensis* (Microphallidae) accounted for 61.5 % of all infections. The prevalences of Philophthalmidae and Echinostomatidae were low to moderate, whereas infection with Heterophyidae and *Microphallus* sp. (Microphallidae) was only rarely observed. At some localities, parasite prevalence increased with increasing snail size, whereas at other localities the prevalence levelled off or peaked at intermediate snail sizes (Fig. 5).

Of the 3 factors (trematode prevalence, snail size and sediment chlorophyll *a*) included in a stepwise multiple regression across 10 localities, only prevalence and size had a significant effect on snail density and only these 2 variables were thus entered in a subsequent multiple regression model that also included the remaining 3 localities. Both snail size and prevalence were significantly and negatively related to snail density (Table 1). No relationship was found between mean snail size and prevalence ( $F_{1,11} = 0.43$ ,  $p = 0.53$ ,  $R^2 = 0.04$ ). Residuals of the regression of mean snail density on mean snail size were, on the other hand, significantly and negatively associated with trematode prevalence ( $F_{1,11} = 13.41$ ,  $p = 0.004$ ,  $R^2 = 0.55$ ) (Fig. 6a). This finding indicates that castrating trematodes may have a strong negative effect on snail density in the field. For biomass data, all 3 factors (trematode prevalence, chlorophyll *a* and snail size), were included in a stepwise multiple regression model, indicating that

they together explained a significant amount of the variation in snail biomass among localities. However, only prevalence was significantly related to snail bio-

mass among the 10 sites where chlorophyll *a* was measured (Table 2). Inclusion of all 13 localities in the analysis strengthened the relationship between prevalence and biomass of *Zeacumantus subcarinatus* (linear regression,  $F_{1,11} = 10.88$ ,  $p = 0.007$ ,  $R^2 = 0.50$ ) (Fig. 6b).

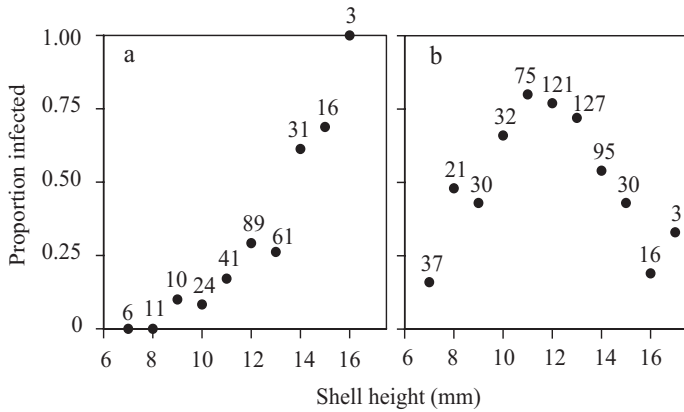


Fig. 5. *Zeacumantus subcarinatus*. Relationship between snail size and proportion of infected snails in each size class at 2 study sites sampled in August and September 2002. Sample sizes denoted above each data point. (a) Papanui Inlet (Site 13), September ( $n = 292$ ); (b) Oyster Bay (Site 8), pooled data from August and September ( $n = 587$ )

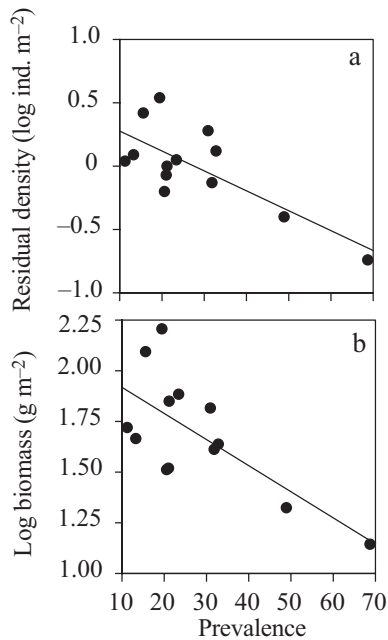


Fig. 6. *Zeacumantus subcarinatus*. Relationship between snail density and biomass and trematode prevalence (arcsine square-root-transformed) across 13 study sites in and around Otago Harbour. (a) Residual density from regression of mean density on snail size (individuals  $m^{-2}$ ) plotted against trematode prevalence; trematode prevalence for each study site is based on a sample ranging between 108 and 370 snails (mean = 223 per locality); density data are mean values of 15 core samples per locality; line denotes best-fit through data points ( $y = -0.016x + 0.434$ ,  $R^2 = 0.55$ ). (b) Calculated snail biomass (g wet wt  $m^{-2}$ ) plotted against trematode prevalence; line denotes best-fit through data points ( $y = -0.013x + 2.047$ ,  $R^2 = 0.50$ )

**DISCUSSION**

Very few studies have documented population-level impacts of parasites in natural systems. Our study used laboratory experiments combined with field measurements to assess the effect of larval trematodes on the snail *Zeacumantus subcarinatus*, one of the dominant grazing gastropods in New Zealand intertidal areas.

