

Parasitism shaping host life-history evolution: adaptive responses in a marine gastropod to infection by trematodes

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Summary

1. Variation in life-history strategies among conspecific populations indicates the action of local selective pressures; recently, parasitism has been suggested as one of these local forces.
2. Effects of trematode infections on reproductive effort, juvenile growth, size at maturity and susceptibility were investigated among different natural populations of the marine gastropod *Zeacumantus subcarinatus*, Sowerby 1855.
3. Reproductive effort was not higher in uninfected snails from populations experiencing a high trematode prevalence, but females from high prevalence populations produced significantly larger offspring compared with their conspecifics from other populations.
4. Juvenile growth rate was significantly higher in laboratory-raised snails originating from females in a high prevalence population compared with other populations.
5. Size at maturity, determined by the appearance of functional gonads, was significantly and negatively related to trematode prevalence, and positively related to mean snail size, across 10 populations in the study area.
6. There was no evidence of different host resistance against trematodes in sentinel snails from high and low prevalence populations exposed to the same infection pressure in the field.
7. Our results strongly indicate that *Z. subcarinatus* adapt to trematodes by reaching maturity early, thereby maximizing their chance of reproducing in populations experiencing a high prevalence of infection by castrating trematodes.

Key-words: *Maritrema novaezealandensis*, parasite, selection, Trematoda, *Zeacumantus subcarinatus*

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Introduction

Life-history theory predicts that natural selection will lead to a maximization of overall lifelong reproductive success (= fitness) (Pianka 1976; Stearns 1989). A key assumption held by life-history theory is that there will be trade-offs between fitness components such as growth, reproduction and survival (Roff 1992). The optimal allocation of energy towards reproduction and growth at any given age will thus be determined by the probability of future reproductive success (Pianka 1976; Kozłowski 1992). That is, if mortality rates increase with age, natural selection should favour indi-

viduals that mature earlier and invest more energy in current reproduction, and vice versa. This has been supported by several empirical studies on predator-mediated mortality, in the laboratory as well as in natural populations (e.g. Reznick 1982; Crowl & Covitch 1990; Reznick, Bryga & Endler 1990; see also Hutchings 1993 for a nonpredation example).

Analogous to predation, parasitism can greatly influence the future reproductive success of hosts. This can be achieved not only by reducing host survival but also by infecting the gonads leading to castration (future reproductive value = 0). Reported host life-history adaptations to castrating parasites often fall into one of the following three categories: (1) host resistance against the parasite; (2) increased host reproductive effort; and (3) earlier maturity (Minchella 1985; Michalakis & Hochberg 1994; Agnew, Koella & Michalakis 2000; Zuk & Stoehr 2002). Host resistance

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involves recognition of the parasite followed by production of antibodies to prevent or strongly inhibit the development of the infection. Mounting an immune response can be costly, and resistant hosts are often reported to have reduced fitness in the absence of parasites compared with their susceptible conspecifics (Minchella & Loverde 1983; Kraaijeveld & Godfray 1997; Languard *et al.* 1998; Webster & Woolhouse 1999; see Sheldon & Verhulst 1996; Kraaijeveld, Ferrari & Godfray 2002 for reviews). Evidence for resistance has mostly been obtained under laboratory conditions, suggesting that resistance is only selected for when the probability and cost of infection are high and constant.

A less costly alternative to resistance is an increase in host reproductive effort (Forbes 1993). This prediction has been supported by a body of evidence from various host–parasite associations, showing that parasites may increase host sexual activity (Polak & Starmer 1998), offspring number (Sokolova 1995; Krist 2001; Kristan 2004), offspring size (Sorci & Clobert 1995; Kristan 2004) and parental effort (Christie, Richner & Oppliger 1996). Likewise, natural selection may favour a greater allocation of energy towards sexual maturity earlier in life in populations where the probability of parasite-induced host mortality and/or castration is high (Lafferty 1993; Agnew *et al.* 1999). However, maturing early and increasing current reproductive effort often comes at the expense of future reproduction (Agnew *et al.* 1999). A higher investment into reproduction is usually traded off against growth, which in turn will reduce the future reproductive potential of the individual (Kozłowski & Uchmanski 1987).

A much studied host–parasite association with respect to host life histories is the snail–trematode association (Minchella & Loverde 1981, 1983; Thornhill, Jones & Kusel 1986; Lafferty 1993; Jokela & Lively 1995; Sorensen & Minchella 1998; Krist 2001). There are at least two good reasons to choose this host–parasite system when looking for parasite-induced changes in host life-history strategies. First, infection by trematodes exerts a strong selective pressure on the snail host because infection almost inevitably leads to host castration, which in evolutionary terms is equivalent to death of the host. Secondly, trematodes are widespread and common parasites in almost every aquatic gastropod species (see: Pechinik, Fried & Simpkins 2001; Poulin & Mouritsen 2003). Snail host life-history adaptations to trematodes may therefore have wider impacts on the coexistence of species and in turn on the structure of aquatic animal communities (see Thomas *et al.* 2000).

