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Parasite-induced trophic facilitation exploited by a non-host predator: a manipulator's nightmare

Kim N. Mouritsen*, Robert Poulin

Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand

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Abstract

Parasites with complex life cycles, relying on trophic transmission to a definitive host, very often induce changes in the behaviour or appearance of their intermediate hosts. Because this usually makes the intermediate host vulnerable to predation by the definitive host, it is generally assumed that the parasite's transmission rate is increased, and that the modification of the host is, therefore, of great adaptive significance to the parasite. However, in the ecological "real world" other predators unsuitable as hosts may just as well take advantage of the facilitation process and significantly erode the benefit of host manipulation. Here we show that the intertidal New Zealand cockle (*Austrovenus stutchburyi*), manipulated by its echinostome trematode (*Curtuteria australis*) to rest on the sediment surface fully exposed to predation from the avian definitive host, is also subject to sublethal predation from a benthic feeding fish (*Notolabrus celidotus*, Labridae). The fish is targeting only the cockle-foot, in which the parasite preferentially encysts, reducing the infection intensity of manipulated cockles to levels comparable with those in non-manipulated, buried cockles. Based on the frequency and intensity of the foot cropping and predation rates on surfaced cockles by avian hosts, it is estimated that 2.5% of the parasite population in manipulated cockles is transmitted successfully whereas 17.1% is lost to fish. We argue that the adaptive significance of manipulation in the present system depends critically on the feeding behaviour of the definitive host. If cockles constitute the majority of prey items, there will be selection against manipulation. If manipulated cockles are taken as an easily accessible supplement to a diet composed mostly of other prey organisms, behavioural manipulation of the cockle host appears a high risk, high profit transmission strategy. Both these feeding behaviours of birds are known to occur in the field.

Keywords: Host manipulation; Partial predation; Transmission success; Echinostomatidae; Parasitism; Intertidal bivalve; Intramolluscan metacercariae

1. Introduction

The ability of parasitic helminths to manipulate the phenotype of their intermediate hosts in ways that enhance their transmission via predation to the definitive host, has become one of the best documented adaptive feature of parasites (Poulin, 1995; Lafferty, 1999; Moore, 2002). In spite of the voluminous literature now available on this aspect of parasite ecology, there are almost no robust quantitative data available on its role in the population dynamics of parasites. The majority of alterations in host phenotype reported in past studies involve simple changes in host activity levels, microhabitat use, or colouration: all these can increase the susceptibility of the intermediate host to a range of predators, not all of which are suitable definitive hosts. The very few population models that have considered the increased rate of predation by definitive hosts on intermediate hosts mediated by parasite manipulation of intermediate hosts, have assumed that suitable definitive hosts are the only predators in the system (Dobson and Keymer, 1985; Dobson, 1988). Larval helminths in intermediate hosts are assumed to either end up in a suitable definitive host, or die within the intermediate host. The models have ignored the possibility that larval helminths, by altering the behaviour or colouration of their intermediate hosts, also increase predation rates by other predators that do not act as definitive hosts. Similarly, models that investigate the evolution of host manipulation by parasites from the perspective of costs and benefits also assume that increased transmission success toward suitable definitive hosts is the only alternative to death within the intermediate host (Poulin, 1994; Brown, 1999). If host manipulation

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* Corresponding author. Department of Marine Ecology, University of Aarhus, Finlandsgade 14, DK-8200 Aarhus N, Denmark. Tel.: +45-89424386; fax: +45-89427584.

E-mail address: kim.mouritsen@biology.au.dk (K.N. Mouritsen).

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leads to increased probabilities of both successful transmission to a definitive host and ingestion by an unsuitable predator, then its potential benefits can be outweighed by the dangers associated with it.

Very few empirical studies have considered the possibility that manipulated intermediate hosts may end up in predators that are not suitable definitive hosts for the parasite. Some authors have shown that the altered anti-predator behaviour induced by the cestode *Schistocephalus solidus* in its stickleback intermediate host is identical whether the predator is a bird definitive host or a predatory fish in which the parasite cannot pursue its development (e.g. Milinski, 1985; Ness and Foster, 1999). However, the quantitative impact of this non-specific response on transmission dynamics of the cestode in the field is unknown. Webber et al. (1987) have shown that in aquaria, the behaviour manipulation induced by the trematode *Plagiorchis noblei* in its mosquito intermediate host makes the host more susceptible to predation by semi-aquatic rodents that can serve as definitive hosts, but not by guppies that cannot be definitive hosts. The obvious weakness of this study, in addition to the fact that it was carried out in artificial conditions, was that the alternative host used, the guppy, is not a natural predator of *Plagiorchis*-altered mosquitoes in their temperate freshwater habitats. At present, even tentative field estimates of how likely manipulated intermediate hosts and their parasites are ending up in predators other than definitive hosts, are completely lacking.

