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RESEARCH ARTICLE

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Parasitism can influence the intertidal zonation of non-host organisms

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Abstract Zonation of organisms in soft-bottom intertidal habitats is mainly a consequence of the tidal sorting of the substrate and how benthic organisms respond to it, with predation and competition also playing a role. Although parasites are ubiquitous in these habitats and known agents of community structure, their impact on zonation is rarely considered. Here, using observational and experimental data, we show that parasitism of the cockle *Austrovenus stutchburyi* by echinostome trematodes has both direct and indirect effects on the zonation of four other animals associated with cockles on a New Zealand tidal flat. The trematodes encyst in the foot of cockles, leaving heavily infected cockles unable to bury and lying exposed on the sediment surface. Whereas cockle density peaks in the mid intertidal zone, both infection intensity by trematodes and density of surfaced cockles increase linearly towards the shore; this influences the vertical zonation of animals attaching to or feeding on cockles. First, the relative abundance of the whelk *Cominella glandiformis* increase exponentially shoreward, as does its rate of predation on cockles; the effect of the parasite on the cockles' burying ability has thus shifted the peak abundance of whelk predators towards the upper tidal level, away from peak cockle densities. Second, the density of the anemone *Anthopleura aureoradiata*, corrected for the availability of its cockle substrate, peaks in the lower intertidal zone, although a transplantation experiment revealed that anemones can survive higher up the shore if attached to buried cockles. Anemones on exposed cockles are susceptible to desiccation, and thus again the parasite-induced surfacing of cockles at higher tidal levels plays a role by decreasing substrate availability for anemones. Third, densities of the limpet *Notoacmea helmsi* increase rapidly toward higher tidal levels, where limpets graze the surfaces of empty cockle shells or live surfaced cockles. The availability of both substrates used by limpets is influenced either directly (surfaced cockles) or indirectly (empty shells, via whelk predation) by parasitism. Fourth, the fish *Notolabrus celidotus* feeds on cockles by cropping their foot. Although the lower intertidal is submerged for longer and cockle densities peak in the mid intertidal, the frequency of foot-cropping by fish peaks in the upper tidal levels, where parasitism maintains a high density of surfaced cockles. Our results are the first demonstration that parasitism of a key species can influence the zonation of non-host organisms, a phenomenon likely to occur in other systems.

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Introduction

The seminal work by Connell (1961), Paine (1966), Dayton (1971), Menge (1976) and Lubchenco (1978) on rocky shore zonation and community structure has provided lessons on factors determining community organisation that reach beyond the intertidal zone. The different upper intertidal limits of various organisms are set by different resistance to environmental stresses, whereas the community structure below is determined largely by biotic interactions. Studies in soft-bottom habitats, in contrast, have been less successful in identifying clear patterns. The wider intertidal zone and the sedimentary nature of mudflats that dampens abiotic stresses during exposure, create a less well-defined zonation (Bertness 1999). The soft-bottom habitat is frequently disturbed by wave-action and bioturbation that together create a mosaic of patches in various stages of recovery (Bertness 1999), thus blurring patterns of distribution along the intertidal gradient. However, zonation *is* evident in the soft-bottom intertidal, and although it is attributed largely to the tidal sorting of the substrate and how benthic organisms respond to it, biotic processes may also play a role (Raffaelli et al. 1991; Snelgrove and Butman 1994; Bertness 1999).

Although parasites are ubiquitous in the intertidal zone, their potential role as determinants of the zonation of other organisms has largely been ignored. Parasitism is now recognised as a potentially important factor structuring natural communities, including intertidal systems (Minchella and Scott 1991; Combes 1996; Pennings and Callaway 1996; Hudson and Greenman 1998; Thomas et al. 1998; Mouritsen and Poulin 2002, 2005). Because parasites are rarely distributed evenly along the tidal gradient (e.g. Callaway and Pennings 1998; Mouritsen and Poulin 2002, 2003a), their impact will vary accordingly and should thus influence the zonation of intertidal organisms. Parasitism may influence zonation in two basic ways: (1) directly through the impact on host abundance or behaviour, and (2) indirectly through parasitic mediation of the interspecific interactions in which host species are engaged. However, empirical evidence for either is still scarce: The trematode *Maritrema arenaria* may be partly responsible for sudden shoreward declines in the abundance of the encrusting barnacle *Semibalanus balanoides* (Carrol et al. 1990). Trematode infections have also been shown to affect the zonation of periwinkles *Littorina* spp. and the mud snail *Ilyanassa obsoleta* through direct parasite-induced behavioural change (Lambert and Farley 1968; Curtis 1987; Mikhailova et al. 1988). The post-settlement seaward migration of the bivalve *Macoma balthica* is viewed in part as an adaptive response to a much higher risk of infection by the detrimental trematode *Parvatrema affinis* in the upper intertidal zone (Beukema 1993; Hiddink 2003). However, the fact that these few cases cover both soft- and hard-bottom habitats, and involve direct and indirect effects, suggests a general role for parasitism that is yet to receive the full attention of ecologists.

