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Los Angeles

From Host to Host:
Interaction of Behavior and the Environment
During Parasite Transmission

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Biology

by

Jonathan Todd Fingerut

2003

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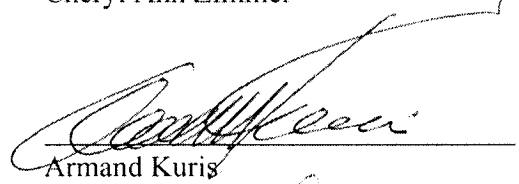
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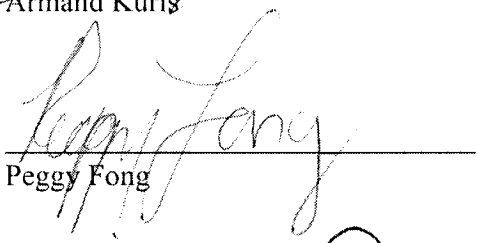
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2003

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DEDICATION

This work is dedicated to Samantha, for her love

and support across so many miles

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ABSTRACT OF THE DISSERTATION

From Host to Host:

Interaction of Behavior and the Environment

During Parasite Transmission

by

Jonathan Todd Fingerut

Doctor of Philosophy in Biology

University of California, Los Angeles, 2003

Professor Richard Zimmer, Chair

Dispersing larvae of marine invertebrates can replenish populations, appropriate new habitat and enhance gene flow among sites. A clear understanding of colonization mechanisms requires studying the entire larval life history, from production to settlement. Here, I closed the larval loop for an estuarine trematode parasite, and revealed the profound role of active behavior compared with passive

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physical processes. Active control of emergence allowed cercariae to leave their natal host only during those times when a hierarchy of conditions acting at different time scales (temperature monthly, light diurnally and water-depth tidally) were most favorable to encountering a new host. Following release, vertical distributions of short-lived (4 h) cercariae were bottom skewed, with most larvae occurring < 5 cm of the bed where their snails hosts live. In the relatively weak (shear velocity ≤ 0.2 cm/s) flows in southern California marshes, most cercariae transported < 2.5 m before settling. Laboratory studies in flume flows replicating those found in the natural habitat showed that downward swimming overpowered turbulent eddies in such flows. In-situ measurement of dispersal distances show that larvae disperse only 1-2 meters before settling, shorter by 1-2 orders of magnitude than would be predicted if there was no active movement towards the bed. Thus, minimal cercarial transport was a direct consequence of larval behavior. Nominal larval dispersal accounts for a significant predictive relationship between cercarial release (production) and settlement, as well as localized amplification of prevalence by a factor of ten. Transit through near-bottom waters is the "last lap" prior to settlement, so processes distributing larvae within this region may have a disproportionate effect on recruitment. In this study, minimal dispersal and maximal transmission are the consequences of larval behavior.

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Chapter 1

PATTERNS AND PROCESSES OF LARVAL EMERGENCE IN AN ESTUARINE PARASITE SYSTEM

Parasite larvae typically disperse prior to finding and infecting a host. As with propagules of free-living organisms, such as crabs (Forward et al. 1986, Morgan 1996), sponges (Amano 1988) and plants (Horn et al. 2001, Schauber et al. 2002), external cues may direct emergence of parasite larvae under favorable conditions. Because dispersal stages of digenetic trematodes have very short (hours) life spans, timing emergence to correspond with host availability would be especially advantageous (Combes et al. 1994, Pechenik and Fried 1995, Toledo et al. 1999). Moreover, the widespread distribution of trematodes in fresh (Pages and Theron 1990, Gerard 2001), and salt water (Martin 1972, Bartoli and Combes 1986, Jonsson and Andre 1992, Curtis 1997) environments allows for cross-habitat comparisons of emergence characteristics.

Trematode emergence has been studied largely in freshwater systems where emergence has been shown to vary on a circadian cycle synchronized with host availability (Combes et al. 1994, Haas 1994). These rhythms are usually entrained by photoperiod or thermoperiod (Mouchet et al. 1982, Theron 1984, Combes et al. 1994). Freshwater parasites moving from aquatic to terrestrial vertebrate hosts

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time their emergence to coincide with waterfront activities of the hosts, on scales of hours (Theron 1989, Raymond and Probert 1991).

