

Equal partnership: two trematode species, not one, manipulate the burrowing behaviour of the New Zealand cockle, *Austrovenus stutchburyi*

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Abstract

Metacercariae of the trematode *Curtuteria australis* (Echinostomatidae) accumulate in the foot of the New Zealand cockle *Austrovenus stutchburyi*, severely impairing the cockle's ability to burrow under the sediments. This results in increased predation by birds on cockles, and thus enhanced transmission rates of the parasite to its bird definitive hosts. This host manipulation by the trematode is costly: fish regularly crop the tip of the foot of cockles stranded on the sediment surface, killing any metacercariae they ingest. A second, previously undetected trematode species (characterized by 23 collar spines) co-existing with *C. australis*, has been found in the foot of cockles in the Otago Harbour, South Island, New Zealand. The relative abundance of the two species varies among localities, with the identity of the numerically dominant species also changing from one locality to the next. Both *C. australis* and the new species have a strong preference for encysting in the tip of the cockle's foot, where their impact on the burrowing ability of the host is greatest, and where they both face the risk of cropping by fish. Results indicate that these two species are ecological equivalents, and their combined numbers determine how the cockle population is affected.

Introduction

The parasitology literature of the past 30 years contains numerous examples of intermediate hosts displaying altered behaviour following infection by larval helminth parasites, with the modified behaviour often facilitating the transmission of the parasites to their next host (see reviews in Combes, 1991, 2001; Poulin, 1995, 1998; Moore, 2002). Helminth species known to be capable of manipulating the behaviour of their intermediate hosts regularly coexist with other helminth species, forming packets of infective worms acquired simultaneously by any predator ingesting the intermediate host (Bush *et al.*, 1993). These situations raise interesting questions. For instance, if two helminth species share two consecutive

hosts in their life cycle and are transmitted from one to the other by predation, have they both evolved the ability of manipulating the behaviour of the first host? Or is one doing all the work, with the other being a hitch-hiker, getting all the benefits at no cost? To date, these questions have only been addressed in a few host–parasite systems (see Thomas *et al.*, 1997, 1998; Lafferty *et al.*, 2000; Mouritsen, 2001), despite how important they are for our understanding of the evolution of parasite transmission strategies.

The trematode *Curtuteria australis* (Echinostomatidae: Himasthlineae) is a common parasite of the New Zealand cockle, *Austrovenus stutchburyi* (Allison, 1979). Its ability to modify the behaviour of the cockle is well documented: metacercariae of *C. australis* accumulate in the foot of cockles, where they impair the normal burrowing abilities of cockles (Thomas & Poulin, 1998; Mouritsen, 2002). Heavily infected cockles are left stranded on the sediment surface, where they face increased predation by

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oystercatchers, the parasite's definitive host (Thomas & Poulin, 1998). This manipulation is thus advantageous for the parasite, but it also comes with a cost: fish regularly crop the foot of cockles lying on the sediment surface and trying to burrow, resulting in the death of any metacercariae they ingest (Mouritsen & Poulin, 2003a,b). The tip of the foot is especially vulnerable to cropping; it is also where metacercariae are expected to encyst if they are to interfere effectively with the cockle's ability to burrow (Mouritsen, 2002; Mouritsen & Poulin, 2003a). Thus the manipulation of the cockle's burrowing behaviour is a two-edged sword: on the one hand it increases the probability of transmission to birds, while on the other it increases the probability that metacercariae will end up in fish, where they cannot develop.