Here, we provide the first field investigation of these issues, using a model system for which there already exists good background information. The trematode *Curtuteria australis* (Echinostomatidae: Himasthlinae) uses the New Zealand cockle *Austrovenus stutchburyi* as second intermediate host, from which it is transmitted by predation to its definitive hosts, i.e. oystercatchers and other shorebirds (Allison, 1979). Cockles are the dominant bivalves of New Zealand intertidal sandflats and mudflats, where they normally live buried under 1–3 cm of sediments. After leaving the snail first intermediate host, cercariae of *C. australis* penetrate cockles through their inhalant siphon and then encyst as metacercariae in the foot of cockles. As a cockle accumulates metacercariae in its foot, its ability to burrow (if dislodged to the sediment surface) decreases, to the extent that heavily infected cockles are forced to lie exposed on the surface (Thomas and Poulin, 1998; Mouritsen, 2002). This reduced burrowing ability in heavily infected cockle is an example of host manipulation by the trematode, which then achieves a much higher probability of successful trophic transmission to avian definitive hosts (Thomas and Poulin, 1998). However, cockles lying partially or fully exposed at the surface may also be more susceptible to predation by other organisms. Recently, high frequencies of foot cropping, i.e. non-lethal predation of the foot of the cockles, have been documented in Otago Harbour, South Island, New Zealand (Mouritsen and Poulin, 2003). The foot cropper is most likely a fish preying on cockles active at the surface at high tide. It appears likely that manipulated cockles, which are on the sediment surface trying in vain to burrow, incur greater risks of foot cropping. From the trematode's perspective, manipulation could then be a two-edged sword: on the one hand increasing the probability of transmission to birds, but on the other causing many metacercariae to be lost permanently from the system due to consumption by an inappropriate non-host animal.

The objectives of this study were: (1) to determine whether manipulated cockles incur a higher risk of foot cropping than non-manipulated (i.e. buried) cockles; (2) to obtain a quantitative estimate of the number of metacercariae lost from the population via cropping of cockle feet; and (3) to identify the foot cropper and confirm that it is not a potential avian definitive host. Overall, this study is the first to assess in the field the relative importance of the different outcomes of host manipulation for the transmission dynamics of a parasitic helminth.

2. Materials and methods

2.1. The study site

The study was carried out in February and April 2002 on a sheltered sand flat in Company Bay, Otago Harbour, South Island, New Zealand (45°51′S/170°37′E). In February, a transect study was carried out to elucidate the general relationships between cropping frequency, surfacing behaviour and the mean infection intensity in the cockle population (Section 2.2). In April, further investigations were carried out in the mid-intertidal zone (1) to clarify the relationships between infection intensity, incidence of foot cropping, and the cockle's vertical position in the sediment, and (2) to obtain data suitable for estimating the proportion of *C. australis* metacercariae lost by foot cropping (Sections 2.3 and 2.4).

2.2. The transect study

A transect of 12 stations separated by 6 m along a tidal gradient from the spring low tide mark to the upper mid-intertidal zone was established. At each station the density of cockles lying fully exposed on the sediment surface was estimated from numbers present within a circular frame (diameter = 61 cm) at five randomly chosen sites. By using a core sampler, 24–27 randomly selected cockles were collected at each station and taken to the laboratory. After measurements of maximum shell length, the cockles were opened and the foot was removed by cutting along the well-defined interface between the foot tissue and the gonad-visceral mass. The foot was squeezed lightly between two microscope slides and the number of *C. australis* metacercariae, easily seen through the transparent foot tissue, was counted 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. Finally we recorded whether or not the feet had been recently cropped, recognisable as distinctive depressions along the otherwise straight foot rim and/or by the presence of newly regenerated tissue. Cockles are able to regenerate lost foot tissue (Mouritsen, unpublished) and new tissue can quite easily be distinguished from older tissues by its thinner and whitish appearance with only very few embedded metacercariae.