The cockle *Austrovenus stutchburyi* is the dominant bivalve of New Zealand intertidal flats, usually attaining densities of several hundred individuals m^{-2}. The cockle serves as second intermediate host for two echinostome trematodes, *Curtuteria australis* and *Acanthoparyphium* sp., which use shorebirds as definitive hosts and the mud flat whelk *Cominella glandiformis* and the mud snail *Zeacumantus subcarinatus*, respectively, as first intermediate hosts (Allison 1979; Babirat et al. 2004; unpublished data). Both species of trematodes infect mainly the foot of the cockle, which renders heavily infected cockles unable to bury and lying exposed on the sediment surface (Thomas and Poulin 1998; Mouritsen 2002; Babirat et al. 2004). The mean intensity of infection generally increases with increasing tidal height (Poulin et al. 2000; Mouritsen et al. 2003; Mouritsen and Poulin 2003a), and the abundance of surfaced cockles may thus follow a similar pattern. The combination of heavy parasite loads and environmental stresses associated with prolonged surfacing at higher tidal levels can then be hypothesised to elevate cockle mortality rate (see Mouritsen and Poulin 2002), in turn affecting their pattern of zonation. These processes may also influence the intertidal distribution of *C. glandiformis*, the mud flat anemone *Anthopleura aureoradiata*, the limpet *Notoacmea helmsi* and the spotty *Notolabrus celidotus* (Pisces: Labridae) that all rely on surfaced cockles as substrate for attachment or as prey. *C. glandiformis* is an important predator of particularly surfaced cockles (Ansell 2001; Mouritsen 2004), and its abundance may therefore increase shoreward. *A. aureoradiata* uses cockles as the only available hard substrate for attachment in the soft-bottom intertidal, but since it is sensitive to desiccation and hence disappears from surfaced cockles (Thomas et al. 1998), its abundance may decrease disproportionally with increasing tidal height where parasitism is high. *N. helmsi* grazes microalgae that grow on hard substrates lying on the sediment, and may therefore increase in density in the shoreward direction due to the higher abundance of surfaced cockles and dead shells at the upper tidal levels. Finally, *N. celidotus* is engaged in an unusual kind of sublethal predation by cropping the feet of particularly surfaced cockles (Mouritsen and Poulin 2003b, 2003c). Hence, the fish may feed preferentially at upper tidal heights where the density of surfaced cockles is highest.

The validity of the above hypotheses was assessed by a combination of experiments and field observations carried out along an intertidal gradient in a New Zealand bay.

Materials and methods

Study site and transect

The study was performed during the austral summer 2002–2003 on a small, sheltered intertidal sand flat in Company Bay, Otago Harbour, South Island, New Zealand (4551′S/170°37′E). This area was selected for study due to a relatively high abundance of all five target species and relatively high parasite loads in the resident cockle population, which should optimise the probability of verifying the hypothesised processes. However, Company Bay is by no means a unique site in Otago Harbour, neither regarding parasite abundance, presence of shell-beds, or the composition of the macrofaunal community. In the middle of the bay, a 20 m wide transect with 12 stations separated by 6 m was established along the tidal gradient from the lower mid-intertidal to the spring tide low water mark. The submersion time ranged linearly between 70% (station 1) and almost 100% (station 12).

As opposed to most exposed, high-energy sand flats in Otago Harbour, sheltered ones like the Company Bay sand flat have no extensive beds of beached cockle shells.

This is not because cockles are rare in such places, but due to the lack of sufficiently powerful waves to beach the shells that accumulate on the sediment surface as cockles dies. In Company Bay, a bed of cockle shells is present in the mid-intertidal zone (see Results), which suggests a relatively high mortality rate among the cockles in that particular zone. To verify that the shells there likely originate from cockles in also that area, the maximum shell length of 152 randomly selected intact cockle-shells on the sediment surface (station 1–2, see Fig. 1) were measured and compared with the size-distribution of live cockles along the transect (see below). We acknowledge, however, that a similar size-distribution of shells on the surface and live cockles at a given tidal level is only suggestive, and does not provide decisive proof of the intertidal origin of all empty shells found.

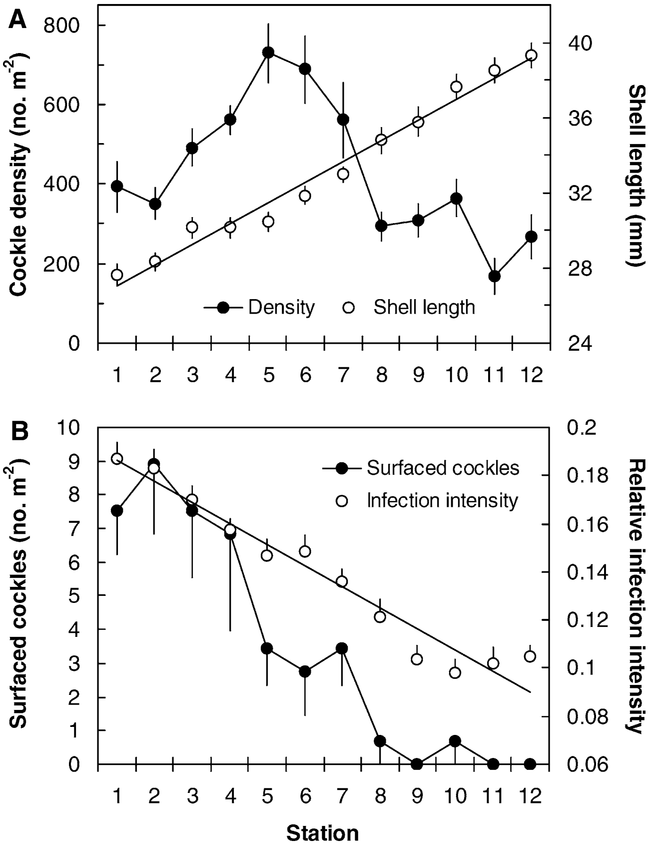

Fig. 1 a,b Cockle *Austrovenus stutchburyi* characteristics as a function of decreasing tidal height in Company Bay. Station 1 and 12 represent the lower mid-intertidal and the spring low tide level, respectively. **a** The mean density of cockles (no. m^{-2} ± SE, $n = 6$ for all mean values, *filled circle*) and the mean shell length of cockles (mm ± SE, $n = 24$–27, *open circle*). The regression line based on mean length per station is shown. **b** The mean density of surfaced cockles (no. m^{-2}–SE, $n = 5$ for all mean values, *open circle*) and the mean relative infection intensity of uncropped cockles (ln-transformed no. of metacercariae per mm cockle + SE, $n = 12$–25, *filled circle*). The relative infection intensities represent real parasite loads of between 150 and 500 metacercariae cockle^{-1} . The regression line based on mean intensity per station is shown

Cockle zonation and parasite infections

To estimate cockle density along the transect, the number of cockles within six core samples (0.012 m^2) taken at each station was determined. Where the abundance of cockles was low, additional samples were taken so that at least 24 individuals were collected from each station. The cockles where brought to the laboratory where 24–27 randomly selected individuals from each station were further processed. After measurements of maximum shell length, the cockles were opened and the foot was removed in order to count the number of contained echinostome metacercariae under a microscope. Only metacercariae present on one side of the feet were enumerated, which means that the infection intensities recorded are approximately half the real values.