This costly manipulation has so far been attributed to *C. australis*, the only echinostome described from the cockle *A. stutchburyi*. However, two recent observations suggest that there may be a second, hitherto unknown, echinostome species sharing cockles with *C. australis*. First, the distribution of the diameters of metacercarial cysts in cockles often appears to be bimodal (Mouritsen, unpublished data). This is not what we would expect if there were only one species infecting cockles. Second, echinostome infections are commonly found in the mud snail *Zeacumantus subcarinatus* (Batillariidae) (B.L. Fredensborg, personal communication), whereas the first intermediate host of *C. australis*, in which cercariae are produced prior to infecting cockles, is the whelk *Cominella glandiformis* (Buccinidae) (Allison, 1979). The two snail species are sympatric throughout our study area, and the cercariae they release have been found encysted only in cockles. Given the high specificity of trematodes for their first intermediate host (Adema & Loker, 1997), cercariae issued from the two different snails are unlikely to be conspecifics. The possibility that two related echinostome species share the same second intermediate host (cockles) and most likely the same avian definitive hosts suggests some interesting scenarios. Are the two species equally contributing to the manipulation of the cockle host, and thus facing the same risk of ending up in a foot-cropping fish? Or is one of them encysting away from the tip of the foot, hitch-hiking a safe ride toward bird hosts at the expense of the other species?

The objectives of this study were: (i) to determine whether there are indeed two distinct echinostome species infecting cockles and, if so, how to distinguish between them; and (ii) to quantify the respective distributions of the two species within the tissues of the cockles, to see whether they are equally likely to be encysted in the tip of the cockle's foot.

Materials and methods

Large numbers of whelks, *Cominella glandiformis*, and mud snails, *Zeacumantus subcarinatus*, were collected at low tide in January 2003 from mudflats in the Otago Harbour, South Island, New Zealand. They were returned to the laboratory and kept individually for several days in small plastic containers filled with seawater, under constant illumination and at room temperature. The containers were checked daily for live echinostome cercariae used for morphological measurements. Live,

mature cercariae were placed in a droplet of seawater on a microscope slide and, under coverslip pressure, were measured individually using a compound microscope. For each cercaria, the number of collar spines, body length, head width, body width, tail length (all at 40× magnification), oral sucker diameter and ventral sucker diameter (the latter two at 100× magnification) were recorded. Data were obtained for 50 cercariae shed by whelks (10 cercariae from each of 5 infected whelks), and 51 cercariae shed by mud snails (6–10 from each of 6 infected snails).

Cockles (*Austrovenus stutchburyi*) were also obtained from mudflats in the Otago Harbour. Thirty randomly-chosen metacercarial cysts were carefully dissected from the foot of 8 cockles (for a total of 240 metacercariae), and their diameter was measured under the microscope (30× magnification). Cysts were then placed under conditions simulating those inside a bird digestive tract, i.e. in Petri dishes in an excystation solution consisting of 5 ml of bicarbonate saline (0.8% weight/volume sodium chloride and 1.5% w/v sodium bicarbonate) containing 0.8% w/v sodium taurocholate and 0.3% w/v trypsin, and 5 ml 0.02 M hydrochloric acid containing 0.8% w/v L-cysteine (Irwin *et al.*, 1984; Irwin, 1997). Metacercariae were left in the solution for 1 h at 40°C and then examined under the microscope. Their collar spines were counted, and the range of cyst diameters corresponding to different numbers of collar spines was determined.

Further cockles were collected for the purpose of comparing the distribution of the two species of metacercariae in the foot of cockles. Cockles were taken from three sites within the Otago Harbour that differed with respect to intensities of infection: Otakou (fewer than 50 metacercariae per cockle on average), Lower Portobello (around 200 metacercariae per cockle), and Harwood (around 400 metacercariae per cockle). Three cockles were dissected from each site. The foot of each cockle was cut into three sections: the tip, middle and hind parts of the foot. The relative surface areas of each section, determined by projecting the outline of the foot on graph paper, were 27.3%, 30.6% and 42.1% of the total foot area, respectively. Based on mass (after weighing foot sections separately to the nearest 0.0001g), the relative sizes of the tip, middle and hind sections were 26.2%, 37.9% and 35.9% of the total foot mass, respectively. The number of metacercariae of each species in each foot section was counted; the two species were distinguished based on the diameter of their metacercarial cysts and on collar spine counts following excystation. To obtain accurate counts, all foot sections were digested separately in a pepsin solution (6 g pepsin and 7 ml HCl in 1000 ml water) at 40°C for 4 h. This procedure allows the recovery and count of all metacercariae under the microscope, including those not visible because they are deeply embedded in the foot tissue (Lepitzki *et al.*, 1994). In addition, the remaining soft tissues were scraped off the cockle shells, placed in a container with digestive fluid, and any metacercariae recovered were identified and counted. The relative frequencies of metacercariae of the two species in different cockle body parts (tip of the foot, middle of the foot, hind part of the foot, or rest of the body) were compared using a contingency table analysis (Chi-squared test).