2.3. Relationships between cropping, cockle behaviour and parasite loads

From a 12-m wide intertidal zone at the mid-tidal level, three types of cockles were collected according to their vertical position in the substrate, and thus to their burying ability: (1) surfaced cockles, having more than 75% of the shell (along the height axis) exposed or lying on one side on the sediment surface; (2) interface cockles, having 25–75% of the shell exposed; (3) buried cockles, having less than 25% of the shell exposed. About 35 similar-sized cockles of each category were collected singly by hand, ensuring that collections of all groups were distributed approximately at random within the 12-m zone. The density of surfaced and interface cockles was estimated from numbers present within the previously used circular frame ($n = 10$) at randomly distributed sites, whereas the density of buried cockles was obtained from numbers recovered in 12 randomly distributed core samples (diameter = 12.3 cm). Collected animals were returned to the laboratory and processed as above, except that the foot length was also measured and that all *Curtuteria* metacercariae present in the foot were enumerated by counting on both sides of the foot. Moreover, because *C. australis* is distributed unevenly along the length-axis of the foot (Mouritsen, 2002), the number of metacercariae present in the tip (the anterior one-third) and in the remaining proximal part of each foot was obtained separately. The foot was then placed on graph paper so that it formed a right-angled triangle, with the cut edge and the front of the foot making up the two sides of the right angle and the outer foot rim as the hypotenuse (Fig. 1a), and the outline of the foot was drawn on the paper. Incidences of newly inflicted wounds and older, fully or partially regenerated damages were treated separately. The former was identified as before, and the proportion of the foot missing was estimated from the relationship between the projected foot area lost and the projected uncropped/intact foot area (obtained by extra or interpolation of undamaged parts of the foot rim) (Fig. 1b). In order to estimate the proportion of the foot recently regenerated, a sharp pencil was used to pierce the borderline between old and new tissues leaving a dotted line on the graph paper when the foot was removed (Fig. 1c). As for the metacercarial load, estimates of the proportion of the foot that has been cropped and/or regenerated were obtained for the tip and the proximal part separately.

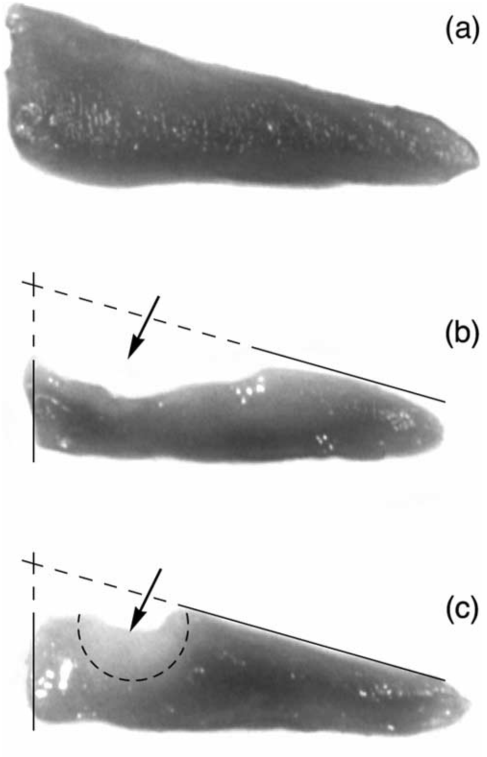

Fig. 1. Cropped and uncropped cockle feet. The foot-tip is to the left and the base is where it has been separated (cut) from the gonad-visceral mass. (a) An uncropped, intact foot. (b) A foot cropped in the tip and mid-part (arrow), showing no signs of regeneration. (c) A tip-cropped foot with partial regeneration of the damage (light tissue, arrow); the dotted line highlights the transition between old and newly regenerated tissue. Straight lines are tangents to the outer foot rim and their broken sections represent extrapolations where foot tissue has been cropped off.

2.4. Estimation of pre-cropped parasite loads

Knowing the proportion of the foot that has been lost by cropping, and the density of metacercariae (number per projected foot area) in the remaining tissue, the parasite load prior to cropping can be estimated. However, to optimise the accuracy of the estimate, the density distribution of the metacercariae along the foot also has to be taken into account. Because the density of *Curtuteria* metacercariae is approximately 4.15-fold higher in the one-third of the foot closest to the tip than in both the mid-part and the proximal third of the foot (Mouritsen, 2002), an estimate of parasite density per unit foot area based on the entire foot will result in an underestimation of the pre-cropped parasite load if the foot has been cropped in the tip, and in an overestimation if the proximal part has been affected. Hence, an estimate of the pre-cropped parasite load, correcting for the within-foot distribution of the parasites, can be calculated according to the formula

$$N_c = N_{obs}/1 - (0.806PC_{tip} + 0.194PC_{hind}) \quad (1)$$

where N_c is the corrected (estimated pre-cropped) number of metacercariae present in the foot, N_{obs} the number of metacercariae actually observed, PC_{tip} the proportion of the foot-tip lost to cropping, PC_{hind} the proportion of the proximal part of the foot lost to cropping, and the constants 0.806 and 0.194 denote the previously observed overall relative density distribution of metacercariae between the tip one-third of the foot and the proximal two-thirds of the foot, respectively.