In southern California intertidal marshes, there is a guild of over 18 digenetic trematode species (Martin 1972). Sexual reproduction takes place in definitive host shorebirds, from which parasite eggs are released into the marsh. The first intermediate host snails are infected by ingestion of, or by penetration by, miracidia larvae that hatch from the eggs. Infected snails cease reproduction while infection persists for the lifetime of the snail (Kuris 1990, Sousa 1990). Asexual reproduction of parthenitae in the snail results in the production of tens to thousands of free-swimming cercariae per snail, per day. These cercariae then encyst in or on a second intermediary host (snail, fish, crab or clam). Ingestion of this 2nd intermediate host by a shorebird completes the trematode's lifecycle. Swimming, non-feeding, cercariae are short-lived, so they must move toward or remain near host habitat for effective transmission. In marshes, with their tidally varying water depth and currents, the ability of cercariae to control where they are transported may be limited.

Previous work in the area of cercarial emergence has been primarily laboratory based (e.g. Raymond and Probert 1987, Shostak and Esch 1990) with work on this particular guild focusing on intraspecific competition (Kuris 1990, Sousa 1990). The only other field-based study of cercarial emergence in these estuarine species (Stevens 1996) used water samples to investigate assemblages of

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planktonic cercariae and the effects of environmental variables on their composition. Stevens proposed that both temperature and light levels effected emergence numbers on seasonal and daily time scales. However, that study did not allow for the time of cercarial emergence to be determined directly or at fine temporal scales, nor did its concentration on fieldwork incorporate a complete set of controlled laboratory experiments. Therefore, there remains a pressing need for further investigation before the extent of environmental control of emergence can be fully understood.

The purpose of this study was to identify factors controlling cercarial emergence in an intertidal estuary, as compared to freshwater systems. Two hypotheses were tested.

(1) *Timing of cercarial release varies over the day.* Light (24 h period) may impose a similar diel periodicity in both systems, but tidal (12 h period) effects (water level, currents) would be unique to the estuary.

(2) *Exogenous factors control cercarial emergence in intertidal estuarine habitats.* Circadian rhythms in freshwater cercariae are often associated with innate activity patterns of intermediate or definitive hosts (Combes et al. 1994). Such predictable host signals may not be as important in an estuarine system, where submergence is limited by the tidal cycle.

Research was conducted in two stages. Field studies measured emerging parasite larvae as a function of external variables (temperature, salinity, tidal

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height). Then, laboratory experiments identified the role of the specific factors in controlling the onset and duration of emergence.

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MATERIALS AND METHODS

Field Observations

In the field, cercariae emerged from snails (*C. californica*) were collected while simultaneously measuring environmental variables. Data were collected on 38 days over 1999-2002. Measurement intervals were selected to span all hours of the day and months of the year. Moreover, six night collections ($\geq$ 1 h after sundown) were paired with daylight collections on the preceding or proceeding tide to compare emergence within the same group of snails.

The field site (tidal channel) was located in Carpinteria Salt Marsh Reserve (CSMR), east of Santa Barbara, California. Biological measurements took place along the centerline of a 400-m long by 5-m wide by 0.6-m deep (high tide) channel. A centrally located 3-m-wide mudflat was carpeted ($460 \pm 16/m^2$) with *C. californica*, the first intermediate host for the trematodes studied here. The snail population extended 100s of meters in the along-channel direction. The hydrodynamic regime in this and similar southern California marshes (e.g., Mission Bay and Newport Beach) was dominated by slow flows (< 5 cm/s), with shear velocities (u_*) $\leq$ 0.2 cm/s occurring > 80% of the time. Rarer, storm-driven currents ($u_* \geq 0.8$ cm/s) occurred only ~ 1 % of the time. The mudflat was located

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1.3 m above MLLW and was inundated twice a day by the semi-diurnal tide. Water depth exceeded this height for an average of 4 h during each 12-h tidal cycle.