This study investigates possible life-history adaptations to larval trematodes in populations of the New Zealand mudsnail *Zeacumantus subcarinatus*. *Z. subcarinatus* is a common inhabitant of soft-sediment intertidal bays, where it feeds on epiphytic and epipellic diatoms. It is characterized by having a direct development of juveniles without a planktonic stage (eggs hatch into crawl-away larvae) (Fredensborg, pers.

obs.). In a previous study, no migration of snails between bays was detected, and populations are thus highly reliant on local reproduction (Fredensborg, Mouritsen & Poulin 2005). With a very limited gene flow between populations, *Z. subcarinatus* is likely to display local adaptation (see Yamada 1989). The prevalence of trematodes is spatially highly variable in snail populations within the study area (4–86%) (Fredensborg *et al.* 2005), and the selective pressure exerted by parasites on the snail host is therefore very different among host populations. Trematode prevalence in populations of snails is roughly constant across seasons and years ($\pm 5\%$, Fredensborg, unpublished data) with very little spatial variation within populations, and snail populations are therefore under an approximately constant selection pressure. Individuals from high prevalence populations able to maximize their early reproductive effort should therefore have a strong selective advantage. The key life-history components, i.e. reproductive effort, measured as offspring number and size, growth, size at maturity, and host susceptibility to trematode infection were examined across snail populations experiencing different trematode prevalences. We were especially interested in the effect of trematodes on the pattern of energy allocation between reproduction and growth, and in possible maternal effects on offspring size, growth and size at maturity. Ours is one of the rare studies to investigate parasite-driven life-history adaptations in natural populations as opposed to laboratory cultures. Based on the reproductive strategy of *Z. subcarinatus* in combination with the varying trematode prevalence among populations, this snail–trematode association provides a useful model to achieve a better understanding of the effect of parasitism on host evolution in natural populations.

Materials and methods

ZEACUMANTUS SUBCARINATUS AND ITS TREMATODE PARASITES

A total of five trematode species have been found inhabiting the gonads of *Z. subcarinatus*, all leading to total castration of the host. The most common trematode is *Maritrema novaezealandensis* (Microphallidae), which accounts for approximately 60% of all trematode infections in *Z. subcarinatus*. The life cycle of *M. novaezealandensis* involves three hosts, with *Z. subcarinatus* acting as the first intermediate host, small crustaceans serving as second intermediate hosts, and shorebirds as the definitive hosts (Fredensborg, Latham & Poulin 2004; Martorelli *et al.* 2004).

REPRODUCTIVE INVESTMENT VERSUS TREMATODE PREVALENCE

In mid-October 2003, several hundred *Z. subcarinatus* were collected from three localities in the Otago Harbour, South Island, New Zealand: Turnbull Bay (TB),

Company Bay (CB) and Lower Portobello Bay (LPB). The three localities were selected based on the total trematode prevalence recorded in the residing *Zeacumantus* populations (including all individuals > 6.0 mm), which was characterized as being low (13%, TB), moderate (30%, CB) or high (86%, LPB). For all three localities the snails were collected by hand in an area of approximately 50 × 50 m in the upper tidal zone. For each locality, all snails were measured to the nearest 0.1 mm (shell length, apex to aperture) using a calliper, and approximately 25 snails from each of eight size classes ranging from 7 to 15 mm were randomly selected and separately transferred to 75-mL containers provided with 2 mm of sediment from the site of collection. The containers were kept at room temperature (16–20 °C) and under the natural photoperiod. Every second day throughout the study (87 days), the containers were checked for the presence of egg-strings and the numbers of eggs were recorded. Water and sediment were replaced every second week throughout the study. At the end of the study, all snails were measured to estimate growth during the study and dissected to examine for trematode infections in the gonads.

To examine maternal investment in offspring size, a subsample of five eggs was randomly selected for measurement from each egg-producing female from all localities during a period of 4 weeks. During that period, egg production was checked daily to ensure an approximately equal age of the eggs at the time of measurement. Because eggs were always produced in a gelatinous mass, often with not very distinctive eggshells, embryos rather than eggs were measured. For each egg, the diameter of the embryo was measured to the nearest 10 µm. Embryo volume was subsequently estimated using the formula for a sphere ($\text{Volume} = 4/3 \times \pi \times r^3$, where r represents the radius of the embryo). The mean volume of embryos from each snail population was subsequently multiplied by egg production to estimate snail reproductive output (i.e. total offspring volume per snail).