Results

Cercariae shed by the whelk *Cominella glandiformis* all had 31 collar spines and, despite being slightly larger in all body dimensions than the ones described by Allison (1979), they corresponded to *Curtuteria australis* (table 1). In contrast, all cercariae which emerged from the mud snail *Zeacumantus subcarinatus* displayed 23 collar spines and were generally smaller than *C. australis* (table 1). These 23-spine cercariae represent an undescribed species.

Metacercariae with 23 collar spines that were obtained by excystation had cyst diameters ranging between 200 and 250 μm (mean 235 μm). Metacercariae with 31 collar spines, i.e. those of *C. australis*, had cysts ranging between 267 and 333 μm in diameter (mean 285 μm). There may have been some overlap between these ranges had more metacercariae been examined, but it would appear to be negligible. For the counts described below, we thus considered metacercarial cysts with a diameter of 250 μm or less to be the 23-spine species, and those with a diameter of 267 μm or more to be *C. australis*.

At the Otakou site, overall intensities of infection were low, and neither species showed a marked numerical dominance over the other. Metacercariae of both species showed a preference for the tip of the foot, and no metacercariae were found outside of the foot (fig. 1). There was no difference between the distributions of the two species among cockle tissues ($\chi^2 = 1.663$, $df = 2$, $P = 0.4354$). If we consider only the tip of the foot versus all other sites of encystment pooled together, there is also no difference between the distributions of the two species ($\chi^2 = 0.171$, $df = 1$, $P = 0.679$).

At the Lower Portobello site, intensities of infection were higher and the 23-spine species was twice as abundant as *C. australis*. The two species differed in their distribution among cockle tissues at this site ($\chi^2 = 109.608$, $df = 3$, $P = 0.0001$). Both species were more frequent in the tip of the foot than elsewhere; however, whereas most remaining metacercariae of the 23-spine species were found in the middle of the foot, a large proportion of remaining *C. australis* metacercariae occurred outside the foot, in other cockle tissues (fig. 1). A closer look revealed that some of these *C. australis* metacercariae were lodged in the siphon and some in the mantle edge, but that the majority were found in the other tissues. If we consider only the tip of the foot versus all other sites of encystment pooled together, the difference

between the distribution of the two species becomes less pronounced ($\chi^2 = 5.427$, $df = 1$, $P = 0.0198$).

Intensities of infection were highest at the Harwood site, and *C. australis* was approximately 10 times as numerous as the 23-spine species at this site. Again, the distribution of the two species among cockle tissues proved to be different ($\chi^2 = 52.101$, $df = 3$, $P = 0.0001$). The pattern was very similar to that at Lower Portobello: the 23-spine species was found mainly in the tip of the foot, followed by the middle section of the foot, whereas for *C. australis* the largest proportion of metacercariae, after the tip of the foot, was found outside the foot, i.e. in the rest of the cockle's body (fig. 1). However, if we consider only the tip of the foot versus all other sites of encystment pooled together, the difference between the distribution of the two species disappears ($\chi^2 = 0.983$, $df = 1$, $P = 0.3775$).

The preference of both trematode species for the tip of the foot becomes slightly more pronounced if we correct the frequencies of metacercariae found in each of the three foot sections for their relative sizes. For instance, pooling all three collection sites, 870 *C. australis* metacercariae were found in the foot of cockles, 78.05% of which were in the tip of the foot; if we weigh this value for the proportion of total foot mass accounted for by the tip of the foot, it becomes 83.5%. Similarly, of 553 metacercariae of the 23-spine species found in the foot of cockles, 55.5% were in the tip of the foot without correction for the relative mass of the tip, and 64.1% after correction.

