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Spatial heterogeneity in parasite loads in the New Zealand cockle: the importance of host condition and density

K.N. Mouritsen*, S. McKechnie, E. Meenken, J.L. Toynbee and R. Poulin

Department of Zoology, University of Otago, PO Box 56, Dunedin, New Zealand. *E-mail: kim.mouritsen@stonebow.otago.ac.nz

Samples of the New Zealand cockle *Austrovenus stutchburyi* (Veneridae) collected at two different intertidal levels were found to be heavily infected by the echinostome trematode *Curtuteria australis* which use the mud flat whelk *Cominella glandiformis* and shorebirds as first and definitive host, respectively. Parasite loads were on average more than 2-fold higher at the high site than at the low site, whereas the density of *Curtuteria australis* metacercariae per square metre showed the opposite trend. This pattern appeared unrelated to the abundance of first intermediate hosts and *C. australis* prevalence within this host that did not differ between sites. At both sites there was a positive relationship between parasite load and cockle condition, a likely by-product of the relationship between condition-based filtration capacity and the rate at which individual cockles encounter the free-swimming trematode larvae. The results suggest that the between-site variation in parasitism is a consequence of the much higher density of cockles at the low site. There, the dense cockle population is able to clear the water column of parasites and consequently individual cockles virtually 'compete' for parasite larvae. This will depress mean infection intensity per cockle but elevate mean parasite density per square metre.

INTRODUCTION

Parasites and particularly trematodes are ubiquitous in the intertidal zone, where their complex life cycles cause most, if not all, members of the animal community to be host to usually several species (Mouritsen & Poulin, 2002). Although our knowledge of the impact of parasites on their hosts is far from complete, mounting evidence suggests that they are often detrimental with implications for host population dynamics and even community structure (Mouritsen & Poulin, 2002). However, parasites are not evenly distributed on any spatial scale, and large variation in parasite recruitment ranging from the level of individual host within site to larger-scale vertical as well as horizontal heterogeneity is the rule in tidal habitats (Mouritsen & Poulin, 2002).

The New Zealand cockle *Austrovenus stutchburyi* is an abundant member of the intertidal soft-bottom community, and second intermediate host to an echinostome trematode (*Curtuteria australis*) that encysts as metacercaria in the foot tissue (Allison, 1979). Shorebirds feeding on the bivalves serve as definitive host, in which the adult parasites infect the digestive tract. Parasite eggs leave the bird host through droppings and hatch into miracidia larvae which penetrate the whelk *Cominella glandiformis*. Here the parasite multiplies asexually into tailed cercariae that emerge from the snails. Swimming in the water column, these infective larvae enter the mantle cavity through the filtration current. *Curtuteria australis* is very abundant in the Otago Harbour (South Island), and its prevalence in mature cockles usually approaches 100%. However, there is still substantial variation in parasite loads among individuals within sites as well as in mean intensities of infection between sites on a larger scale, both vertical and horizontal (Thomas & Poulin, 1998; Poulin et al., 2000). The present study addresses this hitherto unexplained spatial heterogeneity by testing two hypotheses: (i) the intensity of *C. australis* infection (no. metacercariae cockle^{-1}) correlate with cockle condition, as the latter will influence filtration rate, and hence, the rate at which cercariae enter the mantle cavity of the cockle; and (ii) the previously observed vertical pattern of decreasing parasite loads in a seaward direction coincides with a similar pattern in whelk density and/or prevalence of *C. australis* infections in whelks, and hence with the abundance of infective cercariae released in the water column.

MATERIALS AND METHODS

Animals were collected in August (cockles) and November (snails) 2001 on an intertidal sand flat at Gills Corner, Otago Habour, South Island, New Zealand. Two sampling sites were chosen at different tidal levels: a high site having a submersion time of approximately 4.5 h (corresponding to a water column of about 0.75 m at maximum high water), and a low site having a submersion time of 9 h (1.5 m water column at high water). The sites were separated by 175 m along the intertidal gradient. At each site, cockles from ten randomly distributed core samples (314 cm^{-2}) were collected, counted, and brought to the laboratory. An additional qualitative sample of cockles was collected at the high site due to its low density of cockles. Because of their generally low density and aggregated distribution, whelks were collected by using cockles as bait: at both the high and low site, four crushed cockles of similar size were placed at each of five sub-sites separated by five metres along the receding tidal front. After 30 min, just before the exposure of the sediment surface, whelks present within a radius of 10 cm of the