At each station the density of live cockles lying fully exposed on the sediment surface was also estimated from numbers present within a circular frame (diameter = 61 cm) at five randomly chosen sites.

Whelk zonation and cockle predation

The zonation of whelks *C. glandiformis* was established by the use of bait. Four plastic-mesh cages (8 cm in diameter, 16×20 mm mesh size) were pushed into the sediment at each of four stations (2, 5, 8 and 11) with an inter-cage distance of 5 m. During a receding tide, three similar sized and crushed cockles were placed in each of the 16 cages for 30 min, after which time the number of whelks present inside the cages was counted.

A similar collection was carried out on a nearby sand flat at Gills Corner, also in Otago Harbour, at three tidal heights covering approximately the same intertidal range as in Company Bay. At Gills Corner, the parasite load of cockles is low and surfaced individuals are rare in comparison with Company Bay.

In order to estimate the mortality rate of cockles due to whelk predation, the number of feeding parties of whelks on cockles (i.e. two or more snails in tight association with a cockle) was recorded between stations along the tidal gradient (i.e. within 6×20 m areas). This was carried out in eight areas between station 1 and 9 on each of four receding tides. Based on the mean density of cockles between adjacent stations and the mean number of observed feeding parties per tide, the mortality rate of cockles due to whelks was calculated for each of the eight intertidal zones.

Cockle mortality-laboratory experiment

Whelks are predominantly scavengers (Ansell 2001), and their preference for the upper tidal zone may reflect a high abundance of moribund cockles in this intertidal zone, possibly due to a combination of abiotic stress and heavy parasite loads (see Results). The survival rate of cockles from both the upper and lower transect was therefore investigated in the laboratory. We collected 60 surfaced cockles (mean shell length [± SE]: 29.8 ± 0.46 mm) and 60 buried cockles (28.0 ± 0.49 mm) from the upper zone (station 1–2), and 60 buried (33.1 ± 0.69 mm) from the lower zone (station 7–8), and placed 15 individuals in each of 12 tanks arranged as a 2×6 block. Each tank contained cockles collected exclusively from the same microhabitat, hence, four replicates for each of three treatments (surfaced, upper zone; buried, upper zone; buried, lower zone) were established and randomised within the block of tanks. Sea water was supplied to the tanks according to the total cockle weight, which meant that lower and upper zone cockles were supplied with 0.23 and 0.17 l ind^{-1}, respectively. The tanks were left each with an effective oxygen supply at room temperature (18–22°C) for 100 days, after which time the number of survivors was recorded in each tank. During the experiment, dead or dying cockles (i.e. individuals unable to close their valves when touched) were removed daily, and water was replaced five times.

Anemone zonation and field experiment

The mud flat anemone *A. aureoradiata* uses cockles for attachment as the only available hard substrate in the soft-bottom habitat. Hence, in order to determine the anemones' zonation pattern, the number of anemones present on the shells of the cockles collected along the transect was recorded. In addition, the disk diameter of each attached anemone was measured.

Because anemones are associated with cockles, the zonation and size-frequency distribution of the latter will affect the distribution of anemones. To correct for this, the anemone density per unit area of substrate was estimated for each station. By wrapping the dorsal half of cockles of different sizes with folio-paper (from the dorsal shell rim to the 'shoulders' of the shell as the widest part; anemones only exceptionally attach below this point), transforming folio-weights into unit area equivalents, and establishing the linear regression between surface area (SA, cm^2) and shell-length (SL, mm) (for the length range 24–40 mm: $SA = 0.622 \times SL - 9.495$; $r^2 = 0.88$, $df = 34$, $P < 0.0005$), the average anemone density per cm^2 substrate at each station was calculated on the basis of the corresponding number of attached anemones and shell-length of individual cockles.

The abundance of anemones declines in the shoreward direction (see Results), and to assess whether this pattern can be explained solely be reduced survivorship and growth conditions due to increasing exposure time, a transplantation experiment was carried out. Thirty fully buried cockles of similar size, and each harbouring 3–6 anemones, were collected from station 7. The disk diameter of all attached anemones was measured on location, and the cockles were subsequently placed individually in their natural burying depth inside mesh-cages, placed across the transect at station 7 (i.e. transplanted to the zone of origin = control, $n = 15$) and station 3 (i.e. transplanted to a higher intertidal zone with 60% longer exposure time, $n = 15$). The cages were pushed into the sediment, leaving 5 cm above the substrate, and the sediment inside was cleared of local cockles prior to the establishment of experimental animals. The cages were left for 100 days after which the number and disk diameter of anemones present on each cockle was respectively recorded and measured. During the experiment, eleven cages were lost and the analysis includes only data from those that persisted throughout the experiment.

Limpet zonation

The limpets *N. helmsi* are associated with hard surfaces on which they are grazing microalgae. However, as opposed to the anemones, limpets are only rarely found attached to fully buried cockles and shells (personal observation), and the investigation of their intertidal zonation therefore focused solely on surface structures. At each of six stations along the tidal gradient, we counted separately the number of shells and live cockles present on the sediment surface, and the limpets they harboured, within five to ten randomly placed circles (circle area = 0.29 m^2). Only shells with a maximum dimension larger than 15 mm were taken into account.

Partial predation by fish

Because spotties *N. celidotus* crop off the feet of surfaced cockles and because the abundance of surfaced individuals increases in the shoreward direction (see Results), it can be expected that foot-cropping is more frequent on upper than lower shore levels, due to a numerical and/or functional response by the fish. To test this hypothesis, the feet of the collected cockles were inspected for incidences of cropping (see Mouritsen and Poulin 2003b, 2003c).