Discussion

Bivalves commonly serve as second intermediate hosts for more than one trematode species. For instance, the European cockle, *Cerastoderma edule*, harbours metacercariae of many trematodes, including several species of the echinostome genus *Himasthla* (Lauckner, 1983). In such situations, different parasite species may have different impacts on the host, and may play different ecological roles. The results of the present study clearly show that metacercariae found in the foot of the New Zealand cockle, *Austrovenus stutchburyi*, do not represent a single species of echinostome as previously believed, but instead consist of a mixture of two species. Both the 23-spine species from the mud snail *Zeacumantus subcarinatus*, and *Curtuteria australis* from the whelk *Cominella glandiformis*, use cockles as their second intermediate host where they encyst in the foot. The present data show that both species are located

Table 1. Morphological comparison (mean, range in parentheses) of cercariae of the unnamed 23-spine echinostome shed by the mud snail *Zeacumantus subcarinatus* and of *Curtuteria australis* shed by the whelk *Cominella glandiformis*.

Variable	23-spine species (present study)	<i>Curtuteria australis</i> (present study)	<i>Curtuteria australis</i> (from Allison, 1979)
Number of collar spines	23	31	31
Body length (μm)	675 (380–975)	1150 (709–1443)	690 (520–1130)
Head width (μm)	184 (127–228)	251 (203–304)	Unavailable
Body width (μm)	278 (203–365)	413 (304–506)	260 (150–340)
Tail length (μm)	396 (203–684)	609 (405–962)	490 (unavailable)
Oral sucker diameter (μm)	64 (51–81)	86 (81–91)	50 (unavailable)
Ventral sucker diameter (μm)	115 (81–142)	170 (142–213)	150 (unavailable)

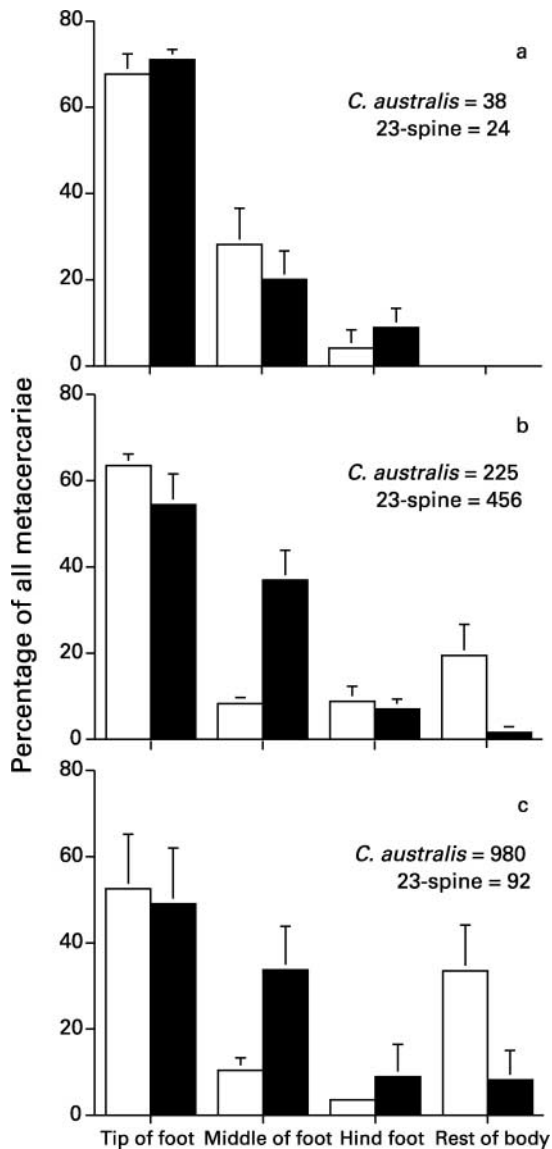


Fig. 1. Percentage (mean \pm SE) of metacercariae of two echinostome species, *Curtuteria australis* (open bars) and the new 23-spine species (black bars), found in four body regions of cockles, at three sites (a, Otakou; b, Lower Portobello; c, Harwood) in the Otago Harbour, New Zealand. The data are from three cockles per site; total numbers of metacercariae of both species are given in the figure.