To verify the relationship between trematode prevalence and offspring size obtained in 2003, snails were collected from another three localities with similar trematode prevalences in October 2004, using identical sampling methods. The three localities were: Latham Bay (LatB, prevalence = 10.5%), Lamlash Bay (LamB, prevalence = 27%) and Oyster Bay (OB, prevalence = 56%). As in the previous year eggs for measurements were collected during a 4-week period.

To investigate juvenile growth rate in populations of snails experiencing different probabilities of becoming infected, approximately 300 eggs from each of the localities sampled in 2003 were transferred to three separate aquariums (30 × 18 × 15 cm). The juveniles were raised under optimal conditions for growth (temperature: 16–20 °C and with epilithic diatoms growing on small rocks and pebbles, and epiphytic diatoms growing on sea lettuce as food sources). Every second week for 275 days, 50 randomly selected juveniles from each

locality were measured (shell length, apex to aperture) to test for differences in growth rates between localities. During the study, two-thirds of the water was replaced weekly, and small rocks and sea lettuce were replaced approximately every second week.

SIZE AT MATURITY

From mid-October to early November 2004, a large sample of 7–12-mm long *Z. subcarinatus* was randomly collected by hand in the upper tidal zone from an area covering approximately 50 × 50 m for each of 10 bays in and around Otago Harbour. In the laboratory, snails from each locality were measured and grouped into 0.5 mm size classes with 10 snails in each size class. Snails were dissected and categorized as being either mature or immature based upon the state of development of gonads. The criterium for maturity in females was based on vitellogenesis (production of yolk) and in males on the production of spermatozoa. The latter was examined by squashing the male gonad between a glass slide and a cover slip and inspecting it through a light microscope (400× magnification).

An investigation of factors determining size at maturity in *Z. subcarinatus* was carried out using a multiple regression analysis (see data analysis). For that purpose, data on trematode prevalence, chlorophyll *a* content in the sediment, mean snail size and snail density were obtained from a recent study (Fredensborg *et al.* 2005). Quantitative and qualitative samples of snails were collected randomly from an area covering 50 × 50 m in the mid-tidal zone from all 10 localities (see Fredensborg *et al.* 2005 for a detailed description of the sampling method). In the laboratory, snail size was measured (apex to aperture), and all snails > 6.0 mm were dissected and examined for the presence of larval trematodes in the gonad. Only uninfected snails have developed gonads, and only data on uninfected snails were thus used. Data on chlorophyll *a* content in the substrate were available for eight of the 10 localities. Sediment samples were obtained in May by random sampling of five core samples (each 0.1188 m², 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.

SUSCEPTIBILITY TO TREMATODE INFECTIONS

To test whether uninfected snails in high prevalence populations have been selected for resistance against trematode infections, approximately 1000 snails, ranging from 9 to 14 mm, were collected from LPB (high prevalence) and approximately 300 snails from TB (low prevalence) using the sampling technique described above. The snails from the two localities were brought back to the laboratory and transferred to separate containers supplied with aerated seawater, and with sea lettuce *Ulva lactuca* and epilithic microalgae as a food

source. Approximately every 10 days for 10 weeks, all snails from the two localities were individually transferred to 10-mL Petri dishes and incubated at 25 °C for 24 h. This method facilitates the temperature-dependent release of cercarial larvae from the snail host and has previously been used to identify infected *Z. subcarinatus* (Fredensborg *et al.* 2005). After 24 h, the Petri dishes were inspected for trematode cercariae. On each of the incubation events, all snails shedding cercariae were excluded from the experiment. After 10 weeks, the remaining snails from the two localities were marked with contrasting colours. Snails were left to dry on a paper cloth for a few minutes after which the apex of the snails were dipped in nail polish. In February 2004, during the austral summer, marked snails from the two localities were transferred to LPB, where they were released into 10 closed mesh cages (area: 314 cm², mesh size: 3 mm), each containing 10 snails from each locality. Snails from the two localities were size-matched both within and between cages to avoid any effect of size on susceptibility to infection by larval trematodes. The 10 cages were positioned along a transect parallel to the shoreline 30 m from the mean high water level, and with 20 m between each cage. After 53 days, all cages were emptied and the snails returned to the laboratory. Recent trematode infections in snails can be difficult to detect, and the snails were therefore kept under laboratory conditions for an additional period of 47 days to enhance the probability of the infection to develop. Subsequently, all snails were dissected and gonads examined for the presence and species of trematodes.

STATISTICS

To test for differences in allocation of energy towards growth and reproduction among the three snail populations (at TB, CB and LPB), the total reproductive output (mean volume of embryos multiplied by number of eggs produced) was plotted against the specific growth rate. A difference in the allocation of energy directed towards reproduction and growth among populations would be detectable as an interaction between the trade-off curves. It is preferable to consider the slope of the trade-off curve rather than the actual value, as the total amount of energy available to snails may differ between snail populations.