Table 1. *Mean ±SD (N) density, size and condition factor of cockles from the two tidal levels, and their mean ±SD (N) infection intensity by* Curtuteria australis. t *and* P *values are summary statistics of* t*-tests.*

	Tidal level			
Parameter	High	Low	*t*	*P*
Density (no. m^{-2})	54.1 ±49.9 (10)	480.1 ±113.8 (10)	10.86	<0.0005
Shell length (mm)	24.7 ±3.0 (78)	26.8 ±3.2 (79)	4.24	<0.0005
Condition factor	51.0 ±8.8 (78)	48.9 ±8.9 (79)	1.47	0.16
Metacercarial load (no. ind^{-1})	134.2 ±80.6 (78)	59.2 ±57.2 (79)	6.72	<0.0005

Table 2. *Summary statistics of analysis of covariance on rank metacercarial load vs condition factor and shell length by tidal level (main effects). A preliminary full model analysis of covariance showed non-significant interactions (*P>0.68*), indicating equality of slopes. Consequently, associated sum of squares and df are included in the error variance of the present analysis.*

Source	df	Mean square	*F*	*P*
Tidal level	1	76363	55.90	<0.0005
Condition factor	1	21856	16.00	<0.0005
Shell length	1	1337	0.98	0.32
Error	153	1366		

bait were collected. Because the whelk data obtained this way only evaluate the relative abundance of snails between sites, 30 randomly distributed core samples (119 cm^{-2}) were collected at the low site in order to estimate actual whelk density.

The animals collected were returned to the laboratory, where their maximum shell dimension was measured prior to dissection. Whelks were dissected to determine the presence or absence of larval stages of *C. australis* in the gonad–digestive gland complex. The foot of each cockle was removed, squeezed between two glass slides, and the number of metacercarial cysts of *C. australis* present in the transparent foot tissue was determined under a dissecting microscope. The dry weight of the soft tissue of the cockle was subsequently measured after 24 h at 110°C. The cockles' condition factor was then calculated according to the formula $CF = 100 \times STDW/L^b$, where $STDW$ is soft tissue dry weight (mg), L is the shell length (mm), and b is the regression coefficient for the relationship between $\ln(STDW)$ and $\ln(L)$ across the entire material. This regression was statistically significant ($r = 0.77$, df=155, $P < 0.0005$) with the regression coefficient b=1.783.

RESULTS

Cockle density and metacercarial load per cockle showed opposite trends: the mean density of cockles was at the low site about 9-fold greater than at the high site, whereas mean parasite intensity was more than 2-fold greater at the high site than the at the low site (Table 1).