predominantly in the tip of the cockle's foot, occurring there in similar proportions compared to other parts of the cockle. This suggests that both species contribute about equally to the manipulation of the host, and that, on average, they both face identical risks of being ingested by a fish via foot-cropping. This means that the two species are ecologically equivalent in this system, and that for all practical purposes, using the total number of metacercariae per cockle, regardless of what species they belong to, can still serve as a measure of the impact of the trematodes on cockle predation by birds or other consequences of infection.

We compared the relative distribution of the two echinostome species among different locations in the foot and in the rest of the cockle. Looking instead at the numbers of metacercariae per gram of tissue would have yielded similar results. It is interesting to note, however, that because of the way in which the foot of cockles was sectioned, the tip section was smaller (both in terms of projected surface area and with respect to mass) than either the middle or hind sections. This means that the disproportionately high counts of metacercariae of both species in the tip of the foot reflects a strong preference by both parasites for this part of the host's body, as seen following a correction for the relative mass of different foot sections. The frequency of encystment in tissues other than the tip of the foot tends to increase with increasing total parasite load. Approximately 70% of metacercariae are found in the tip of the foot at Otakou, where intensities of infection are low, whereas only about 50% are found in the tip at Harwood, where intensities are much higher (fig. 1). One possibility is that both echinostome species have evolved a strong preference for the tip of the foot, only settling elsewhere as a consequence of crowded conditions. However, there is no evidence of density-dependent constraints in this system, and, even in highly-infected cockles, much of the volume of the tip of the foot remains available for further metacercariae. A second explanation may be that the parasites settle away from the tip of the foot when the latter is missing. Foot-cropping fish remove the tip of the foot in many cockles, especially in the most heavily-infected ones (Mouritsen & Poulin, 2003a,b), and metacercariae arriving in these cockles before the tip is regenerated would have to establish elsewhere.

The small apparent difference in the distribution of metacercariae of the two echinostome species among cockle tissues, observed at the two sites with highest intensities of infection, may in fact be less pronounced than the present results suggest. There may be a third echinostome species encysting mainly outside of the foot. Metacercariae recovered from tissues outside of the foot were often at the upper end of the range of diameters associated with *C. australis* cysts. In one of them, 36 collar spines were counted, and not the 31 expected from *C. australis*. It may thus be that some of the metacercariae found outside of the foot, and here classified as belonging to *C. australis*, are in fact members of a third species. If this is indeed the case, the true distributions of *C. australis* and of the 23-spine species among foot sections and the rest of the cockle's body may be rather similar.

Our measurements of cercarial dimensions for *C. australis* differ somewhat from those reported by Allison (1979). Considerable variation was observed among cercariae of both *C. australis* and the 23-spine species according to which snail they came from, with some snails consistently producing larger cercariae than others. We believe this explains the differences between our measurements and those of Allison (1979).

The newly-discovered 23-spine species is likely to be subjected to other processes previously thought to apply only to *C. australis*. For instance, predation by anemones has been identified as a significant source of mortality for cercariae leaving their snail host in search of cockles (Mouritsen & Poulin, 2003c). The anemone *Anthopleura*

aureoradiata is often found attached to the shell of cockles, reaching high abundances in some places; its presence results in lower numbers of cercariae successfully infecting cockles. Also, whelks, *C. glandiformis*, are active predators of cockles (Ansell, 2001). Metacercariae ingested by whelks as the latter feed on cockles can survive up to three days inside whelks before passing out in the faeces (Mcfarland *et al.*, 2003). Although whelks may serve as paratenic (transport) hosts because shore-birds occasionally feed on them, the short residence time of metacercariae inside whelks means that this acts instead as a source of considerable losses for the trematodes. The present study suggests that losses caused by anemones or whelks are incurred equally by both *C. australis* and the 23-spine species. In all respects, the two echinostome species appear to play identical roles in the New Zealand soft-sediment ecosystem.

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