Collections of emerged cercariae — Using a specially designed larval collector, cercarial emergence from a specified group of snail hosts was measured non-intrusively. The main body of the collector was a flat, clear Plexiglas chamber (50-cm wide by 50-cm deep by 2.5-cm tall) with 34 μm mesh side panels and a solid bottom. Penetrating the top of the chamber was a 4 by 4 array of 8-cm-diameter holes (covering 32% of the surface area), each fitted with an inverted funnel (i.e., small end pointing away from the chamber) intake. Contents of all funnels were united into a single 12.5-mm (ID) tube that fed into a peristaltic pump (Masterflex IP variable speed). At a rate of ~5 liters/min, the pump continuously filtered water from the chamber over 34 μm mesh. The chamber volume was flushed eight times during each 10 min sampling interval.

During each sampling event, 200 large (>2.5 cm long) snails were placed in the collector. Once on the mudflat, the chamber was gradually inundated by the incoming tide at the same time as the free-ranging snail population. Captives were provided with oxygenated water at ambient temperature. Size ranges of marsh cercariae were 50-100 μm (width) and 200-1000 μm (length) (Martin 1950, Adams and Martin 1963, Martin 1972), so the mesh retained all species. As there was no emigration or immigration of cercariae into or out of the chamber cercariae collected on the filter could be ascribed solely to the enclosed host population.

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The system self-primed when the water level reached the bottom of the funnels (5 cm water depth), usually 5-10 min after the tide first rose to the level of the mudflat. Every 10 min the filter was removed (and replaced), dipped in 90% ethyl alcohol, and placed in a sealed Petri dish for later staining (0.5% Lugol's solution) and counting. Samples were also examined, qualitatively, to identify dominant species (by number) throughout the year and day. Each collection lasted 4 h, matching the average period of snail inundation. A cumulative frequency distribution was assembled from the total number of emerged cercariae during each 10-min collection. The time for 95% of all cercariae to emerge was compared between collections. Total numbers captured from field collections tend to be less than predicted from laboratory shedding of infected snails (see Results). The discrepancy is most noticeable in the number of small cercariae, such as *Euhaplorchis californiensis*. Very small cercariae may be pushed through the filter or more often hidden from counting amongst the sediment and detritus that is unavoidably captured along with cercariae in the field. While limiting the ability to determine the exact number of smaller cercarial species emerging, the large numbers of cercariae that are caught with this technique still provide a reliable (albeit conservative) measure of abundance that is comparable between days.

Environmental measurements – Properties of the physical environment during tidal inundation (4 h interval during flood or ebb) were measured for the 38 periods of quantified cercarial emergence. The mudflat was exposed to air for ~8 h

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between each tidal inundation. Maximal water depth was 60 cm during each tide. Using an ORION Model 140 probe, seawater temperature and salinity were recorded 1 cm above the bed every 10 min. An underwater quantum sensor (LICOR Model 190SA) was flush mounted at the mudflat surface before tidal inundation to register light intensity at 1 Hz over the duration of the flood and ebb tides.

Laboratory Experiments

Field measurements (see Results) indicated that the following factors may control cercarial emergence: temperature, host inundation (water depth), light, and time of day. Because only infected snails (rather than a random sample of the host population) were used in laboratory experiments, numbers emerging were higher than those reported for field observations. With one exception, all laboratory experiments were conducted during summer months (June–September). Trials to evaluate cercarial emergence as a function of water temperature were performed during the spring (March–April).

Temperature — To determine the effects of temperature on emergence, two time series were conducted with each using the same 53 snails (*C. californica*). The animals were placed individually in chambers (27 cm^3) filled with seawater. In the first 7-day series, the same group of snails was held at a higher (+1 ^{o}C) water temperature on consecutive days, over the range 13 - 19 ^{o}C. Chambers were filled

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with water at the new temperature at 10:00 each day, and cercariae collected 4 h later. (All times are reported as Pacific Daylight Savings). Snails were kept dry at a constant 18 °C between runs (average spring air temperature for daylight hours). The second 7-day series followed the same protocol, except that temperature was decreased 1 °C per day, from 19 to 13 °C. At the end of a 4-h incubation period, contents of individual chambers were processed separately to determine species (Martin 1972) and number of emerged cercariae.