Data analysis

The data analysis was carried out in SPSS and all tests were preceded by evaluation of assumptions. If violated, data were transformed to meet the assumptions or non-parametric tests were applied. Because the parasite load of cockles generally increases with size in a non-linear manner, infection intensities are analysed and presented as ln-transformed and size (shell-length)-corrected values, i.e. relative infection intensity, unless otherwise stated. Moreover, only parasite loads of cockles that have not been foot-cropped by fish were included in the analysis. This was done in order to improve the accuracy of the estimate of parasite recruitment along the tidal gradient because foot-cropping removes a substantial part of the metacercariae harboured in the feet of cockles (Mouritsen and Poulin 2003c). To keep the individual cockle as the unit for analysis in the anemone transplantation experiment, the size distribution of anemones on each cockle was expressed as median disk diameter. The average value of the anemone sizes within treatments was therefore expressed as mean median disk diameter.

Results

Cockle zonation and infection intensities

Cockles are not evenly distributed along the tidal gradient (One-way ANOVA, $F_{11,60} = 9.04$, $P < 0.0005$), but show a density peak between the mid and low intertidal (station 5–6) of about 700 ind m^{-2}, from which the abundance is rapidly decreasing in both shoreward and seaward direction (Fig. 1a). The mean shell-length of cockles increases linearly in the seaward direction, evidencing a growth rate of this suspension-feeding bivalve determined by water coverage time: a linear regression between mean cockle size and mean exposure time was highly significant ($r^2 = 0.99$, $F_{1,10} = 993.99$, $P < 0.0005$). Both the relative infection intensity and the density of surfaced cockles also show a strong tidal gradient with almost linear increases in the shoreward direction (Fig. 1b). Hence, there is a highly significant positive linear regression between the mean density of surfaced cockles and the mean relative parasite load in the cockle population within the investigated tidal range ($r^2 = 0.91$, $F_{1,10} = 104.62$, $P < 0.0005$).

In the present investigation, the proportion of cockles found on the sediment surface at the upper part of the transect is relatively modest (about 2% of the population). However, the proportion vary on a seasonal basis and have been observed to reach 12% at this tidal height (unpublished data). In addition, a relatively large proportion of the cockles is also found partly emerged (the less heavily infected individuals, see Mouritsen and Poulin 2003c), and they will tend to influence the distribution of e.g. the mud flat anemone *A. aureoradiata* in a similar way as the fully surfaced cockles (see below and Discussion).

Whelk zonation and cockle mortality

The abundance of whelks increased exponentially in the shoreward direction (ln-transformed data: One-way ANOVA, $F_{3,12} = 28.42$, $P < 0.0005$), with more than a 13-fold increase over just 54 m of the transect (on average 2.3 snails bait^{-1} at station 11 and 30.3 snails bait^{-1} at station 2). For comparison, no significant zonation of whelks was evident on the nearby sand flat at Gills Corner (mean no. of snails bait^{-1} : 42.0–53.8; One-way ANOVA, $F_{2,12} = 2.06$, $P > 0.17$). Although trematode infections have been found in whelks from Company Bay previously (unpublished data), dissection of the 196 snails caught in the cages here revealed no infections.

The average number of whelk parties feeding on cockles also increased in the shoreward direction (ln-transformed data: One-way ANOVA, $F_{7,24} = 3.20$, $P = 0.015$; Fig. 2). Because of the whelks' ability to track down potential food sources by chemoreception, leaving no incompletely eaten, damaged or otherwise moribund cockles unattacked, these data can be used together with the density data (Fig. 1a) to calculate relative *in situ* mortality rates of cockles at different tidal levels (Ansell 2001). These estimates clearly demonstrate a significantly increasing rate of mortality in the shoreward direction, especially at the uppermost tidal levels ($r_s = 1.000$, $n = 8$, $P < 0.0005$), corresponding to an almost 20-fold increase over just 42 m of transect (Fig. 2).

The mean length of shells collected from the shell-bed at the upper fringe of the transect was 29.1 mm ± 0.2 (SE) ($n = 152$), which corresponds to mean sizes of live, buried cockles found at station 2 and 3 (Fig. 1a). Post hoc tests following One-way ANOVA on shell-length versus tidal height, which also included the dead shells as a separate group, demonstrated that the shells were significantly smaller on average than live cockles found on all tidal heights below station 4 ($P < 0.028$ for all comparisons), but could not be separated from cockles at the upper four stations ($P > 0.085$).

The laboratory experiment evaluating the mortality rate of cockles in absence of predation, demonstrated significant differences according to the experimental cockles' vertical position at the time of collection (arcsin-transformed data: One-way ANOVA, $F_{2,9} = 11.15$, $P = 0.004$; Fig. 3). Post hoc tests showed that whereas the mortality rate of buried individuals from the high (station 1–2) and low (station 7–8) intertidal was similar

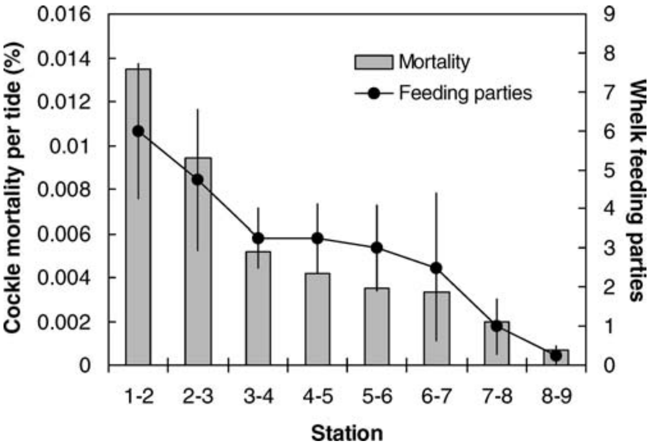

Fig. 2 The mortality of cockles *Austrovenus stutchburyi* as a function of tidal height in Company Bay. *Filled circle*: The mean no. of feeding parties of whelks *Cominella glandiformis* (± SE) preying on cockles per receding tide ($n = 4$ for all values). Bars: the mortality rate of cockles per receding tide (% of standing population at each tidal level), calculated on the basis of the number of feeding parties and cockle density (Fig. 1a)