2.5. Identification of the cropper

An underwater video camera was anchored on the sediment surface in Company Bay during low water at approximately mid-intertidal level. Two 0.5 × 1 m fields were established in front of the camera, one having about 200 randomly selected cockles on the surface, the other having no cockles on the surface. When the incoming tide had submerged the camera it was switched on and left running for 52 min (battery lifetime). From the resulting recordings, the identity of the foot-cropping organism was established and the maximum number of croppers present at any one time as well as the number of feeding attempts in each of the two fields was recorded.

2.6. Statistical analysis

All analyses were carried out in SPSS except for multiple comparisons between groups after Kruskal–Wallis tests, which followed Siegel and Castellan (1988). Parametric tests were preceded by tests of their assumptions. If violated, the data were either transformed or non-parametric statistics were applied. Because parasite loads in cockles generally increase non-linearly with cockle size, all infection data were ln-transformed and corrected for (i.e. divided by) shell length prior to analysis thereby producing relative infection intensities. Because the size range of cockles was narrow and their mean sizes were similar between groups in the April samples (Table 1), transformation of infection intensities had only a small effect on the means. Hence, in order to improve clarity only untransformed infection data are presented in Fig. 3.

3. Results

The transect study demonstrated a significant positive relationship between the cropping frequency and mean relative infection intensity of cockles (linear regression: $r^2 = 0.82$, $F_{1,10} = 45.0$, $P < 0.0005$) as well as between the density of surfaced cockles and mean infection intensity ($r^2 = 0.92$, $F_{1,10} = 52.0$, $P < 0.0005$) (Fig. 2). It follows that the density of surfaced cockles is significantly and positively related to the frequency of foot cropping in the population of cockles ($r^2 = 0.71$, $F_{1,10} = 24.5$, $P = 0.001$). This suggests that the croppers are specifically targeting heavily infected cockles that either are present on the sediment surface already or are left there as a consequence of past cropping activity.

The cropping frequency depended on the cockle's vertical position in the substrate and increased significantly from 14% among fully buried animals to 83% among surfaced individuals (Fisher's exact test, $P < 0.028$ for all possible comparisons; Table 1). The average proportion of the foot cropped was significantly higher (three to fourfold) in surfaced cockles than among interface and fully buried cockles (Table 1).

The proportion of cockles that showed clear signs of recent foot-tissue regeneration was high in all cockle groups, but particularly among interface cockles (Table 1). Whereas surfaced and buried cockles did not differ (Fisher's exact test, $P = 0.46$), interface cockles showed a significantly higher regeneration frequency than the two other groups considered together (Fisher's exact test, $P = 0.018$). The proportion of the foot that had regenerated did not differ significantly between cockle groups (Table 1).

Croppers targeted the tip of the cockle's feet in particular although this part of the foot constitutes only

Table 1
Density, cropping and infection characteristics of the three behavioural categories of cockles *A. stutchburyi* from Company Bay, Otago Harbour, South Island, New Zealand[a]

Parameter	Cockle position in substrate			*P*
	On surface	Interface	Fully buried	
Density (no. m^{-2})	7.7 ± 1.2 (10)	4.5 ± 0.9 (10)	645.2 ± 46.7 (12)	
Cropping frequency (%)	82.9 (35)	38.2 (34)	13.9 (36)	<0.0005[b]
Proportion of foot cropped (%)[c]	21.6 [3–76] (29)	6.5 [2–22] (13)	4.9 [2–11] (5)	<0.0005[d]
Regeneration frequency (%)	42.9 (35)	64.7 (34)	38.9 (36)	0.057[e]
Proportion of foot regenerated (%)[c]	15.9 [4–57] (15)	17.0 [5–41] (22)	19.4 [2–53] (14)	0.88[f]

[a] Except for cropping and regeneration frequency, values are means ± SE [range] (*n*).
[b] Chi-square test, $\chi^2_2 = 35.01$.
[c] Affected individuals only.
[d] Kruskal–Wallis test, $\chi^2_2 = 18.99$.
[e] Chi-square test, $\chi^2_2 = 5.35$.
[f] Kruskal–Wallis test, $\chi^2_2 = 0.25$.