Growth of juvenile *Z. subcarinatus* fitted the Gompertz growth curve, for which the specific growth rate $\ln(S_2/S_1)/\Delta t$ is a linear function of $\ln S$ (where S_1 and S_2 represent the initial and final size in the time interval Δt , and S is the geometric mean of S_1 and S_2) (Kaufmann 1981). The specific growth rates of juvenile snails from the three populations (TB, CB and LPB) were compared using an ANCOVA, with the specific growth rate as the dependent variable and $\ln S$ as the covariate.

The influence of trematode prevalence on size at maturity in *Z. subcarinatus* was tested for following the method of Lafferty (1993). The relationship between

the proportion of snails with functional gonads and snail size was tested by a linear regression, and size at maturity was estimated as the mid-point of the regression line indicating the size at which 50% of snails had reached maturity. The influence of environmental factors and parasites on size at maturity was tested in a step-wise multiple regression analysis using the estimated size at maturity as the dependent variable and trematode prevalence, chlorophyll *a* content in sediment, snail density and mean snail size as the predictor variables. Trematode prevalence was arcsin sqrt-transformed, and snail density and size at maturity were log-transformed prior to analysis to mitigate violations of normality and heteroscedasticity.

Statistical analyses were performed using the statistical packages SPSS for Windows (10.1), and Statistica (6.0) and used the 5% level of significance.

Results

REPRODUCTIVE EFFORT

Egg production was positively and linearly related to snail weight in all three populations (TB: $y = 6.997x + 0.9479$, $R^2 = 0.518$, $P < 0.0001$; CB: $y = 3.672x + 1.229$, $R^2 = 0.141$, $P = 0.0003$; LPB: $y = 7.497x + 0.594$, $R^2 = 0.303$, $P = 0.0001$) (Fig. 1). A test for homogeneity of slopes revealed an interaction of regression lines between CB and the other two populations (General Linear Model, $F = 3.14$, d.f. = 2, $n = 162$, $P = 0.046$). This was mainly due to a higher variance in egg-producing females among the larger snails in CB (Fig. 1). A GLM not assuming homogeneity of slopes followed by a *post hoc* test showed that snails from TB produced significantly more eggs than the other two populations at any given size (Unequal N HSD test, $P < 0.0001$), whereas egg production did not differ between CB and LPB ($P = 0.975$).

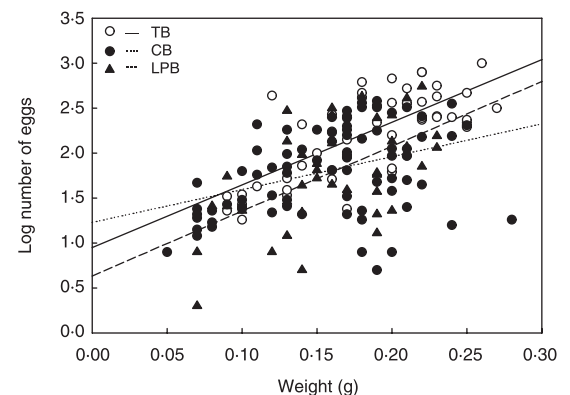


Fig. 1. Relationship between initial snail wet weight and egg production in snails from three populations experiencing different trematode prevalences. The three snail populations are: Turnbull Bay (TB; low prevalence; $n = 47$), Company Bay (CB; moderate prevalence; $n = 80$) and Lower Portobello Bay (LPB; high prevalence; $n = 41$).

Table 1. Results of two one-way ANOVAs, evaluating embryo size across three snail populations with different trematode prevalence in 2003 and 2004. In 2003, the three investigated snail populations were: Turnbull Bay (TB), Company Bay (CB) and Lower Portobello Bay (LPB). In 2004, the investigated snail populations were: Latham Bay (LatB), Lamlash Bay (LamB) and Oyster Bay (OB). Data were log-transformed prior to analysis

Year	d.f.	SS	MS	F-ratio	P-value
2003	2	0.001	0.001	12.012	< 0.001
2004	2	0.012	0.006	25.576	< 0.001

Intriguingly, embryos from the three populations measured in 2004 were consistently larger than embryos from the three populations measured in 2003. Food availability, measured as chlorophyll *a* content in the sediment, did not differ among populations in either 2003 or 2004 (one-way ANOVA: $F_{2,14} = 0.400$, $P = 0.679$, and $F_{2,14} = 1.246$, $P = 0.202$, in 2003 and 2004, respectively). It is unlikely that the relatively large difference in embryo size observed among populations sampled in 2003 and 2004 was solely due to habitat. Data on offspring size were therefore analysed separately for the 2 years.