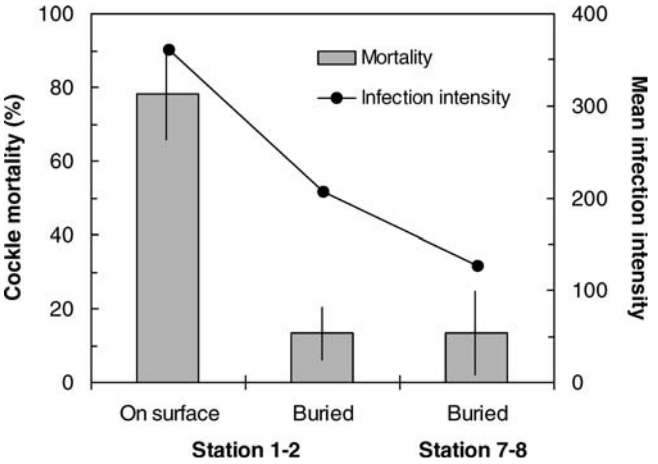

Fig. 3 The mean mortality rate (% dead after 100 days of starvation in the laboratory ± SE) (*bars*) and average infection intensity (*filled circle*) of cockles *Austrovenus stutchburyi* collected at different vertical positions in Company Bay. Infection intensities are mean of station means and include only uncropped cockles (see Data analysis). Whereas the infection data on buried cockles originate form the present study, data on surfaced individuals from station 1–2 are taken from Mouritsen and Poulin (2003c). Note that recorded parasite loads are approximately half the real values (see Materials and methods)

($P = 1.000$), surfaced cockles from the upper zone had a significantly (6-fold) higher mortality rate than the two groups of buried individuals ($P = 0.008$ in both cases). The infection intensity was substantially higher among surfaced than buried cockles (Fig. 3), suggesting that the parasites may be an additional factor causing the higher mortality of surfaced individuals.

Anemone zonation and transplantation experiment

The anemones show a well-defined zonation pattern with densities peaking between station 5 and 10 (data not shown, but see Mouritsen and Poulin 2003a). However, this distributional pattern is to a large extent governed by available substrate for attachment (buried cockles) that peaks around station 5 and 6 (Fig. 1A). A correction for this effect reveals a narrower zone of optimal living conditions around station 9, from where the anemone density per cm^2 available substrate decreases almost linearly (by 84%) in the shoreward direction (linear regression: $r^2 = 0.95$, $F_{1,7} = 146.51$, $P < 0.0005$; Fig. 4a). Interestingly, no similar decline was observed in the maximum number of anemones per unit substrate ($r_s = -0.33$, $n = 7$, $P = 0.42$), and consequently, the coefficient of variation (CV = 100×SD/mean) increased rapidly shoreward from 65% at station 9 to 245% at station 1 ($r_s = 0.96$, $n = 7$, $P < 0.0005$). The size distribution of the anemones was unaffected by tidal height (Kruskal-Wallis test, $\chi^2 = 15.58$, $df = 11$, $P = 0.16$), but maximum recorded disk diameter showed a weak declining trend in the shoreward direction (linear regression: $r^2 = 0.45$, $F_{1,10} = 8.29$, $P = 0.016$) (Fig. 4b).

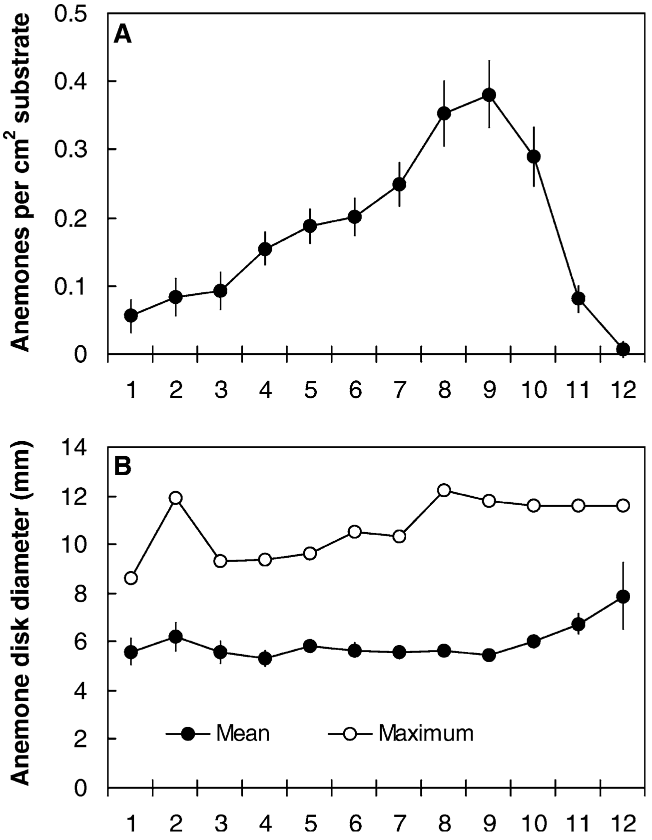

Fig. 4 a,b The abundance and size distribution of mud flat anemones *Anthopleura aureoradiata* as a function of decreasing tidal height in Company Bay. **a** Number of anemones per cm^2 available substrate (i.e. surface area of the upper half of buried cockles) (± SE, $n = 24$–27 per station). **b** Mean (± SE) and maximum disk diameter (mm) ($n = 5$–114 per station, $n_{tot} = 652$)

The transplantation of anemones to a higher tidal height with 60% longer exposure time had no or only little impact on survival and growth (Fig. 5). At the start of the experiment, the average number of anemones per caged cockle was similar at the two tidal heights (Student's *t*-test, $t_{28} = 0.92$, $P = 0.36$), and a Repeated-measures ANOVA showed lack of significant two-way (day-by-station) interaction ($F_{1,17} = 1.46$, $P = 0.24$) and no effect of day ($F_{1,17} = 0.22$, $P = 0.64$) (Fig. 5a). Hence, the survival rate of the anemones seems unaffected by a substantially longer exposure time for a prolonged period of time (100 days). The disk diameter of experimental anemones at the start of the experiment did not differ significantly between station 3 and 7 (Student's *t*-test, $t_{28} = 0.21$, $P = 0.83$, Fig. 5b), but during the experiment sizes tended to increase at station 3 and decrease at station 7, evidenced by a significant two-way interaction (Repeated-measures ANOVA, $F_{1,17} = 17.23$, $P = 0.001$). However, the changes were small, and no statistically significant difference in median disk diameter could be demonstrated between tidal heights after 100 days of experimentation (Student's *t*-test, $t_{17} = 1.95$, $P = 0.07$).

