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SUBSTRATUM PREFERENCE OF *PHILOPHTHALMUS* SP. CERCARIAE FOR CYST FORMATION UNDER NATURAL AND EXPERIMENTAL CONDITIONS

Allison T. Neal and Robert Poulin*
Department of Biology, University of Vermont, Burlington, Vermont 05405. *e-mail: aneal@uvm.edu*

ABSTRACT: Selection on parasites should favor adaptations that maximize the probability of transmission to the definitive host, such as the preference for and use of intermediate hosts or encystment substrata that are likely to be consumed by the definitive host. Eye flukes in the genus *Philophthalmus* are passed to their definitive avian host through the ingestion of metacercariae encysted on hard substrata. The life cycle of these parasites is generally well understood; however, there is almost no information on substratum use or preference of the cercariae of these parasites. In this study, we combine a survey of naturally occurring substrata with experimental, laboratory-based choice tests to determine the preferred substratum of *Philophthalmus* sp. and whether this preference is affected by the presence and density of pre-existing cysts. A concordance between natural and experimental data show a preference for the shells of multiple species of snail over other hard substrata that are common at the field site, including seaweed, other molluscs, and crustaceans. In addition, we found that cercariae preferred substrata with pre-existing cysts and that this preference seemed to increase with increasing cyst density. Such a preference should lead to an aggregated distribution of cysts among snail shells that may benefit the parasite by increasing the number of potential mates that become established in the definitive host. The identification of a preferred substratum also may help to identify potential definitive hosts that were previously unknown.

In parasites with complex life cycles, natural selection should favor a preference for, and a disproportionate use of, the few host species that maximize parasite reproduction or transmission. For example, in trematodes, cercariae released from the snail first intermediate host should preferentially infect the 1, or a few, second intermediate host species in which subsequent transmission to the definitive host is most likely. The same is true of trematode taxa, like species of philophthalmids, whose cercariae encyst on external substrata instead of inside a second intermediate host. Adult flukes in this family are typically found in the eye orbits of birds, and eggs passed in the tears hatch in water. Miracidia inject the preformed redia stage into the gastropod intermediate host and, after multiple redia generations, cercariae are released. Cercariae encyst on hard substrata, which are consumed by birds to complete the life cycle (Nollen and Kanev, 1995).

Despite the fact that the general life cycle of species of *Philophthalmus* is fairly well understood (Nollen and Kanev, 1995), little is known about the substratum preference of the cercariae. In particular, several papers mention that the cercariae encyst on hard substrata (Alicata, 1962; Nollen and Kanev, 1995), but we were only able to find 1 paper mentioning a specific substratum (West [1961] reported that cercariae of *P. gralli* generally encyst on the exoskeleton of arthropods, especially crayfish, although he did not give a source for this information).

We therefore aimed to determine the substrata used by a species of *Philophthalmus* in New Zealand, where the parasite has been identified from mudsnails (*Zeacumantus subcarinatus*) (Howell, 1965; Martorelli et al., 2008) and infects up to 30% of snails in some areas (Martorelli et al., 2008). Its cercariae are strongly positively phototactic (R. Poulin, personal observation) and readily encyst on glass or plastic surfaces in the laboratory (Lei and Poulin, 2011), but which substrata it uses in nature is unknown. In addition to helping us understand whether the parasite has a strong preference for certain substrata, knowing which substrata are used by the parasite could help identify likely definitive hosts based on their dietary preferences. Parasites in this genus seem not to be overly host specific in their definitive hosts (Thakur and Cheng, 1968), so we may not be aware of the full extent of bird species parasitized by these trematodes. Howell (1965) reported eye flukes in black-backed gulls (*Larus dominicanus*) in the Wellington, New Zealand, area, but it is unclear whether this is the only avian host used by this parasite. The specific objectives of this study were to (1) identify encystment substrata used in nature and determine which harbor the most cysts relative to their surface area; (2) test for substratum preference by cercariae in choice tests performed under controlled laboratory conditions; and (3) determine whether substratum choice is density-dependent, i.e., whether the density of preexisting cysts influences the substratum choice of cercariae.