Mean snail size, for which a subsample of five embryos had been measured, did not differ among populations in any of the two years (one-way ANOVA: $F_{2,70} = 1.246$, $P = 0.294$, and $F_{2,41} = 0.040$, $P = 0.961$, in 2003 and 2004, respectively). There was no relationship between maternal size and offspring size across snails from all populations in any of the two years ($r = 0.097$, $P = 0.416$, and $r = -0.004$, $P = 0.979$, in 2003 and 2004, respectively). Embryo size differed significantly between snail populations in both years (Table 1, Fig. 2). In 2003, embryos from LPB were significantly larger than embryos from CB and TB (Tukey's test: $P = 0.025$, and $P < 0.001$, respectively). Embryos from CB and TB did not differ significantly in size ($P = 0.052$). No significant relationship between egg size and numbers was detected for any of the three populations (all, $P \geq 0.080$). However, there was a significant correlation between offspring size and number across snails from all populations combined ($r = -0.36$, $P = 0.002$), indicating that the optimal allocation of resources between offspring number and size differed among populations. In 2004, embryos from OB were significantly larger than embryos from the other two localities (Tukey's test: $P < 0.001$, for both comparisons). There was no significant difference in embryo size between LatB and LamB ($P = 0.072$). Thus in both years, snails originating from populations with the highest trematode prevalence (i.e. a high risk of castration) produced significantly larger offspring compared with other populations.

ENERGY ALLOCATION

For all three populations sampled in 2003, the reproductive output, measured as mean embryo volume

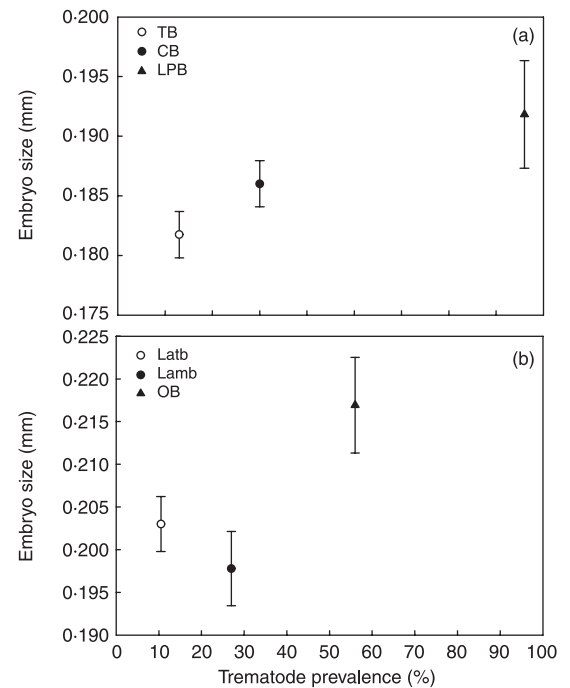


Fig. 2. Relationship between trematode prevalence and progeny size across populations of *Zeacumantus subcarinatus*. Data represent mean ($\pm 95\%$ confidence interval) embryo size per snail for each locality (based on subsamples of five embryos per snail). (a) Data from Turnbull Bay (TB; low), Company Bay (CB; moderate) and Lower Portobello Bay (LPB; high) obtained in 2003. Sample sizes are: TB = 28, CB = 26, LPB = 19. (b) Data from Latham Bay (LatB), Lamlash Bay (LamB) and Oyster Bay (OB) obtained in 2004. Sample sizes are: LatB = 20, LamB = 9, OB = 13.

multiplied by number of eggs, was negatively associated with specific growth rate across individual snails, indicating a trade-off between the two life-history traits (TB: $y = -1008.655x - 0.027$, $R^2 = 0.375$, $P < 0.0001$; CB: $y = -512.947x - 0.5345$, $R^2 = 0.199$, $P < 0.0001$; LPB: $y = -906.263x - 0.4843$, $R^2 = 0.281$, $P = 0.0002$) (Fig. 3). Locality had a significant effect on the relationship between the reproductive output and the specific growth rate (General linear model: $F_{2,162} = 14.60$, $P < 0.001$). Overall, snails from TB showed higher values for both variables compared with snails from the other two localities (*post hoc* Unequal N HSD test: $P < 0.001$, for both comparisons), indicating that more resources were available for snails in that population. The relationship between reproductive output and specific growth rate was similar between TB and LPB (i.e. no interaction between regression lines, Fig. 3). Snails from the two populations thus had a similar allocation of energy between the two life-history traits, suggesting that snails did not shift their allocation of energy towards reproductive output where the risk of becoming infected by castrating trematodes was highest. A weak interaction of regression lines between CB and the two other populations was observed, suggesting that snails from CB with a low specific growth rate also had a low reproductive output and vice versa (Fig. 3).