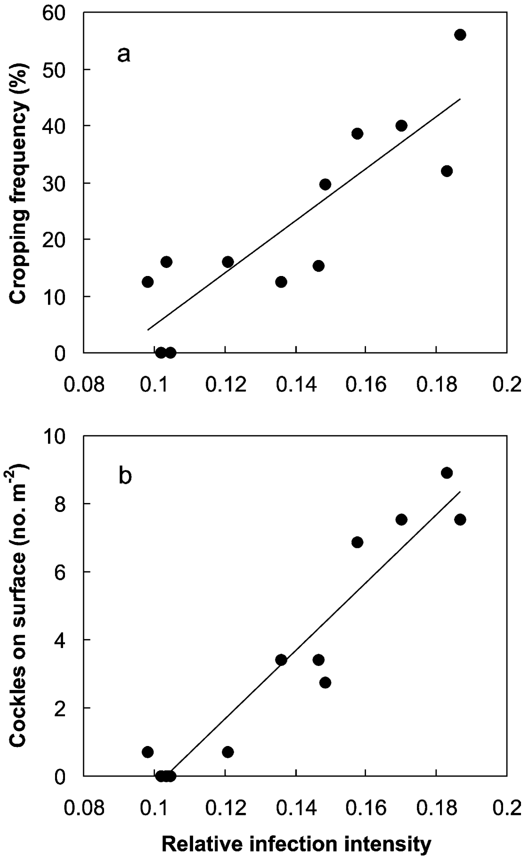

Fig. 2. The cropping frequency in the cockle population (a) and the mean density of surfaced cockles (b) as a function of the mean relative infection intensity of cockles across 12 stations in Company Bay, Otago Harbour, South Island, New Zealand. The relative infection intensities are ln-transformed number of recorded metacercariae per cockle corrected for shell length. The range of values corresponds to a real mean parasite load of about 145–522 metacercariae per cockle. Note that cropping frequency here includes individuals with fresh wounds as well as regeneration of earlier damage.

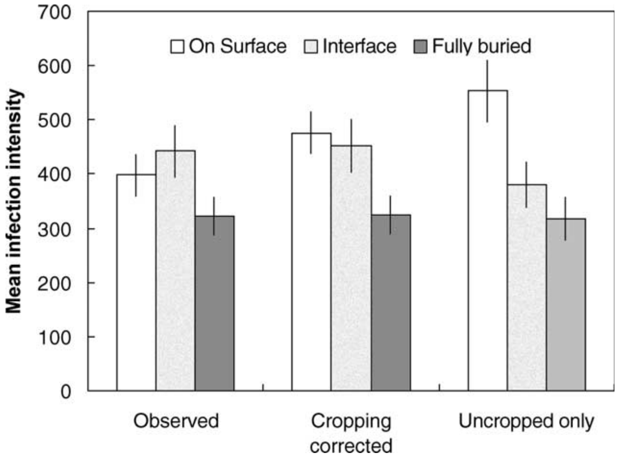

Fig. 3. The mean infection intensity (number of metacercariae per individual) ± SE of surfaced, half buried (interface) and fully buried cockles. Observed: infection intensities based on all collected cockles irrespective of cropping-status. There is no significant difference in mean infection intensity between cockles with different vertical position (one-way ANOVA, $F_{2,102} = 2.21$, $P = 0.12$). Cropping-corrected: as 'Observed', but infection intensities are N_c values calculated according to formula (1) (see Section 2). The mean infection intensity of cockles with different vertical position differs significantly (one-way ANOVA, $F_{2,102} = 6.25$, $P = 0.003$). Uncropped only: observed infection intensities of cockles that have not been cropped recently. The mean infection intensity of cockles with different vertical position differs significantly (Kruskal–Wallis test, $\chi^2_2 = 9.43$, $P = 0.009$). Sample sizes vary between 34 and 36 for both observed and cropping-corrected individuals, whereas for the group of uncropped cockles, n equals 6, 21 and 31 for surfaced, interface and fully buried cockles, respectively. Statistical tests were carried out on ln-transformed values corrected for cockle size.