MATERIALS AND METHODS

Survey of naturally occurring cysts

Potential substrata were obtained at low tide from Lower Portobello Bay, Otago Peninsula, New Zealand (45°49′56.26″S, 170°40′21.69″E) in late June to early July 2011. This site was selected because of the parasite's high prevalence at this location. In a sample of 523 *Z. subcarinatus* collected at the site concurrently, approximately 17% were infected with *Philophthalmus* sp. Eleven substrata were selected for a survey of naturally occurring cysts. These substrata comprise practically all common natural surfaces, living or non-living, available for encystment; they included spotted topshell (*Diloma aethiops*), mudflat topshell (*D. subrostrata*), mudflat whelk (*Cominella glandiformis*), uninfected New Zealand mudsnails (*Z. subcarinatus*), New Zealand cockle (*Austrovenus stutchburyi*, both live individuals and empty shells), wedge shells (*Macomona liliana*, empty shells only), eelgrass (*Zostera capricorni*), sea lettuce (*Ulva* sp., probably *Ulva lactuca*), small rocks, and 2 crab species (Brachyura, *Hemigrapsus crenulatus* and *Macrophthalmus hirtipes*). Approximately 30 individuals of each substratum (or an amount of sea lettuce or eelgrass of comparable surface area) were collected haphazardly across the mid- to upper intertidal zone; they were transported in seawater to the laboratory, where they were examined using a dissecting microscope for presence and abundance of *Philophthalmus* sp. cysts. For any substrata on which cysts were found during the preliminary survey (with the exception of crabs), an additional 30 individuals were collected and scanned to improve our estimate of the prevalence and abundance of cysts on that substratum. Due to the difficulty of gently restraining live crabs, only their carapace and the base of their legs were examined thoroughly, although the legs and claws also were scanned when possible. Because it was impossible to determine which individual mudsnails were infected while in the field, hundreds were collected and transported back to the laboratory in individual containers. Uninfected individuals were identified by placing each snail in a well of a 12-well polystyrene plate with approximately 5 ml

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* Department of Zoology, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand.
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TABLE I. Cysts found on substrates collected from the field.*

Substrate	Examined	With cysts	Total cysts	Max cysts	Max dim. (mm)	Total SA (cm^2)
Diloma subrostrata	69	32	51	4	16–23	614
D. aethiops	67	29	51	5	18–24.5	735
Cominella glandiformis	68	9	30	8	15–22	167
Zeacumantus subcarinatus	61	15	23	5	9–15	36
Brachyura	30	1	1	1	10–20	100
Austrovenus stutchburyi (live)	32	0	0	0	21–31	471
A. stutchburyi (shell)	30	0	0	0	20–39	1,174
Macomona liliana (shell)	31	0	0	0	20–27	637
Rock	30	0	0	0	23–42	633
Zostera capricorni	—	0	0	0	—	461
Ulva sp.	—	0	0	0	—	827

* "Examined" and "with cysts" are, respectively, the number of individuals of each substrate that were scanned for and found with *Philophthalmus* sp. cysts. "Total cysts" and "max cysts" are the total number of *Philophthalmus* sp. cysts found on all individuals of that substrate combined and the maximum number found on any one individual, respectively. "Max dim." gives the range in length of the longest dimension of the individuals of each substrate scanned. "Total SA" is the sum of the areas of all individuals of the substrate. See Materials and Methods section for common names of substrate species.

of seawater and maintaining them at 25 C with constant illumination for at least 5 hr to promote cercariae shedding (Fredensborg et al., 2004). Mudsnails that failed to release *Philophthalmus* sp. cercariae during this period were considered uninfected and were scanned for cysts immediately.

The surface area of individual items from each substratum type was estimated by selecting regular geometric shapes that approximated the shape of the substratum: the 2 topshell species were approximated by hemispheres, the whelk by 2 cones stuck bottom to bottom, the mudsnail by a single cone, clams and cockles by fractions of circles, and pieces of seaweed and crab carapaces by rectangles. The surfaces of rocks were split into circles, ovals, rectangles, and triangles for surface area estimation.

The total number of cysts on each substratum and the total scanned surface area of each substratum were calculated. A chi-square test compared the cyst counts from each substratum, with the number expected based on the surface area available for cyst formation.

Experimental substratum choice tests

After field-collected substrata were scanned, cysts were individually scratched off, when present, to provide cyst-free substrata for choice tests. Choice tests were performed in 13-cm-diameter circular plastic containers with 380 ml of water and approximately 0.5-cm-deep mud, both collected from the field site. The mud was added to prevent cercariae from encysting on the bottom of the container. Due to the limited space available in the container, 8 substrata were chosen for the choice tests, i.e., single individuals of both species of topshell, the whelk, the mudsnail, the cockle (alive and shell only), the eelgrass (25 cm total length), and 1 rock.