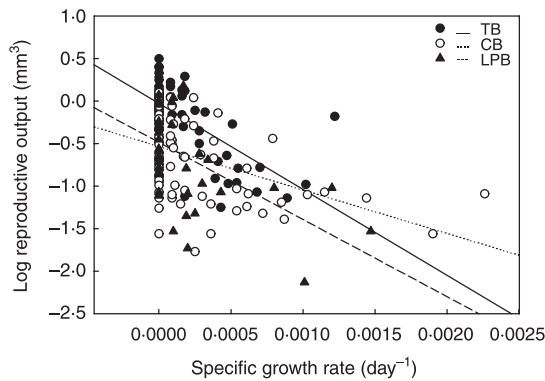


Fig. 3. Relationship between specific growth rate and the reproductive output in snails from three snail populations. Populations and sample sizes as in Fig. 1.

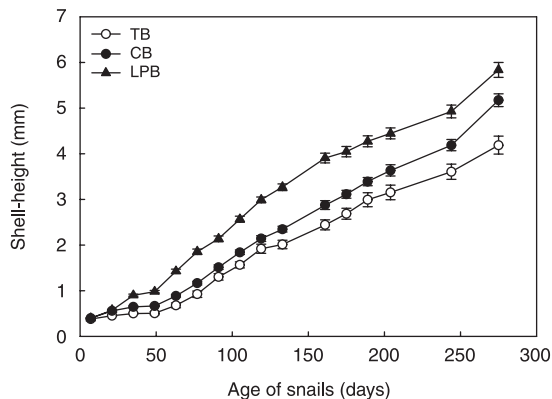


Fig. 4. Relationship between age and size of juvenile *Zeacumantus subcarinatus* from the three snail populations: Turnbull Bay (TB; low prevalence), Company Bay (CB; moderate prevalence), and Lower Portobello Bay (LPB; high prevalence). Each data point denotes the mean ($\pm 95\%$ confidence interval) shell height (mm) of 50 snails.

JUVENILE GROWTH

Shell-height of juvenile *Z. subcarinatus* differed significantly among snails from the three populations after 275 days (one-way ANOVA, $F_{2,147} = 93.648$, $P < 0.001$, Fig. 4). Mean size of juvenile snails from LPB was larger than snails from the other two populations (*post hoc* LSD test, $P < 0.001$, for both comparisons), and snails from CB were significantly larger than their conspecifics from TB ($P < 0.001$). The specific growth rate of juveniles declined linearly with size from day 49 and onwards, and differed significantly between populations (TB: $y = -0.015x + 0.021$, $R^2 = 0.714$, $P = 0.002$; CB: $y = -0.011x + 0.019$, $R^2 = 0.854$, $P < 0.0001$; LPB: $y = -0.015x + 0.025$, $R^2 = 0.880$, $P < 0.0001$) (Table 2). Juvenile *Z. subcarinatus* from LPB had a significantly higher growth rate for any given size compared with snails from the other two populations (ANCOVA: $F_{1,19} = 9.281$, $P = 0.007$, and $F_{1,19} = 5.093$, $P = 0.035$ for pairwise comparisons with TB and CB, respectively). Specific growth rates of juvenile snails did not differ between TB and CB (ANCOVA: $F_{1,19} = 0.932$, $P = 0.347$).

Table 2. Results of an ANCOVA, examining the specific growth rate in juvenile snails in relation to population of origin with snail size as the covariate. Growth rates were compared among populations after day 49 ($n = 11$ measurements per population). Snail size was log-transformed prior to analysis

Factor	d.f.	SS	MS	F-ratio	P-value
Snail size	1	0.0011	0.011	120.342	< 0.0001
Population	2	0.0001	< 0.0001	4.752	0.0164
Error	29	0.0003	< 0.0001		

SIZE AT MATURITY

Similar to Lafferty (1993) the relationship between the proportion of mature snails in each size class and size could be explained by a linear regression, where the lower bound was the largest size class where all snails were immature, and the upper bound was the smallest size class where all snails were mature (mean $R^2 = 0.838$). For each of the 10 snail populations, the mid-point of the regression between size class and the proportion of mature snails was used as an estimate of the size at which 50% of snails in a population had attained sexual maturity. Data on size at maturity, trematode prevalence and environmental variables obtained from 10 snail populations in and around Otago Harbour are presented in Table 3. Chlorophyll *a* was not significantly related to size at maturity across the eight snail populations where chlorophyll *a* data were obtained ($r = -0.10$, $P = 0.847$), and did not affect the result of the analysis. Therefore, only the results of the multiple regression analysis including all 10 sites are presented here. Trematode prevalence and mean snail size were both significantly related to size at maturity across all 10 snail populations (Table 4). This result indicates that snails in general mature at a larger size where growing conditions, measured as mean snail size, are favourable. However, the analysis also shows that snails from populations experiencing a high trematode prevalence mature at a significantly smaller size, independently of growing conditions (Table 4).