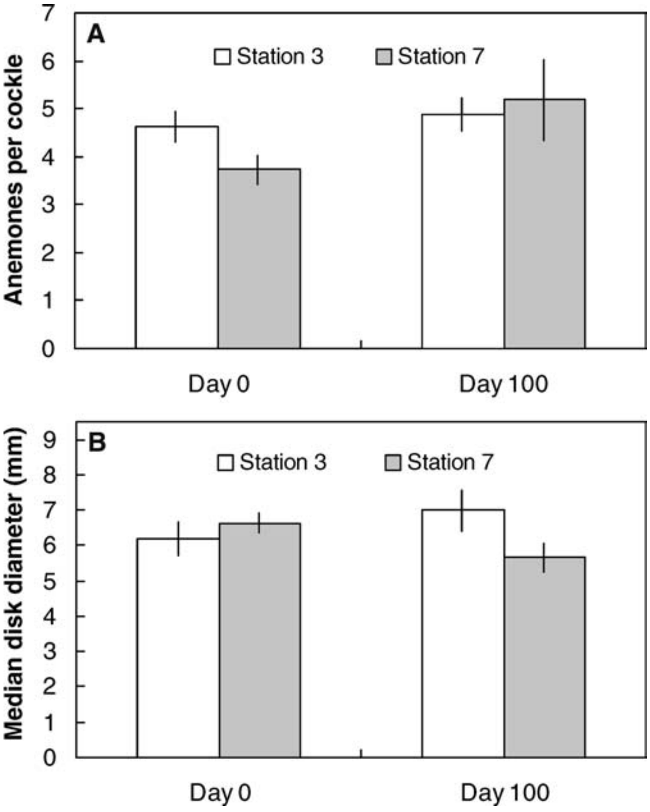

Fig. 5 a,b The abundance and size distribution of mud flat anemones *A. aureoradiata* in the transplantation experiment. **a** The mean no. of mud flat anemones per caged cockle *A. stutchburyi* (± SE). **b** The mean median disk diameter (mm ± SE) of anemones per caged cockle at the start (Day 0) and the end (Day 100) of the field experiment in Company Bay. *White bars*: cockles transplanted from the lower intertidal (station 7) to a higher tidal height (station 3) ($n = 8$). *Shaded bars*: cockles re-transplanted at the lower intertidal (control) ($n = 11$)

Limpet zonation

Although limpets were found throughout the transect, densities increased rapidly in the shoreward direction at the upper quarter of the transect (Fig. 6). Hence, the vast majority of limpets were found in association with the lower fringe of the mid-intertidal shell-bed, and their distribution reflects a similar pattern to the density of both shells (linear regression: ln[no. m^{-2}] = 3.90–0.39 station, $r^2 = 0.79$, $F_{1,48} = 180.61$, $P < 0.0005$) and cockles (ln[no. m^{-2}] = 1.56–0.084station, $r^2 = 0.18$, $F_{1,48} = 10.48$, $P < 0.002$) on the sediment surface used as substrate. Based on double ln($x + 1$)-transformed data (to optimise linearity), a multiple regression including station and the density of shells and surfaced cockles as predictors and limpet density as dependent variable, showed a significant total regression ($r^2 = 0.75$, $F_{3,46} = 46.79$, $P < 0.0005$) and a significant effect of station (partial: $t = -2.85$, $r = -0.39$, $P = 0.007$) and shells (partial: $t = 2.60$, $r = 0.36$, $P = 0.012$), but no effect of surfaced cockles (partial: $t = 1.47$, $r = 0.21$, $P = 0.15$). The separate effect of station may reflect that the number of limpets per unit substrate increases in the shoreward direction, whether the substrate was surfaced cockles (linear regression: $r^2 = 0.83$, $F_{1,4} = 19.46$, $P = 0.012$) or dead shells ($r^2 = 0.76$, $F_{1,4} = 12.41$, $P = 0.024$) (Fig. 7). A full model ANCOVA on these data demonstrated no two-way interaction (substrate type by station, $F_{1,8} = 0.41$, $P = 0.54$), but highly significant effects of substrate type ($F_{1,9} = 15.17$, $P = 0.004$) and, of course, station ($F_{1,9} = 30.38$, $P < 0.0005$) in a reduced model. Hence, the density of limpets was generally larger (about 50% based on back-transformed grand means) on shells than on surfaced cockles (Fig. 7).

The shoreward increase in limpet density per unit substrate could be interpreted as a disproportionately strong numerical response to the high abundance of substrate at the upper tidal levels. However, after

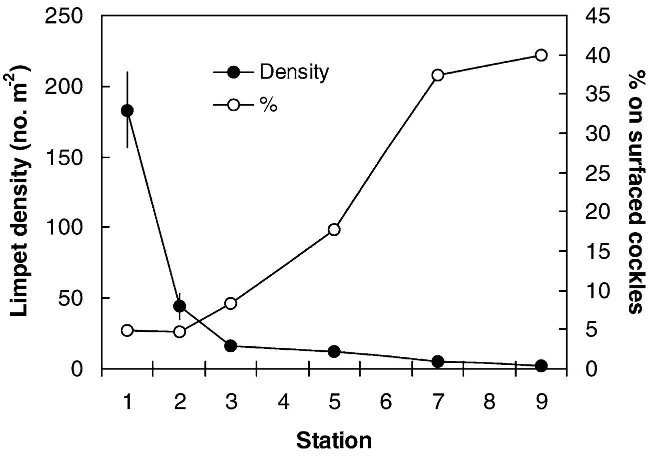

Fig. 6 The mean density of limpets *Notoacmea helmsi* m^{-2} (± SE) (*filled circle*; $n = 5$–10) and the proportion (%) associated with surfaced cockles (*open circle*) as a function of decreasing tidal height in Company Bay