45% of the entire projected foot area. Considering both cockles that were cropped at the time of collection (i.e. tissue missing) and cockles with regenerated foot tissue, on an average 31.5% of the projected foot-tip was or had been affected compared with 15.2% of the proximal part of the foot (Wilcoxon signed rank test, $n = 67$, $Z = 4.07$, $P < 0.0005$).

No statistically significant difference in mean relative infection intensity by *C. australis* could be demonstrated among the three groups of cockles (Fig. 3). However, correcting the observed parasite loads for the estimated number of *Curtuteria* metacercariae lost through cropping, resulted in a significantly higher mean pre-cropped parasite load in surfaced than in fully buried cockles (Fig. 3; Bonferroni post hoc test, $P = 0.002$). Similarly, considering only uncropped cockles, those found on the sediment surface harboured significantly more metacercariae than fully buried specimens (Fig. 3; Multiple comparison post hoc test, $P < 0.05$). Moreover, no significant difference in mean relative parasite load was evident between cropping-corrected (cropped individuals only) and uncropped cockles from the sediment surface (Student's t-test, $t_{33} = 1.27$, $P = 0.21$), which shows that formula (1) succeeded in correcting adequately for the loss of metacercariae to croppers.

The relative foot length (i.e. length corrected for cockle size) differed significantly among uncropped cockles depending on their position in the substrate (one-way ANOVA, $F_{2,57} = 9.83$, $P < 0.0005$): cockles on the surface had smaller feet than fully buried specimens (Bonferroni post hoc test, $P = 0.02$). Considering together all uncropped individuals, there was a significant negative relationship between relative foot length and the size-corrected parasite load ($r_{57} = -0.29$, $P = 0.026$): heavily infected cockles had shorter feet than lightly infected ones.

Based on the difference between the observed and cropping-corrected mean infection intensities, 17.1% of the metacercariae that initially infected the surfaced cockles had been lost to croppers. The proportion of metacercariae lost at the population level, i.e. including all groups of cockles, can also be estimated. In order to be meaningful the estimate should include also the proportion of regenerated tissue that constitutes a substantial part of the foot of many of the cockles. Because regenerated tissue represents a part of the foot that in the recent past has been lost to cropping it contains relatively few metacercariae, and those missing in comparison to the surrounding unaffected tissue hence represent an additional loss to cropping. Based on a significant negative regression between cropping-corrected parasite loads (N_c) and the proportion of regenerated tissue in the foot ($F_{1,50} = 21.23$, $P < 0.0005$), the number of parasites expected in feet without regeneration and with 17.4% regeneration, the latter being the observed cockle group grand mean (see Table 1), can be calculated. The ratio between these two values suggests that on average 40.2% of metacercariae are missing in feet with regeneration in comparison to feet without regeneration. Hence, based on the observed mean parasite load, regeneration frequency and mean density of cockles in the three cockle groups (see Fig. 3 and Table 1), an estimated 14.5% of the standing stock of *Curtuteria* metacercariae is lost due to sublethal predation on the cockle's feet.

The underwater recordings showed unequivocally that the Spotty, *Notolabrus celidotus* (Pisces: Labridae), is quantitatively the most important cropper of cockle feet. Except for a single passing Barracouta (*Thyrsites atun*), Spotties were the only fish species recorded. The first Spotty arrived after 4 min of recording, and from then on, up to 18 individuals patrolled and fed on the bed of surfaced cockles. On average, 4.2 feeding attempts per minute were recorded on the cockle bed, and on several occasions the attacked cockles were temporarily lifted from the seabed. In contrast, a maximum of five patrolling Spotties were observed over the adjacent cockle-free area, and only three feeding attempts towards the bottom were seen, corresponding to 0.063 attempts per min on an average. These figures clearly demonstrate that feeding Spotties are strongly attracted to surfaced cockles.