SUSCEPTIBILITY TO TREMATODE INFECTION

A field experiment on recruitment of larval trematodes to sentinel snails from a high and a low prevalence population was conducted. Snail sizes and infection data did not differ among the 10 cages used in the experiment, and the analysis therefore involves snail data pooled across cages. Of the 200 sentinel snails transferred to mesh cages in LPB, 111 (55.5%) were still alive at the time of dissection 100 days later. Of the 111 snails dissected, 30 were infected by one of three trematode species: *M. novaezealandensis*, *Acanthoparyphium* sp. and a philophthalmid. The proportion of infected snails was significantly larger for LPB compared with TB ($N_{infLPB} = 23$, $N_{infTB} = 7$, Yates' corrected chi-square test: $\chi^2 = 15.87$, $P = 0.0001$). Most of the

Table 3. Site characteristics used in a multiple regression analysis to explain the variation in size at maturity across populations of *Zeacumantus subcarinatus*. Size at maturity represents the size (mm) at which 50% of snails had functional gonads (based on dissections of 10 snails per 0.5 mm size class ranging from 7.0 to 12.0 mm). Prevalence is the percentage of snails > 6.0 mm that were infected by trematodes ($n = 100\text{--}370$ per population). Chlorophyll *a* represents mean amount of chlorophyll *a* ($\text{mg}^{-1} \text{m}^{-2}$) based on five core samples per locality. Density represents the mean number of snails per m^2 , based on 15 core samples (each, 0.1188 m^2 , 5 cm deep) per locality. Size denotes the mean snail size (mm) of snails from the 15 core samples per locality

Locality	Size at maturity	Prevalence	Chlorophyll <i>a</i>	Density	Size
Lower Portobello Bay	8.76	86.8	534.4	65	11.1
Lamlash Bay	10.83	27.0	453.6	348	13.7
Latham Bay	9.45	10.5	489.4	2185	9.0
Oyster Bay	9.19	58.3	496.4	299	8.3
Edwards Bay	9.26	13.4	343.9	1924	4.7
Turnbull Bay	9.99	13.0	540.2	413	8.7
Company Bay	9.05	30.0	584.5	1140	5.6
McAndrew Bay	8.98	17.4	414.7	1962	5.1
Blueskin Bay	10.19	9.3	–	1853	8.5
Papanui Inlet	9.72	31.1	–	546	10.6

Table 4. Results of a stepwise multiple regression analysis, evaluating factors of importance to the variation in size at maturity across 10 snail populations

	Partial correlation	<i>P</i> -value coefficient
Included variables		
Mean snail size	0.890	0.001
Trematode prevalence	–0.873	0.002
Model: $F_{2,7} = 18.198$, $P = 0.002$, $R^2 = 0.793$		
Excluded variable		
Snail density	–0.358	0.384

infections (66.7%) were nonpatent (i.e. no cercarial production) and consisted of relatively few sporocysts or rediae, indicating that the infections were obtained during the experiment. It is possible that patent infections could have originated before the experiment, remaining undetected during the incubation tests. However, excluding all patent infections did not change the outcome, and all infections were therefore included in the above analysis.

Discussion

Studies on host life-history adaptations in response to parasites in natural populations are rare. This study provides an insight into the effect of trematodes on several key life-history traits in the marine snail *Zeacumantus subcarinatus*.

REPRODUCTIVE EFFORT

Life-history theory predicts that hosts infected by parasites that negatively influence survival and reproduction, should increase their current reproductive effort to minimize the fitness cost of parasitism (e.g. Forbes 1993). Because trematodes inevitably lead to castration of the snail host, it was expected that *Z. subcarinatus* would invest more energy in reproduction in popula-

tions with a high risk of becoming infected. In spite of the obvious negative impact of trematodes on host fecundity, our results do not provide any evidence that uninfected snails from high prevalence populations invested more energy into reproduction (similar slopes of regression lines between growth and reproduction for high and low prevalence populations; Fig. 3). It is possible that a greater energy allocation towards reproduction was not observed because it may be more beneficial for the snails to grow bigger, as this would enable a higher future egg production. Several studies have reported a parasite-induced increase in the reproductive effort in animals (often termed fecundity compensation) (Minchella & Loverde 1981; Thornhill *et al.* 1986; Gérard & Théron 1997; Adamo 1999). In this study, only the reproductive strategy of uninfected individuals was investigated. Experimental infections are needed to clarify further the role of parasite-induced responses in allocation of energy in *Z. subcarinatus*.