**Reproduction and trematode infection**

Local reproduction, in combination with immigration, determines the degree to which populations can balance or outnumber mortality and emigration. For snail species lacking a planktonic stage, migration is restricted to crawling of adults or floating of juveniles (Race 1981, Adachi & Wada 1999). No floating juveniles were found in this study, suggesting that drifting does not contribute significantly to populations of *Zeacumantus subcarinatus*. Thus for this species it seems that populations are maintained by local reproduction.

Table 1. *Zeacumantus subcarinatus*. Regression summary of effect of trematode prevalence and mean snail size on snail density from 13 localities

Factor	Beta	<i>t</i> (10)	<i>p</i>
Intercept	16.902		<0.0001
Trematode prevalence	-0.560	-3.767	0.0043
Mean snail size	-0.574	-3.674	0.0037
Model: $F(2, 10) = 17.496$ , $p < 0.0001$ , $R^2 = 0.733$			

Table 2. *Zeacumantus subcarinatus*. Regression summary of effect of trematode prevalence, mean snail size and chl *a* on snail biomass from 10 localities

N = 10	Beta	<i>t</i> (6)	<i>p</i>
Intercept	6.409	<0.0001	
Trematode prevalence	-0.822	-3.478	0.0132
Mean snail size	-0.323	-1.536	0.1755
Chl <i>a</i>	0.303	1.302	0.2410
Model: $F(3, 6) = 6.074$ , $p = 0.030$ , $R^2 = 0.628$			

Parasitic castration has been documented in many snail–trematode associations (Sousa 1983, Lafferty 1993, Mouritsen & Jensen 1994, Sokolova 1995, Sorensen & Minchella 1998). The mechanisms by which trematodes castrate the snail host include chemical interference with host hormonal production in the pre-patent stage followed by physical destruction of host reproductive tissue in mature infections (Hurd 1990). This study has demonstrated that parasitic castration occurs in *Zeacumantus subcarinatus* infected with larval trematodes. All trematode species were found in the gonad of the snail, seemingly leading to replacement of host reproductive tissue by redia or sporocyst stages in mature infections. This observation fits well with the significant negative relationship between egg production and trematode prevalence found among replicates of snails from the 3 *Z. subcarinatus* populations studied. Snail fecundity is mainly determined by size and the nutritional status of the uninfected snail (Hughes & Roberts 1981, Hughes & Answer 1982, Sokolova 1995). Thus, a difference in the reproductive output between localities could mirror a difference in mean size and/or habitat quality. Although there was a small difference in mean shell height of snails from the 3 localities, no significant relationship between egg production and snail size could be demonstrated, suggesting that the observed size variation may have had little impact on the different overall reproductive output among sites. Also, the availability of food, and hence probably the nutritional status prior to experimentation, appears to be unimportant. The chlorophyll a content of the sediment was lowest in Dowling Bay, where the snails had the largest reproductive output. Therefore, the difference in reproductive output between localities is best explained by parasitic castration.

#### Parasite-induced mortality

In snail–trematode associations it is commonly observed that trematode prevalence increases with increasing snail age (Whitlatch 1974, Hughes & Roberts 1981, Hughes & Answer 1982, Curtis & Hurd 1983, Sokolova 1995, Kube et al. 2002). The simplest explanation is that older snails have been exposed to trematode infections for a longer period of time and therefore have a greater probability of being infected. However, the field survey revealed that in some *Zeacumantus subcarinatus* populations the prevalence levelled off or even decreased as a function of size in the largest size classes (Fig. 5). Based on previous studies of snail–trematode associations (Curtis 2003), it is assumed that trematode infections in *Z. subcarinatus* are not lost over time. Instead, the observed infection

patterns at some localities suggest that infected individuals are being removed from the population. If mortality of infected individuals is higher than that of uninfected conspecifics, the former disappear from the population over time, producing a curvilinear infection pattern as observed in Oyster Bay (Fig. 5b). A decrease in infection level with host age is usually interpreted as parasite-induced mortality and has been recorded for a number of invertebrates (Thomas et al. 1995, Rousset et al. 1996, Latham & Poulin 2002).