Two choice test experiments were performed, each with 10 replicates. In the first, a small plastic string was glued (Selley's Quick Fix Supa Glue, NSW, Australia) to each substratum with a short (~2-mm) section of longitudinally halved toothpick as an anchor. The other end of the string was used to tether the mobile substrata with tape at evenly spaced points around the edge of the circular container such that every substratum was kept approximately the same distance from the center and from other substrata. The positions of the substrata were randomized to ensure that the results were not biased by the substratum's location or proximity to other substrata. Shedding of cercariae from mudsnails infected with *Philophthalmus* sp. was induced in 12-well plates using the conditions detailed above. Approximately 50 cercariae originating from at least 5 different snails were pipetted into the center of each container. Containers were left under constant overhead illumination at room temperature for at least 24 hr, sufficient time to allow all cercariae to encyst. Each substratum was then scanned again thoroughly using a microscope, and the number of cysts present was noted.

In the second choice test experiment, substrata were not tethered, and snails were allowed to move freely around the container. If snail activity affects the site chosen by cercariae for cyst formation (such as mudsnails and substrate species sharing habitat preferences or behavioral patterns), such a design might lead to a different distribution of cysts than the tethered test described above. Non-mobile substrata (i.e., empty cockle shells, eelgrass, rocks) were placed near the center of the container. Infected mudsnails (1 per container) were used as a source of cercariae in this experiment, and containers were placed in an incubator at 19 C (a cooler temperature than generally used for inducing cercariae shedding to reduce stress on live substrata) with constant illumination (to maximize cercariae shedding) for 6 days. At the end of the 6 days, the infected mudsnails were removed, and the number of cysts on each substratum was counted.

For each experiment, the total surface area of, and number of cysts formed on, each substratum was determined. A chi-square test compared the number of cysts on each substratum with the number expected based on the surface area of each substratum.

Density and substratum choice

To examine the effect of pre-existing cyst density on substratum choice of cercariae, 2 substrata of the same type were paired in an arena, 1 with cysts already attached to it and 1 without. We selected 2 substrata for this experiment, mudsnails and whelks. Preliminary results suggested that both were substrata favored by the cercariae, and their small size relative to the topshells reduced the chances of errors in counting the number of cysts initially present. Thirty individuals of each species were selected for the experiment and naturally occurring cysts, if any, were removed. Conspecific snails were paired by size, and 1 of each pair was assigned to a group that would have cysts at the beginning of the experiment, whereas the other was assigned to a group that would not. Both groups were placed in sets of 3 in 200-ml round containers with 23-cm^3 mud (~0.5 cm in depth) and seawater at 19 C with constant illumination for 5 days. Each container that held snails designated to have pre-existing cysts also housed an infected mudsnail for this time.

At the end of the 5 days, the number of cysts on each snail was counted and recorded, and the snails with cysts were tethered (as described above) with their size-matched cyst-free counterpart to opposite sides of a 200-ml round container with 23-cm^3 mud and seawater. The side of the container (with respect to the room) occupied by the cyst-free individual alternated in every container. Approximately 20 cercariae shed from infected mudsnails were pipetted into each container, and the containers were left undisturbed for at least 24 hr at room temperature with constant illumination. At the end of this time, the number of cysts on each individual snail was counted, from which the number of new cysts was calculated. The total number of new cysts on snails with and without pre-existing cysts was tallied and a chi-square test tested whether the distribution differed from expected (50% on each). In addition, we tested whether there was a correlation between the number of pre-existing cysts and the number of new cysts acquired, separately for each snail species.

RESULTS

Survey of naturally occurring cysts

Many *Philophthalmus* sp. cysts were found on all 4 snail species collected at the field site, i.e., mudflat topshells, spotted topshells,

TABLE II. Location of cysts on different species of snails from a natural survey and 2 laboratory experiments.