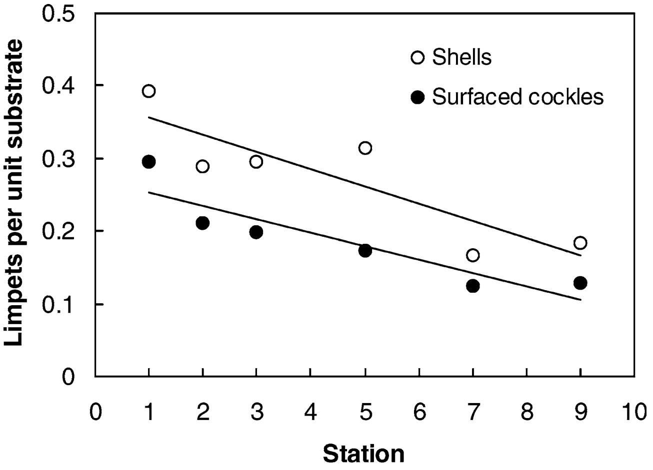

Fig. 7 The ln-transformed mean no. of limpets *N. helmsi* shell^{-1} (*open circle*) and surfaced cockle^{-1} (*filled circle*) as a function of tidal height. The regression lines are shown

correcting for the effect of station no significant relationship could be demonstrated between limpet density per unit substrate and substrate density (linear regression on station residuals: $F_{1,47}=0.84$, $P=0.36$), which rejects this possibility. Rather, the pattern might be explained by an increasing abundance of microalgae on the substrate, and hence food density, in the shoreward direction due to an increasing level of sun radiation (longer exposure time).

Shells on the sediment surface clearly is the main determinant of limpet abundance in the upper quarter of the transect, where surfaced cockles may account for less than 10% of the population (Fig. 6). However, probably as a consequence of shortage of the preferred shells, surfaced cockles becomes increasingly important as substrate in the seaward direction, accounting for up to 40% of the limpet population at the lower tidal heights (Fig. 6).

Foot-cropping as a function of tidal height

The frequency of foot-cropping by spotties in the population of cockles increased linearly in the shoreward direction (linear regression: $r^2=0.89$, $F_{1,10}=77.82$, $P<0.0005$; Fig. 8). Whereas this pattern obviously was unrelated to the overall density distribution of the target organism that peaked at intermediate levels (Fig. 1a), the cropping frequency is significantly and positively related to the density of surfaced cockles (linear regression: $r^2=0.78$, $F_{1,10}=36.36$, $P<0.0005$).

Discussion

All investigated species show a well-defined pattern of intertidal zonation, with densities either peaking (cockles), increasing (whelks, limpets, spotties) or mostly decreasing (anemones) as a function of increasing tidal height. In all cases cockle parasitism appears to be a significant influencing factor, either directly (cockles) or indirectly (the others).

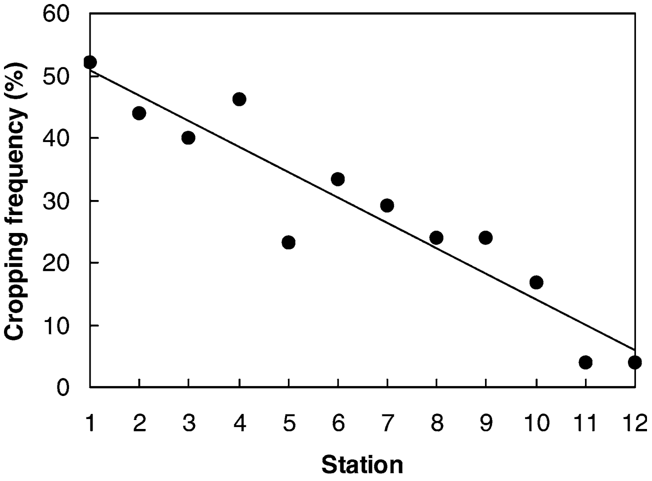

Fig. 8 The frequency (%) of cockles *A. stutchburyi* that have been foot-cropped by spotties *Notolabrus celidotus* as a function of decreasing tidal height in Company Bay. The regression line is shown

Cockles *A. stutchburyi*

In the upper part of the transect the density of buried cockles decreases rapidly in the shoreward direction and the abundance is reduced by half over just 18 meters of intertidal zone (station 5 to 2, Fig. 1a). Because the submersion time in the upper quarter of the transect ranges between 70 and 80%, it is doubtful that reduced larvae settling (see Jensen 1992), starvation or elevated temperature and desiccation stress can justify a population decline of this magnitude. Accordingly, the mortality rate of buried individuals from the upper and lower transect did not differ when exposed to prolonged starvation (Fig. 3), which does not suggest that buried cockles from upper tidal heights are especially weakened. Also, the cockles' growth rate decreased with increasing tidal height in a linear fashion, and in complete accordance with decreasing water coverage time. A greater than linear decline would be expected in the upper intertidal if the population here were under severe physiological stress. Nevertheless, cockles in the upper tidal zone die relatively fast as evidenced by (1) the presence of the extensive bed of cockle shells whose sizes suggest an origin in the upper quarter of the transect, and (2), the relatively high mortality rate here, estimated *in situ* from whelk feeding parties (Fig. 2). The ultimate reason for this appears to be parasitism. The cockles in Company Bay are heavily infected by echinostomes. These parasites preferentially infect the cockles' foot and thereby interfere with the bivalves' burying ability, making them the single most important factor causing cockles to lie stranded on the sediment surface (Thomas and Poulin 1998; Mouritsen 2002; Babirat et al. 2004). Because the infection intensity of cockles increases with increasing tidal height, so does the abundance of surfaced cockles (Fig. 1b). Lying exposed on the sediments, these heavily infected individuals will be subject to temperature and desiccation stress that, combined with high parasite load, in time may weaken them to an extent where they become easy prey for whelks. The laboratory experiment in which cockles were exposed to starvation clearly demonstrates that surfaced cockles from the upper tidal zone in fact are weakened or moribund to a much larger extent than buried individuals. On top of this mortality comes direct predation on also more healthy surfaced cockles by whelks and oystercatchers *Haematopus* spp. Both these predators seize newly surfaced cockles five times as often as buried specimens (Mouritsen 2004). Other mortality factors unrelated to trematode infections may of course also be influential.