Parasite-induced mortality has been recorded for other snail–trematode associations. Huxham et al. (1993) and Lafferty (1993) demonstrated higher *in situ* mortality rates for infected versus uninfected *Littorina littorea* and *Cerithidea californica*, respectively. In addition, several studies have reported a higher susceptibility of infected snails to environmental stress factors frequently experienced by fauna in the intertidal zone (McDaniel 1969, Tallmark & Norrgren 1976, Lauckner 1980). The mechanisms behind a higher mortality of trematode-infected snails are unclear, however. In *Biomphalaria glabrata*, mortality of snails infected by *Schistosoma mansoni* apparently resulted from the physical damage of host tissue following the release of cercariae, as survival in the pre-patent stage did not differ from that of uninfected specimens (Pan 1965). As cercarial production is highly temperature-dependent (Fig. 3), parasite-induced mortality is expected to be more pronounced at higher temperatures when cercarial emergence is facilitated. The results of the survival study show that survival is reduced for snails incubated at higher temperatures. The similar but temporally out-of-phase survival curves for uninfected and infected snails at 25°C suggest that with the restricted amount of food available after Week 6, the reduced survival of infected individuals is more likely to be caused by higher nutritional demands of infected individuals, leading to earlier death by starvation, than by a direct effect of emerging cercariae. This explanation is supported by the delayed effect at 18°C, where the metabolic rate was lower and starvation therefore was expected to occur later in the experiment. However, the effects of higher nutritional demands and cercarial emergence are not mutually exclusive, and may both contribute to the observed differences in survival between uninfected and infected *Zeacumantus subcarinatus*.

#### Implications for *Zeacumantus subcarinatus* populations

While the negative effect of infection by castrating trematodes on the fecundity and mortality of hosts is well known, only a few studies have dealt with the

impact of trematodes on snail populations. Lafferty (1993) and, to a lesser extent, Sokolova (1995) found a negative association between snail density and prevalence of castrating trematodes in *Cerithidea californiensis* and *Littorina saxatilis*, respectively, both species with a reproductive strategy similar to that of *Zeacumantus subcarinatus*. In a small-scale study, Ewers (1964) found a negative relationship between trematode prevalence and snail density in *Velacumantus australis*, but explained the higher prevalence in low-density patches with a higher probability of snails becoming infected from an equally dense distribution of trematode eggs on the sediment surface. In this study we did not find any within-locality relationship between density of snails in the core samples and trematode prevalence (unpubl. results). A direct impact of trematodes on snail fecundity and survival thus seems to provide a more plausible explanation for the observed relationship between density and trematode prevalence in *Z. subcarinatus* populations. However, as with any study on correlations, we cannot exclude the possibility that trematode prevalence is correlated with an unknown factor highly associated with snail density and biomass in the field.

The data presented here indicate that the potential effect of larval trematodes on *Zeacumantus subcarinatus* populations is mainly via castration of the snail host, substantially reducing the reproductive output of the population. The relatively low mortality rate for infected snails in the laboratory experiment and the high prevalence of *Maritrema novaezelandensis* recorded in the field survey suggest that the difference in mortality between uninfected and infected individuals has a secondary effect on snail density. Furthermore, the effect of trematode prevalence on snail density and biomass was most obvious at high trematode prevalence. In low-prevalence populations, other factors may play a greater role in determining snail density and population structure. To gain a better understanding of how trematodes affect populations of direct-developing snails, future studies should address the effect of other factors affecting snail density such as establishment success of newly recruited juveniles, predation and physical parameters. The negative impact of trematodes on the density and biomass of one of the dominating grazers in the soft-bottom animal community is likely to go beyond the level of the snail host population. Whereas trematodes do not have a direct effect on non-host species, their functional effect on the epibenthic community (i.e. increasing food abundance for other grazers and changing the prey availability for predators) may still be significant (see Mouritsen & Poulin 2002).

**Acknowledgements.** This study was funded by a grant from the Marsden Fund to R.P. and by a grant from The Danish Natural Science Research Council to K.N.M. We wish to thank A. Armstrong for technical assistance and F. Lefebvre and M. Hardman for comments on an earlier draft of the manuscript.

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Editorial responsibility: Otto Kinne (Editor-in-Chief), Oldendorf/Luhe, Germany

Submitted: April 28, 2004; Accepted: October 7, 2004  
 Proofs received from author(s): March 14, 2005