Snail	Source of data	Shell	Aperture	Operculum	Total
Diloma subrostrata	Natural survey	0	22	29	51
	Tethered choice test	15	4	5	24
	No-tether choice test	32	16	26	74
D. aethiops	Natural survey	0	13	38	51
	Tethered choice test	19	11	8	38
	No-tether choice test	38	31	13	82
Cominella glandiformis	Natural survey	3	23	4	30
	Tethered choice test	8	0	0	8
	No-tether choice test	17	11	8	36
	Density experiment	143	43	33	221
Zeacumantus subcarinatus	Natural survey	22	1	0	23
	Tethered choice test	10	0	0	10
	No-tether choice test	22	0	0	22
	Density experiment	114	0	5	119

mudflat whelks, and mudsnails. A single cyst also was found on a crab. No cysts were found naturally on any of the other substrata (Table I). This distribution of cysts was significantly different than expected if cercariae encysted randomly relative to available surface area ($P < 0.0001$, $\chi^2 = 844$), with all 4 snail species having more cysts than expected, and all other substrata having fewer.

Cysts were exclusively found inside the rim of the aperture and on the operculum of both species of topshells: no cysts were found naturally on the rest of the shell. On the whelks, cysts were primarily found in the aperture (23/30), although a few were found on the operculum (4/30) and outer shell (3/30). In contrast, cysts on the mudsnails were found predominantly on the outer shell (22/23), with only a single cyst found in the aperture (Table II). Many cysts found in the aperture seemed to be at some stage of becoming embedded in the shell, with thin layers of shell material partially covering the cyst.

Experimental substrate choice tests

Of the approximately 500 cercariae transferred to the containers for the tethered choice test (~50 per container), 96 were recovered as cysts from the substrata under study (1–19 per container; median, 9.5). Of these, the majority (80/96) was found on the 4 species of snail, all of which had more cysts than expected if the cercariae encysted randomly relative to available surface area ($P < 0.0001$, $\chi^2 = 94.5$; Table III). Unlike the natural distribution of cysts on the topshells, the cysts observed on the topshells in this experiment were found on all parts of the shell (Table II), i.e., outer shell (34), aperture (15), and operculum (13). Cysts on the whelks and mudsnails were found only on the outer shell. The single cyst found on a cockle was on the smoother, dark-colored end of the shell, and the cysts on empty cockle shells were all on the smooth inner surface.

In the no-tether choice test, 280 cysts were recovered from 10 containers (4–74 per container; median, 25.5). Again, the distribution of cysts differed from that expected based on surface area ($P < 0.0001$, $\chi^2 = 420$), with all 4 snail species having more cysts than expected, and the other substrata having fewer (Table III). Cysts were found on all parts of the shell on both species of topshell and the whelks, whereas they were only found on the outer shell of the mudsnails (Table II). The majority of the cysts found on empty cockle shells (36/38) were found on the inner, smooth surface, and the 2 cysts that were on the outer surface of the shells were on the smoother, dark half of the shell.

Because a greater proportion of cysts were found on cockle shells in the no-tether experiment than in the tether experiment,

TABLE III. Cysts found on substrates after 2 choice tests. In the "tether" test, substrates were each tethered an equal distance from the center, and live cercariae were transferred via pipette into the center of the container. In the "no tether" test, substrates were allowed to move freely around the container, and infected intertidal snails were used as a source of cercariae. All values in the table are sums from substrates in 10 containers. The expected number of cysts was calculated based on the surface area available for encysting. See Materials and Methods section for common names of substrates.

	Tether			No tether		
Substrate	Total area (cm^2)	Expected cysts	Cysts	Total area (cm^2)	Expected cysts	Cysts
Diloma subrostrata	80	10.9	24	89	33.4	74
D. aethiops	102	13.9	38	119	44.7	82
Cominella glandiformis	25	3.4	8	22	8.2	36
Zeacumantus subcarinatus	8	1.0	10	7	2.5	22
Austrovenus stutchburyi (live)	121	16.4	1	130	48.8	0
A. stutchburyi (shell)	126	17.0	4	150	56.3	38
Zostera capricorni	100	13.6	2	100	37.5	21
Rock	146	19.8	9	129	48.6	7
Total	707	96	96	747	280	280