Host inundation — Experiments evaluated the effect of host inundation on cercarial emergence. Two groups of 100 snails each were randomly assigned to one of two treatments, and were placed in 21-cm-long by 11-cm-wide by 3-cm-deep trays, at 20 snails per tray. Both groups were then situated in an environmental chamber at 21 °C (average summer water temperature) with constant light (40 μmol/m^2/s). For one group, trays were kept dry for 4 h, and for the other group, trays were filled with water from CSMR. At the end of 4 h the dry trays were briefly filled to suspend any emerged cercariae, and the water from both groups was filtered through a 34 μm mesh. The number of emerged cercariae from each treatment was determined using the same processing methods as for the field samples. The following day, the treatments were reversed for the two snail groups. This 2-day experiment (one 4-h test period each day) was replicated three times, with a new batch of snails each time.

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Light — Experiments determined the effect of light intensity on cercarial emergence. Snails were exposed to a midday intensity (~2000 μmol/m^2/s) or to simulated dawn/dusk intensity (~40 μmol/m^2/s), created by reducing midday intensity with a neutral density filter. Snails (100 per group) placed in water-filled trays (see Host inundation) were randomly assigned to one of the light conditions. Every 15 min, trays were overturned onto a 1-mm mesh panel. Snails remained in the trays, but water and cercariae passed through. Trays were immediately refilled. Water and larvae were filtered over 34 μm mesh and processed as in the field study. To control for the warming effect of the stronger sunlight, snail trays were held in a water bath maintained at 21 °C (average for summer months). Trials lasted 2 d, with each group exposed to both conditions on consecutive days, and was replicated three times, each with different snails.

Time of day — Experiments evaluated whether the duration of cercarial emergence varies over the course of a day. Two series of experiments were performed, one testing time of day (TOD) on emergence duration and the other testing for the existence of an endogenous rhythm. In the first series, 100 snails were placed in water-filled trays (see Host inundation) at constant temperature (21 °C) and light (40 μmol/m^2/s) for 4 h, starting at a different time each day (9:00, 12:00, 15:00, and 18:00). During each 4-h interval, emergence duration was determined as in the light experiments. Three replicate trials, each with new snails, were tested at all four start times.

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The goal of the second series of experiments was to determine if an endogenous rhythm, stimulated by light, may control emergence duration. This research is necessary but not sufficient to establish a circadian rhythm (Aschoff 1960, Pittendrigh 1993, Dunlap 1999). The experiment held light:dark ratio constant while varying the onset of daylight. The prediction was that emergence duration would track with an internal clock set by incipient dawn, independent of the absolute time of day.

Two temperature and light-controlled chambers were set at 21 °C and 40 $\mu mol/m^2/s$. The control chamber was set on a light:dark (14:10) cycle, with the natural (6:00) sunrise. The experimental chamber used the same light:dark ratio but with sunrise shifted either 8 h forward to 14:00 or backward to 22:00. In the first experiment, 100 snails were placed in the control and another 100 in the forward-shifted light chamber for 1 wk before the trial began. After this acclimation period, emerging cercariae in both chambers were monitored over 4 h, beginning at 18:00, as in the light experiments. In the second experiment, new snails were acclimated to the control and backward-shifted light regime for 1 wk, and snails were monitored for 4 h beginning at 10:00. In both experiments, the control and light-shifted snails were tested on sequential days. Moreover, as a check on the repeatability of the results, the same set of snails was monitored over an additional 4 d. Both experimental series were conducted once in 2000 and once in 2001.

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RESULTS

Field observations

The magnitude of cercarial emergence (all trematode species combined) correlated significantly with water temperature, but not with salinity or total irradiance (Table 1-1 and Fig. 1-1). Snails *(C. californica)* first appeared in tidal channels in February, when temperatures rarely exceeded 13 °C (Figs. 1-2A). Emergence did not begin until March, however, when water temperatures in the marsh reached ≥ 15 °C (Fig. 1-1). Despite continued warming during April and May, emergence was moderate until June. A relatively close correspondence between temperature and emergence is evident for the spring cercariae. Temperature measurements showed that during these months ocean water entering the marsh was initially cooler than 15 °C, slowly warming up over a period of minutes to hours. Cercarial emergence following snail inundation was typically delayed with the start of emergence coinciding with water temperature reaching ~15 °C. In some cases this translated to a delay of up to 2h from the start of tidal inundation.