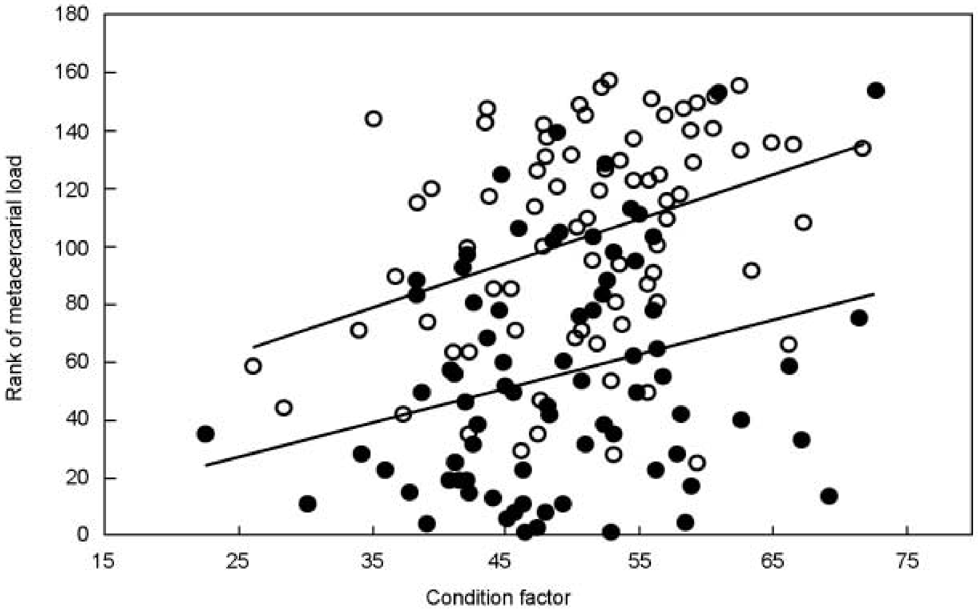

Figure 1. Relationship between rank metacercarial load per cockle vs cockle condition for the high site (○, upper line) and the low site (●, lower line). Both regressions were statistically significant ($F > 5.87$, $P < 0.018$).

Table 3. *Mean ±SD (N) relative abundance and size of whelks from the two tidal levels, and the prevalence of* Curtuteria australis *in these whelks.*

Parameter	Tidal level: High	Tidal level: Low	*P*
Abundance (number)	42.6 ±10.0 (5)	53.8 ±10.7 (5)	0.13[1]
Shell height (mm)	17.7 ±3.3(210)	16.0 ±3.7 (269)	<0.0005[2]
Prevalence of *C. australis* (%)	5.7 (210)	7.1 (269)	0.58[3]

[1], *t*-test, $t=1.71$; [2], Mann–Whitney test, $Z=5.38$; [3], Fisher's exact test.

Individual metacercarial loads varied between 20 and 387 at the high site, and between 1 and 304 at the low site. However, in terms of mean density of cysts per square metre (mean cockle density × mean parasite load per cockle), *Curtuteria australis* was much more abundant at the low site (28,422 m^{-2}) than at the high site (7260 m^{-2}). On average, cockles were slightly larger (8.5%) at the low than at the high site, whereas the condition factor did not differ significantly between sites (Table 1).

An analysis of covariance on rank-transformed parasite loads (in order to stabilize the error variance) confirmed significantly higher parasite intensity per cockle at the high site than at the low site (Table 2, Figure 1), and demonstrated a significant positive relationship between parasite load and cockle condition that was similar at both sites. Cockle size, on the other hand, had no significant impact on parasite loads in the present samples (Table 2). That cockle condition proved more important than cockle length may be due to the relatively narrow size range of cockles combined with a rather high variation in condition factor within size-classes. Overall, the effect of site and cockle condition explained 26.7 and 9.5% of the variance in parasite loads, respectively.

In contrast to the cockles, whelks were slightly larger (10.6%) at the high site, and snail abundance as well as the prevalence of *C. australis* in snails did not differ between sites (Table 3). At the low site, the spatial distribution of whelks was aggregated with a mean density of 0.40 (range: 0–4, N=30) per core sample, corresponding to 33.6 snails m^{-2}.

DISCUSSION

The significant spatial heterogeneity in infection intensity by *Curtuteria australis* in cockles, with the highest level of parasitism on the upper part of the intertidal, is in accordance with findings from other intertidal flats in the Otago Harbour (Poulin et al., 2000; K. Mouritsen & R. Poulin, unpublished data). However, the hypothesis that this pattern is determined by a corresponding heterogeneity in the abundance of infective larval stages (cercariae) is not supported by the data: neither whelk abundance nor the prevalence of *C. australis* in whelks differed significantly between the two sites. The relatively small difference in snail size between sites is (via a presumed positive relationship between snail size and cercarial emission rate) also unlikely to account for the more than twice higher parasite load in cockles from the high than the low site. Clearly, the overall 4-fold higher density of encysted metacercariae per square metre at the low site is difficult to reconcile with the idea of a greater availability of cercariae released in the water at the high site.