4. Discussion

The tip of a cockle's foot is of critical importance to the cockle's ability to bury as it allows the foot to anchor in the substrate (Mouritsen, 2002). Because the Spotties are targeting the tip of the cockle's feet, such sublethal predation is likely to leave attacked animals on the sediment surface, unable to rebury. Foot cropping occurs on all tidal flats hitherto studied in Otago Harbour (Mouritsen and Poulin, 2003), and since the phenomenon is associated with surfaced and short-footed cockles, it seems at first glance to weaken the parasite manipulation hypothesis predicting such cockles to be the result of trematode infections (Thomas and Poulin, 1998). Foot cropping, and not parasites, could be the very mechanism forcing cockles to remain exposed on the sediment surface. However, the available data suggest that foot cropping is the consequence rather than the reason for surfacing in cockles. First, the cropping frequency as well as the density of surfaced cockles is positively related to the infection intensity of cockles in general. Secondly, the cropping-corrected infection intensity of surfaced cockles is significantly higher than in buried individuals. Finally, the population of surfaced cockles includes a fraction of uncropped, short-footed and heavily infected individuals in which foot-size correlates negatively with parasite load. So, foot croppers appear to specifically attack heavily infected, surfaced individuals. This scenario takes place not because the parasites directly force cockles to the surface and expose their feet, but because of a combined effect of disturbance and reduced performance of the cockle's foot due to *Curtuteria* infections. Cockles regularly relocate to escape disturbance of their site of burial due to, e.g. bioturbation or coverage by macroalgae (Mouritsen, unpublished). Whereas relocation does not pose a problem to lightly infected cockles, heavily infected specimens are hampered from reburying after they have surfaced because of a smaller foot and reduced ability to anchor the foot in the sediment as well as to contract it (Thomas and Poulin, 1998; Mouritsen, 2002; this study). Such cockles will be particularly susceptible to attack from Spotties because of their repeated unsuccessful attempts to bury. In effect 17% of the parasite load of manipulated (surfaced) cockles are lost during cropping resulting in a similar infection level among surfaced and buried bivalves.

Cropped cockles are able to regenerate lost foot tissue and hence regain their burying ability. Depending on the magnitude of the damage this may take up to 8 weeks, but on an average 18 days (Mouritsen, unpublished). Since *C. australis* accumulate at a relatively low rate of about 0.5 metacercariae cockle^{-1} day^{-1} (Mouritsen, 2002), the number of new infections acquired during the 18-day period will be insufficient to keep the regenerated foot immobilised and the cockle will eventually rebury. This suggests that the cockle population is engaged in a behavioural cycle in which metacercariae accumulate in buried individuals until they eventually surface, become cropped, followed by regeneration and reburial. The high proportion of cockles that show sign of foot regeneration irrespective of behavioural group (see Table 1) clearly supports this. It follows that interface cockles are composed of two distinct groups of individuals: those on their way down that recently have regenerated their foot and partly regained burying ability (therefore the particularly high regeneration frequency in interface cockles), and heavily infected individuals on their way up (due to the parasites) with little or no longer detectable tissue regeneration.

Because metacercariae accumulate at a similar rate in surfaced and fully buried cockles (Mouritsen, 2002), surfaced individuals unable to rebury due to a critically high parasite load are, in the absence of foot croppers, likely to accumulate on the sediment surface. However, in the presence of foot croppers that not only depress the parasite load of manipulated cockles but via the regeneration potential of the foot also reverse the manipulation process, the density of surfaced cockles will tend to decline. Since surfaced cockles are preferentially preyed upon by the definitive bird hosts (Thomas and Poulin, 1998; Mouritsen, unpublished), this could prove an important additional factor reducing the overall transmission rate of larval trematodes beside the direct loss to fish, because it may lower the number of definitive hosts choosing to feed in the habitat, or make those present focus on alternative prey (see e.g. Bryant, 1979; Goss-Custard et al., 1992; Maagaard and Jensen, 1994; Ens et al., 1996; Meire, 1996).

Foot cropping is not the only risk trematodes have to face in manipulated cockles. Mud whelks *Cominella glandiformis* that serve as first intermediate hosts to *C. australis* also happen to prey preferentially on surfaced cockles (Mouritsen, unpublished). Based on the daily predation rate and the average residence time of 18 days on the surface until full burying ability is regained (Mouritsen, unpublished), an estimated 3.7% of the metacercariae harboured by manipulated cockles are lost to whelks. However, since these snails can also act temporarily as paratenic hosts this amount may be slightly overestimated (see McFarland et al., 2003). A similar calculation based on the predation rate on surfaced cockles by shorebirds (Mouritsen, unpublished), suggests that 2.5% of the metacercariae are transmitted successfully. So, the loss to non-host predators directly profiting from the parasitic manipulation is more than eightfold higher than the amount of metacercariae transmitted to the definitive hosts.