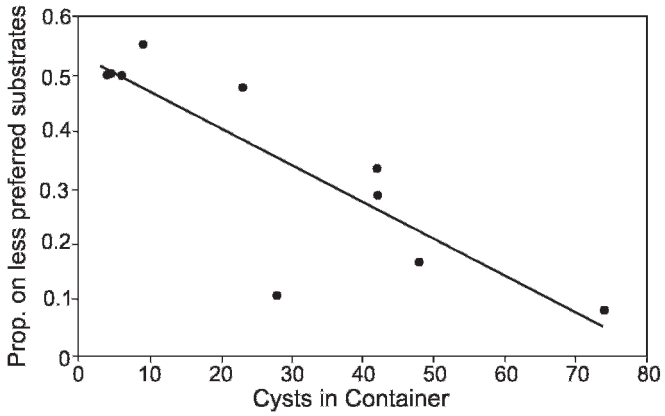

FIGURE 1. Relationship between the total number of cysts recovered from a container and the proportion that were found on the less preferred substrata (i.e., live cockle, empty cockle shell, eelgrass, rock). Each point represents data from 1 container.

the greater abundance on the cockle shells may have been due to substratum saturation on the more preferred substrata. We therefore tested for a correlation between the number of cysts in a container and the proportion of those cysts on cockle shells. If cercariae were avoiding preferred substrata already crowded with cysts, there should be a positive correlation between these variables. Instead, we found a negative trend, though the pattern was not significant ($P = 0.246$, $R^2 = 0.164$). However, when we related the number of cysts in a container with the proportion on the 4 less preferred substrata combined, there was a significant negative correlation ($P = 0.002$, $R^2 = 0.712$; Fig. 1).

Density and substratum choice

The number of pre-existing cysts on mudsnails ranged from 1 to 29 (median 4). One snail in this group had no cysts and was therefore dropped from the experiment, bringing the total number of containers for the experiment to 14. After adding approximately 280 cercariae to the 14 containers, 22 were recovered, 17 from snails that had pre-existing cysts and 5 from snails that did not. The snails with pre-existing cysts gained significantly more new cysts than those without ($P = 0.01$, $\chi^2 = 6.55$). One snail that previously had cysts lost 3 during the experiment, and this was recorded as 0 new cysts (rather than –3). There was a significant positive correlation between the number of pre-existing cysts on a snail and the number it gained during the experiment ($P = 0.012$, Spearman's $\rho = 0.467$; Fig. 2a), even if the most influential point (the snail that started with 29 cysts) was excluded ($P = 0.042$, Spearman's $\rho = 0.394$).

The number of cysts on pre-exposed whelks used in the experiment ranged from 1 to 22 (median 6) after exposure to the infected mudsnail for 5 days. Two whelks were excluded, 1 whelk because it failed to acquire cysts from the infected snail, and the other whelk because its tether came unglued just before the start of the experiment, leaving 13 containers. Of the approximately 260 cercariae added to the 13 containers, 131 were recovered. Significantly more were found on whelks with previous cysts (82) than those without (49; $P = 0.004$, $\chi^2 = 8.31$). There was no significant correlation between the number of pre-existing cysts on a snail and the number of new cysts it gained ($P = 0.050$, $R^2 = 0.379$), although there is a tendency toward a positive relationship, as with the mudsnails (Fig. 2b).