During the warm summer months, June-September, when water temperature was > 18 °C (average of 21 °C), the number of emerged cercariae increased substantially (Fig. 1-1). Larvae left their hosts as soon as the snails were inundated (Fig. 1-2C). Despite similarity in temperature (1-2 °C difference)

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between paired day/night collections, few or no cercariae were collected at night (Table 1-2). Seawater cooled by ~ 3 °C in October, but emergence remained high until November.

Observations were made on the relative abundance of the most common cercarial species in the samples. *H. rhigedana* and *Parorchis acanthus* were the first species collected during the cool spring months (March-May), making up ~ 90% of the cercarial population. Other species, such as *Renicola buchanani* and *E. californiensis* first appeared in late May/early June. Due to its high prevalence (Kuris 1990) and fecundity, *E. californiensis* numerically dominated the June-September samples. Together, *H. rhigedana* and *E. californiensis* comprised >75 % of all cercariae collected throughout the day during the warm summer months.

Whereas cercarial emergence was high and relatively constant from June through September, duration of emergence events varied considerably among the 21 summer/daylight samples. A stepwise multiple regression analysis of emergence duration against temperature, salinity, time of year and time of day, yielded only one significant factor: time of day (Table 1-3). Duration of cercarial emergence decreased significantly throughout the day (Fig. 1-3A). Emergence intervals ranged from 4 h in the morning to ≤ 1 h by dusk (Figs. 1-3A and 1-4). This trend was present regardless of the threshold used to determine emergence duration (i.e., 95 % of all cercariae emerged, as used here, versus 75 % or 50 %). Decreasing emergence duration over the day was significantly associated with an

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increase in the rate of emergence (number cercariae/h) (Fig. 1-3A, and least squares regression: $F = 27.36$, df = 1/19, $P < 0.0001$; $r^2 = 0.73$), and not with changes in the total number of cercariae released from host snails (Fig. 1-3B, and least squares regression: $F = 0.006$, df = 1/19, $P < 0.93$; $r^2 < 0.001$). Long (~4 h) emergence durations during the early morning resulted from a relatively low but constant larval emergence over the entire period of inundation. As the day progressed, more cercariae emerged per unit time, but over a contracted interval (Fig 1-4). Approaching dusk, large numbers of cercariae emerged in a quick burst as inundation commenced. The same patterns were seen in all years of the study.

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Table 1-1. Simple linear regression and stepwise multiple regression analysis of the effect of environmental variables on the number of cercariae to emerge during a four hour period in Carpinteria Salt Marsh.

Source	*F*-value	*P*	r^2	*F*-value of SMR	df	*P*	r^2
Temperature	66.5	<0.001	0.68	37.14	1/31	< 0.001	0.68
Total Irradiance	3.5	0.07	0.11	0.05	1/31	0.82	< 0.0
Salinity	1.2	0.29	0.06	0.34	1/31	0.56	< 0.01

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Table 1-2. Number of cercariae shed during paired day/night collections from 200 snails.

Date	Day or night	Average temperature (°C)	Number emerged per 4 h collection
8/01/2000	Day	21.4	1043
	Night	20.1	22
8/18/2000	Day	20.9	1543
	Night	19.9	11
7/24/2001	Day	22.3	1684
	Night	20.5	28
8/12/2002	Day	19.3	537
	Night	18.2	3
8/22/2002	Day	22.1	832
	Night	21.7	1
9/06/2002	Day	19.3	349
	Night	17.9	0

Water temperature was averaged over the entire 4 h collection. Mean light intensity during nocturnal collections, even under full moon conditions, was always < 0.01 μmol/m^2/s compared to > 1035 μmol/m^2/s during daylight collections.