Energy allocation between growth and reproduction did not vary among snail populations, yet offspring size differed significantly across populations in both years. Often, offspring size in invertebrates is influenced by maternal size and/or the number of offspring produced (Fox & Czesak 2000). In our study there was no relationship between maternal size and offspring size. In addition, there was no evidence for a trade-off between progeny size and numbers within populations. There was, however, a significant correlation between the two traits across populations, indicating that snails from different populations utilize different reproductive strategies. The two snail populations with the highest trematode prevalence produced significantly larger offspring compared with populations experiencing a lower prevalence. All populations were assumed to have equal access to resources, measured as the content of chlorophyll *a* in the sediment. However, the higher growth rates in combination with a higher reproductive output observed in snails from TB suggest that those snails may have had more resources (Fig. 3). The effect of resource availability on offspring size in invertebrates

is not clear. Hence, both larger and smaller offspring have been recorded from different studies investigating the impact of resources on offspring size (see Fox & Czesak 2000 for a review). Resource availability may thus have had an effect on the smaller offspring size observed in snails from TB.

It is generally accepted that larger offspring perform better early in life (Calow 1983; Doherty 1994; Fox & Czesak 2000). Production of large offspring would therefore clearly be beneficial to snails living in populations where trematode infections are very common, as it increases the chance of producing offspring that would themselves reach maturity before becoming infected. Previous studies on vertebrates have revealed that production of large offspring is favoured in environments where parasitism is likely to impose a significant negative impact on host fitness (Kristan 2004; Thomas *et al.* 2004). Moreover, it has been shown that females living in an environment with many parasites can produce offspring that are better adapted to parasites than offspring from unexposed females (Sorci, Massot & Clobert 1994; Sorci & Clobert 1995; Kristan 2004). Those findings are in line with our results on juvenile growth in *Z. subcarinatus* from three snail populations, showing that juveniles from a high prevalence population grew significantly faster and achieved a significantly larger size by the end of the study compared with the other populations. Because the juvenile snails were raised under identical conditions and had never experienced field conditions, the higher growth rate in snails from LPB indicates that the adaptation could be genetically based. However, it cannot be excluded that maternal effects may also have contributed to the observed results.

SIZE AT MATURITY

A large range of physiological and environmental factors are believed to influence the onset of reproduction (Kozłowski 1992; Roff 1992; Stearns 1992; Bernado 1993). A major determinant of age at maturity is the age-specific mortality rate. Life-history models generally predict that high adult mortality should select for earlier age at maturity (Hochberg, Michalakos & de Meeüs 1992; Roff 1992). For snail hosts, infection by trematodes equals mortality because infections inevitably lead to castration, which is permanent (Curtis 2003). In this study, *Z. subcarinatus* matured earlier in populations where trematode prevalence was high, therefore supporting life-history theory. It was apparent that the quality of the habitat also had an effect on the size at which snails matured. Hence, in good quality habitats, maturing at a larger size was beneficial to snails. Our findings are similar to Lafferty (1993), who found that *Cerithidea californica* Haldeman, 1840, living in saltmarsh ecosystems matured at a smaller size where trematode prevalence was high. *Z. subcarinatus* and *C. californica* share very similar life histories, both producing eggs with direct development (i.e. eggs hatch into miniature adults without a planktonic larval

stage). Snails with direct development of larvae are generally more likely to adapt to local selection pressures because immigration is rare and the intrapopulation gene pool therefore is highly reliant upon local recruitment (Janson 1982, 1987; Yamada 1987, 1989). The similar effect of trematodes on the life history of those two species living in different geographical areas and climatic zones, suggests that the effect of trematodes may apply generally to all gastropods with the same reproductive strategy.

SUSCEPTIBILITY

Our results showed that snails from a high prevalence population did not show a higher resistance against trematode infections compared with snails from a low prevalence population. In fact, the opposite was observed. The lack of resistance could be due to a range of factors. First, *Z. subcarinatus* may simply lack genetic variation for resistance to evolve. Secondly, mounting an immune response may be costly and be negatively associated with other life-history traits (Minchella & Loverde 1983; Rigby & Jokela 2000). Especially when infected by several trematode species, general resistance towards them all may not be possible or beneficial. Different trematode species have different transmission routes to snails (either passive via ingestion or active via penetration), and a response against one species may not protect the snail from other species. As a consequence, studies showing host resistance against trematodes in natural populations are rare, and are more often due to a lack of compatibility between different strains of parasites and hosts than active resistance against the parasite (Webster & Davies 2001). There are no obvious reasons why snails from LPB (prevalence = 86%) should be more susceptible to trematode infections than their conspecifics from TB (prevalence = 13%). Owing to the high dispersal ability of bird definitive hosts, the trematodes infecting *Z. subcarinatus* are not likely to be locally adapted to their intermediate hosts, and other factors related to the two populations may determine the observed differences.

In conclusion, this study shows that *Z. subcarinatus* adapts to a high selection pressure exerted by parasites by changing a range of key life-history traits to minimize the negative impact of parasites on important fitness components. The results of this study warrant further investigation into the effect of parasites on life-history evolution in natural populations of hosts with limited dispersal abilities.

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