The above account, summarised in Fig. 4, raises the question of whether manipulation of host behaviour is a worthwhile transmission strategy for *C. australis* in the presence of foot croppers. To answer this thoroughly, a population dynamic model of the parasite's life cycle has to be elaborated. However, based on the available information, the answer depends critically on the feeding behaviour of the oystercatcher definitive host. Depending on habitat, for instance, oystercatchers often specialise on a certain prey species (Dare and Mercer, 1973; Jones, 1983; Boates and Goss-Custard, 1992; Coleman et al., 1999), and in cases where the bird's daily energy requirement is met almost exclusively by cockles, presence of foot croppers becomes critical for the parasites. Because the mean parasite load does not differ between the three behavioural groups of cockles (degree of burying), the transmission rate of the parasites will be similar no matter what group is preferred by the birds. Moreover, because the overall parasite population is reduced (about 14.5%) due to the action of the foot croppers, their presence will inevitably result in a decreased transmission rate in comparison to a situation where croppers are absent. Under the assumption that the transmission rate between the intermediate and definitive host is critical for the parasite's population dynamics, there would be selection against manipulation in effect, i.e. against infecting the cockle's foot tissue rather than other tissues. However, in case the birds subsist on a diet of, say, polychaetes that is topped up by easily accessible cockles at a rate proportional to their abundance, the prospects of the manipulating parasites are very different. The predation pressure on surfaced cockles is five to sevenfold higher than on buried specimens (Thomas and Poulin, 1998; Mouritsen, unpublished), suggesting that cockles lying on the sediment surface are very easy prey. So, although a substantial proportion of the trematodes will be lost to croppers by forcing the cockles to the surface, the fact that they actually are there will ensure a larger fraction of cockles in the birds' diet. Under this scenario, behavioural manipulation of cockles will increase the parasite's transmission rate no matter what proportion is lost to non-host predators.

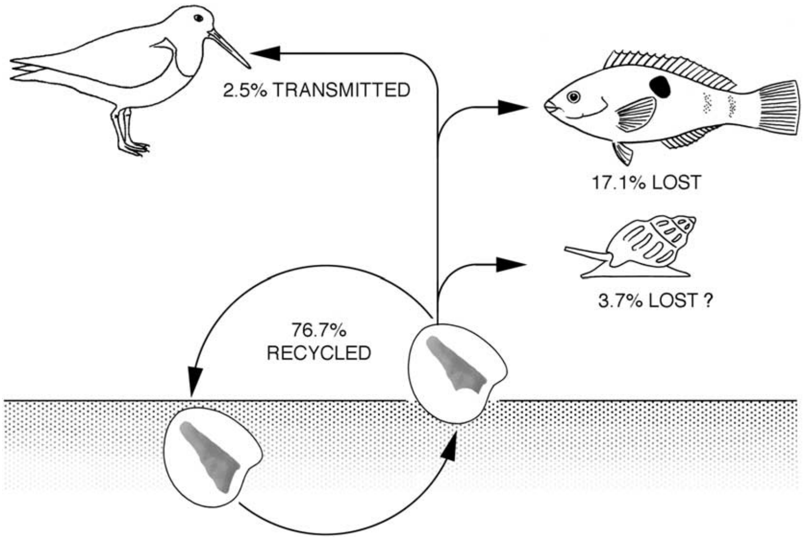

Fig. 4. The fate of metacercariae of *C. australis* infecting surfaced cockles in Company Bay, Otago Harbour, South Island, New Zealand. The proportions of the parasite population that are transmitted successfully to shorebird definitive hosts (e.g. oystercatchers), lost to foot-cropping fish (Spotties) and whelks, or recycled as a consequence of regained burying ability, are shown. Note that since whelks can act as paratenic hosts, metacercariae ingested by these snails cannot all be considered lost. From top to down: oystercatcher *Haematopus ostralegus*, Spotty *N. celidotus* (wrasse), whelk *C. glandiformis*, outline of foot-cropped cockle *A. stutchburyi* on the sediment surface, outline of buried cockle with regenerated foot-tip. See text for further details.

The first of the above two scenarios may apply to, for instance, the Avon-Heathcote Estuary some 350 km north of Otago Harbour. There, cockles comprise 90–95% of the oystercatcher's diet (Jones, 1983), and behavioural manipulation will in the presence of foot croppers be a bad transmission strategy. In Otago Harbour, however, our own direct observations of the oystercatchers feeding behaviour suggest that the second scenario may apply, and the parasites appear here to be engaged in a high risk, high profit transmission strategy by manipulating their intermediate host.

Overall, our results provide the first field measurements of the relative costs and benefits of parasite manipulation. Our findings suggest that manipulation can result in predation by non-hosts, likely with important consequences for the parasite's population dynamics as well as for the selective advantage of manipulation.

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