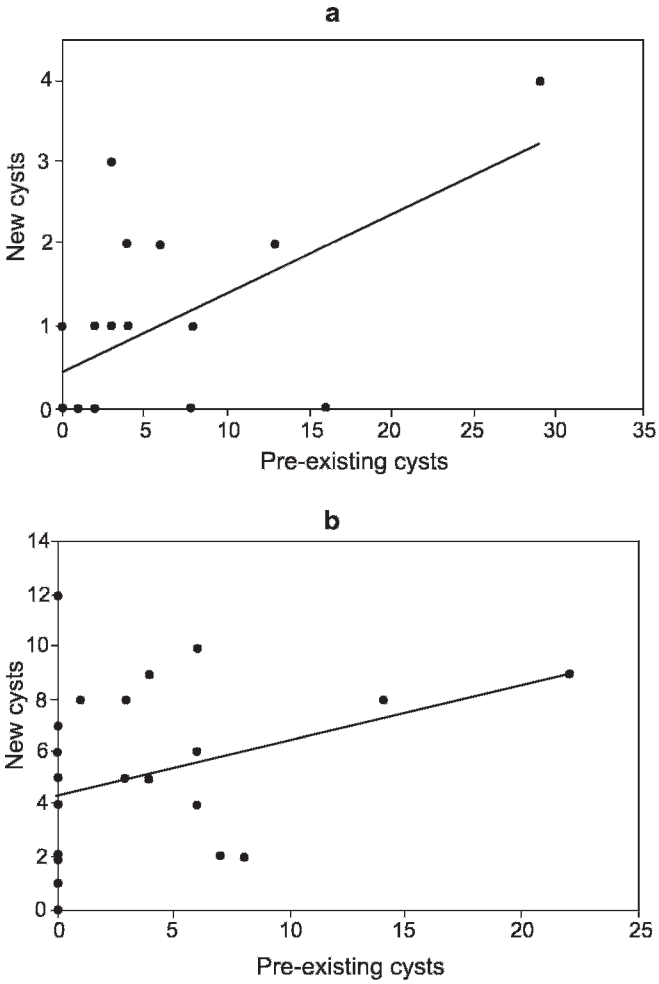

FIGURE 2. Relationship between the number of *Philophthalmus* sp. cysts at the beginning of the experiment and the number of new cysts formed on mudsnails (**a**) and whelks (**b**). Each point represents an individual snail.

DISCUSSION

Our results elucidate a previously unknown step in the life cycle of *Philophthalmus* sp., a common parasite of New Zealand intertidal systems. By identifying the substrata used by this parasite in its transition from snail-to-bird hosts, we also can narrow down the list of potential avian host species. In addition, our results demonstrate a clear density dependence in substratum selection by this parasite's cercariae that may benefit its reproduction as an adult.

Substratum preference and definitive host

Philophthalmus sp. cercariae encysted on various species of snails in preference to other substrata that are common on a mudflat, including bivalves, seaweed, and rocks. Molecular data show that the *Philophthalmus* sp. found in the Otago Harbor are all a single species (Keeney et al., 2009); thus, the use of multiple snail species does not represent a few cryptic species, with each preferring a different substratum. Also, it has been noted that cercariae from species of *Philophthalmus* will readily encyst on any hard substratum (Alicata, 1962; Howell and Bearup, 1967; Díaz

et al., 2002), but our results show that despite this readiness, the cercariae show substratum preferences. This is not surprising because many of the hard substrata available in the field would be almost certain transmission dead ends, e.g., rocks and empty shells. Miracidia from many trematode species use chemical cues to locate snail intermediate hosts, and cercariae that infect snails often show attraction toward these hosts as well (Sukhdeo and Sukhdeo, 2004). It therefore does not seem unreasonable to think that *Philophthalmus* sp. might have this capability.

Although we did not include any crustaceans in our choice tests, the natural survey suggests that, unlike West's (1961) observation for *P. gralli*, arthropods such as crabs are not the most commonly used substratum for cyst formation in this system. However, it is possible that, were this survey conducted during the summer rather than the winter, the results would differ. Warmer temperatures would probably increase the total number of cysts observed (because more cercariae would be produced and released) but also might affect the relative numbers of cysts on substrata, such as crabs, that might be more active during the summer.

Our results on the substratum preference of this species also may clarify the parasite's definitive bird host. Howell (1965) reported that *Philophthalmus* sp. adults had been recovered from the eye orbit of a black-backed gull in Wellington. In general, the diet of *L. dominicanus* is variable (Bertelloti and Yorio, 1999). Near Wellington, >20 species of molluscs, including gastropods, have been found in the regurgitated pellets of *L. dominicanus*, and >40% of the pellets examined contained gastropod remains (Fordham and Cormack, 1970). It seems reasonable then that *L. dominicanus* may become infected with *Philophthalmus* sp. by consuming gastropod shells or shell fragments. *Larus dominicanus* is also the second most abundant shorebird in Lower Portobello Bay, where *Philophthalmus* sp. is common (Fredensborg et al., 2006).

However, because gastropods are among the most common prey items of shorebirds in the Western Hemisphere (Skagen and Oman, 1996), and probably elsewhere, and because some species of *Philophthalmus* are not host specific (Thakur and Cheng, 1968), *L. dominicanus* may not be the only definitive host of *Philophthalmus* sp. Several bird species that have been seen at Lower Portobello Bay and elsewhere on the Otago Peninsula (surveyed by Fredensborg et al., 2006) are known to eat molluscs, including gulls (*L. novaehollandiae scopulinus*, *L. dominicanus*); oystercatchers (*Haematopus ostralegus*, *H. unicolor*); and, seasonally, godwit (Scolopacidae) (Heather and Robertson, 2005). Many of these mollusc-consuming species feed mostly on bivalves, so it at first seems surprising that *Philophthalmus* sp. cysts are not found on cockles. It may be that the shells of the snail species, being smaller, are more commonly ingested than the larger shells of the cockles, which are often opened or broken before the soft insides are consumed (Heather and Robertson, 2005). Surveying other common gastropod-consuming shorebirds would help determine whether *Philophthalmus* sp. infects other bird species.