It is crucial to the present analysis that surfacing of the New Zealand cockle is due mostly to trematode infections, but other factors such as unfavourable environmental conditions can also be envisaged to contribute. This has been shown in the European cockle *Cerastoderma edule* (e.g. Richardson et al. 1993; Desclaux et al. 2002). However, in Otago Harbour the level of surfacing, both between tidal flats and along the tidal gradient within flats, as well as the cockles' vertical position in the substrate at a given tidal level, correlate consistently with the parasite load of the cockles (Thomas et al. 1998; Mouritsen and Poulin 2003c, 2005, present study), emphasising trematode infection as the single most important factor causing surfacing. Moreover, surfacing induced by abiotic factors is temporary, whereas trematode infections are likely to result in long-term, permanent presence of cockles at the surface, which is required in order to alter the zonation pattern of other organisms (see below).

Whelks *C. glandiformis*

Because whelks preferentially prey on surfaced cockles (Mouritsen 2004), their shoreward exponential increase in abundance likely represents a numerical response to increasing food accessibility. This is supported by the lack of significant zonation at the nearby Gills Corner sand flat where the parasite load of cockles is several-fold lower (Mouritsen et al. 2003) and surfaced individuals are rare. The parasites therefore influence the zonation of this epibenthic predator indirectly by shifting its peak abundance shoreward, away from the overall density peak of its main prey (see Fig. 1a).

Mud flat anemones *A. aureoradiata*

Unlike molluscs, mud flat anemones are particularly vulnerable to desiccation during low tide and the observed decrease in their density per unit substrate with increasing tidal height is therefore expectable. However, the transplantation experiment carried out during the season of maximum solar radiation showed that 60% longer exposure time had no severe negative impact on survival and growth of anemones on fully buried cockles. Although the maximum disk diameter tended to decrease shoreward, this agrees well with the lack of change in the overall size distribution of anemones along the tidal gradient (Fig. 4b). If increased environmental stress and shorter feeding time at higher tidal levels poses a serious problem to anemones, a significant decrease in average size with increasing exposure time would be expected. Besides, the shoreward increase in the coefficient of variation (CV) of anemone density per unit substrate also invalidates increasing exposure time per se as the decisive factor. The associated environmental stress and shorter feeding time should cause an even thinning out of the anemones among cockles, resulting in decreasing maximum number of anemones per unit substrate as well as decreasing or unchanging CV as a function of increasing tidal height. This was not observed. The resilience to longer exposure time by anemones of fully buried cockles is likely due to the fact that they retract to just below the sediment surface during low tide (personal observation), which mitigates environmental stress considerably.

Again, anemone zonation may be governed to a large extent by the parasites' effect on the behaviour of their substrate. Anemones attached to cockles that surface will be fully exposed to desiccation (Thomas et al. 1998), and hence are bound to decline in numbers as the surfacing activity increases in the shoreward direction. The variation in parasite load is considerable even among cockles from the same tidal height (see Mouritsen and Poulin 2003a), and since only the more heavily infected tend to surface, the CV of anemone abundance per unit substrate will increase as a consequence.

Limpets *N. helmsi*

Most limpets found in the transect were associated with dead shells lying on the sediment surface, especially at the highest tidal level. There, because the high cockle mortality appears mediated by parasitism, the parasites become a decisive indirect factor determining the intertidal zonation of the limpets. Nevertheless, limpets were also associated with surfaced cockles, and parasite-mediated surfacing of cockles could account for up to 40% of the limpet population at tidal levels where shells are rare (Fig. 6). It can be argued that in absence of parasites, the population of limpets would just congregate on the few empty shells always present on the sediment surface. However, the lack of a significant relationship between limpets per unit substrate (adjusted for tidal level) and substrate density, rejects this possibility by demonstrating that at any given tidal level the abundance of limpets is determined mainly by the abundance of available substrate.

Spotties *N. celidotus*

Spotties can only feed on the tidal flat during high water, and a priori the frequency of foot-cropping should be greatest at lower tidal levels where the available time for feeding is longer. However, the opposite pattern prevails: the incidence of partial predation increases in the shoreward direction. Underwater video recordings have demonstrated that feeding spotties are strongly attracted to surfaced cockles that expose their feet during their repeated attempts to bury (Mouritsen and Poulin 2003c). Hence, the significant positive relationship between the frequency of partial predation and the density of surfaced cockles in the shoreward direction clearly represents a causal relationship, indirectly established by the action of the parasites.

The present results clearly indicate that parasitism has the potential of indirectly determining the intertidal zonation of a taxonomically and functionally diverse group of epibentic animals through its direct impact on the survival as well as the behaviour of a keystone species. The impact of the parasites may reach even further. The abundance of *A. stutchburyi* has previously been shown to affect the occurrence of many infaunal species, including bivalves, polychaetes and crustaceans (Whitlatch et al. 1997). To the extent that cockle density is depressed at higher tidal levels due to parasitism, the structure of the infaunal community may change accordingly. We have, in support, recently demonstrated that the mere presence of cockles on the sediment surface significantly changes the infaunal community structure, probably via changed near sea-bed hydrodynamics and sedimentation patterns (Mouritsen and Poulin 2005).

To our knowledge, the present results represent the first demonstration that parasitism can indirectly determine the intertidal zonation of also non-host animals. Unravelling such cryptic effects seems to be a fruitful avenue to follow in order to better understand the zonation as well as the overall community structure of intertidal organisms (see Mouritsen and Poulin 2002).

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