Assuming an approximately homogeneous distribution of released cercariae along the intertidal gradient, how then can the spatial and individual pattern in parasite loads and overall density of *C. australis* metacercarial cysts per m^2 be explained? *Austrovenus stutchburyi* likely acquires its parasites through the filtration current (Allison, 1979). Because the filtration rate of bivalves is a positive function of the soft tissue dry weight (Riisgård, 2001), a positive relationship can be expected between the condition factor and the number of trematode larvae encountered, and hence, infection intensity in a given cockle specimen. This is confirmed by the present data, although other yet unidentified factors must also contribute significantly to the individual variation in parasite loads (Figure 1). Whereas cockle condition can explain at least some of this variation, it cannot account for the difference between sites, as the average condition factor of cockles did not differ. However, the density of cockles was very different, which means that the populations of cockles at the two sites differ tremendously regarding their ability to clear the water column above the seabed and deplete it of cercariae. The filtration capacity of *A. stutchburyi* is unknown, but it is likely within the range of *Cerastoderma echinatum* ($F=4.22STDW^{0.62}$) and *C. edule* ($F=11.60STDW^{0.70}$, where F is filtration rate [$\mathrm{L\ h^{-1}}$] and $STDW$ is soft tissue dry weight [g]), that encompasses filtration rates observed in most coastal bivalves (see Riisgård, 2001). Based on (1) this range of filtration rates, (2) the mean soft tissue dry weight and density of cockles at the two sites, and (3) the average water column height during submersion at each site, the cockle population at the low site will be able to filter the water column 8–19 times per submersion period. Cockles from the high site, on the other hand, will only manage to do this 0.8–2 times. These figures suggest that cockles at the low site are engaged in rather severe intraspecific exploitative competition, supported by the lack of difference in cockle condition between sites and only a slightly larger mean shell length at the low site despite a twice-longer submersion time at that site. While competing for suspended food particles, the cockles may at the same time 'compete' for swimming *Curtuteria australis* cercariae released by infected whelks. Hence, as a consequence of the high cockle density at the low site, the finite number of cercariae in the water column is divided between the many hosts present that rapidly clear the water for parasites. At the high site, in contrast, the few cockles present do not manage to deplete the water column of trematode larvae that continue to accumulate in cockles throughout the high water period at a relatively high rate. Together these processes will give rise to the observed higher parasite loads per cockle at the high site and a larger density of metacercariae per square metre at the low site.

The above interpretation requires (1) that larval echinostomes are relatively efficient at invading the soft tissue of the cockle once they have entered the mantle cavity, and (2) that the number of *C. australis* metacercarial cysts found per square metre at the low site roughly correspond to the number of cercariae actually released there, as evidence for a high infection success. Indeed, these requirements are likely met. Firstly, in the *Cerastoderma–Himasthla* association, as the European parallel to the presently studied *Austrovenus–Curtuteria* association, the success by which released cercariae accumulate as metacercariae in the host is surprisingly high: 60% of cercariae added to a re-circulation water flume containing 90 cockles (6–12 mm in shell length) were recovered as metacercariae in the cockle tissue after 20 hours (Montaudouin et al., 1998). During this time the experimental cockle population had the potential of filtering the running water body only 0.6 times. The infection success is likely to be even higher in a population of larger cockles (e.g. the ones in this study) due to a much larger filtration capacity. Secondly, based on (1) a documented longevity of echinostome cysts of about one year (Dönges, 1969; Mouritsen, unpublished data), (2) significant cercarial transmission only during the six warmest months of the year, evidenced by a lack of significant emergence of *C. australis* cercariae below 15°C (Allison, 1979), and (3) the observed whelk density of 33.6 ind m^{-2} and 7.1% prevalence of *C. australis* in whelks at the low site, a cercarial emergence rate of 66–110 cercariae per infected snail per day is necessary to reach the average density of 28,442 metacercariae m^{-2} found at the low site (under the assumption of 60–100% infection success). Interestingly, this estimated daily cercarial emergence rate is well within the range observed in *C. australis*-infected whelks (Allison, 1979).

The above considerations may also have implications for the decision-making of cockle larvae settling within the intertidal zone. Although settlement in areas supporting high densities of adult cockles is associated with a high mortality risk (e.g. Guillou & Tartu, 1994), it will provide safety in numbers not only against predators but also parasites. This can prove selectively advantageous because high *C. australis* intensity is known to impair the mobility of the cockle foot, and as a result the ability to re-bury if dislodged to the sediment surface (Thomas & Poulin, 1998; Mouritsen, 2002). Cockles stranded on the sediment surface will experience an approximately 7-fold higher risk of bird predation than their buried conspecifics (Thomas & Poulin, 1998).

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