Substratum use

The experimental results suggest that cercariae readily form cysts on all available surfaces of the snails, though the cysts found in the natural survey seemed more localized. The discrepancy in cyst location between the experimental results and the natural survey could be due to cyst longevity or the ease of forming cysts (due to factors such as water movement) on different parts of the shell. Mudsnails and whelks kept in the laboratory tended to burrow in the mud, as they do in natural habitats, whereas both species of topshells tended to climb the sides of containers. The latter's behavior in the wild is similar, as they tend to climb on, and cling to, eelgrass or rocks at low tide. Therefore, cysts on the outer shell of topshells would probably be more prone to desiccation than those on the outer shells of whelks and mudsnails, allowing cysts to persist on the outer shells of the latter, but not the former. The almost complete lack of cysts in the aperture and on the operculum of the mudsnails in both the natural survey and choice tests was probably due to the small surface area of these parts of the snail. Cysts were observed on these areas in the density experiments when there were more cysts on the mudsnails overall, e.g., the snail with 29 cysts had 3 on the operculum.

Also, the relative abundance of cysts in the aperture of topshells and whelks in the natural survey could be in part due to the snail laying new shell material over the cysts shortly after they are formed. This would probably preserve them from damage (desiccation and abrasion), but being embedded even slightly would probably prevent the metacercariae from escaping through the cyst opening. Therefore, although the aperture of the topshells and whelks had a large number of cysts preserved by the new shell, they were probably a dead end for the parasites within.

Aggregation of cysts

Here, we presented evidence that cercariae prefer to encyst on substrata that already harbor conspecific cysts and that this preference may increase with increasing cyst density. Such behavior should lead to an aggregated distribution of parasites. Aggregation is a widely documented phenomenon among various taxa of parasites on, and within, their hosts (Shaw and Dobson, 1995). Aggregations of parasites are often attributed to differences in host susceptibility due to variability in factors such as genetic-based resistance, spatial or temporal exposure, host behavior (Poulin et al., 1991), or attractiveness to vectors (Medel et al., 2004). The aggregation of *Philophthalmus* sp. on snail shells adds an interesting dimension to the discussion of parasite aggregation on hosts because it seems that differences in host susceptibility are not the driving force. It is hard to imagine shells differing in their susceptibility, especially because our experimental design should have eliminated variation in exposure.

Another mechanism that has often been implicated in aggregation is the use of chemical cues (reviewed in arthropods by Wertheim et al., 2005). For example, ticks use pheromones to attract other ticks to the host on which they are located (Norval et al., 1989). Chemical cues are among the mechanisms used by cercariae (Haas, 2003) and miracidia (Sukhdeo and Sukhdeo, 2004) to locate hosts, so it is feasible that chemical cues could be used to guide cercariae to substrata that already have cysts. However, for such a system to evolve, the benefits of aggregation must outweigh the costs, for both the sender of the cue and its receiver.

For organisms in general, cost of aggregation can be measured in terms of competition, increased disease transmission, conspicuousness to enemies or predators, and the cost of signal production (Wertheim et al., 2005). In contrast, aggregation can

provide a number of benefits, including facilitation of mate finding and protection from predators, e.g., by a dilution effect (Parrish and Edelstein-Keshet, 1999; Wertheim et al., 2005). Indeed, the aggregation of numerous metacercariae of different genotypes within second intermediate hosts is seen as a driving force in the evolution and maintenance of complex life cycles (Brown et al., 2001; Rauch et al., 2005). The same arguments apply to transmission via substrata instead of second intermediate hosts. Data on other *Philophthalmus* species suggest that crowding has little effect except at very high densities and that some species are not self-compatible and do not even mature in single-worm infections (reviewed by Nollen and Kanev, 1995). Therefore, the potentially greater ensuing competition within a bird's eye due to aggregation on substrata may be offset by greater mating opportunities. Identifying the proximate mechanism responsible for the attraction of cercariae to substrata already harboring cysts would allow determination of whether this aggregation is adaptive or a consequence of some other phenomenon.

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