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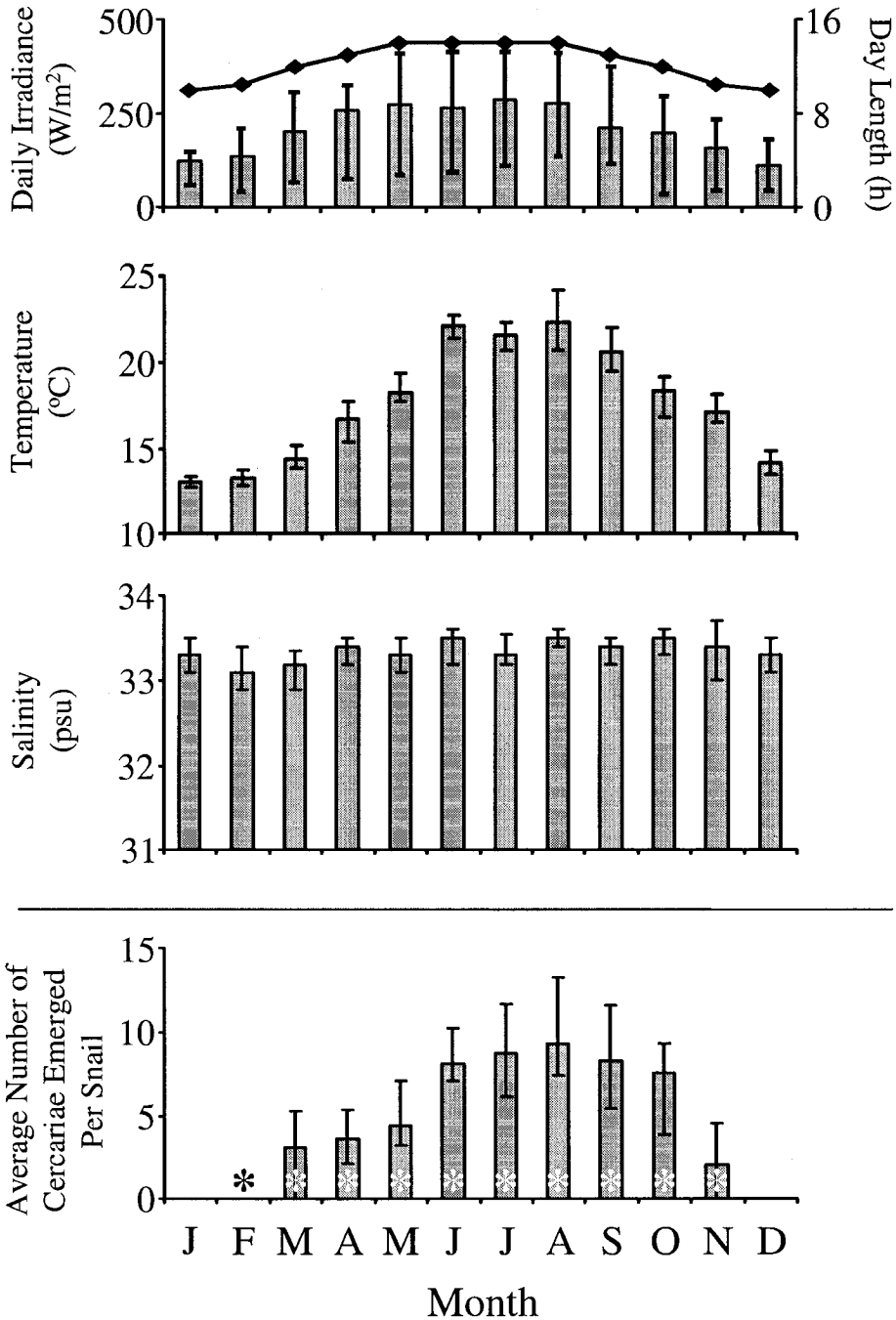
Table 1-3. Simple linear regression and stepwise multiple regression analysis of the effect of abiotic variables as sources of variation in emergence duration.

Source	F-value	*P*	r^2	F-value of SMR	df	*P*	r^2
Time of Day	54.2	<0.001	0.74	47.96	1/19	< 0.001	0.73
Time of Year (month)	0.001	0.98	<0.01	0.03	1/19	0.86	< 0.01
Temperature	0.046	0.83	0.02	<0.01	1/19	0.95	< 0.01
Salinity	1.2	0.2	0.06	1.78	1/19	0.19	0.08

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Figure 1-1. Variation throughout the year in average monthly (A) daily irradiance (histograms) and day length (diamonds) (B) temperature, (C) salinity, (D) cercarial emergence and host presence on the mudflat (*). Cercarial emergence is shown as the total number of cercaria counted / the total number of snails placed in the collector (200) including both infected and uninfected individuals. Histograms represent monthly means and vertical bars plot the ranges. For day length, ranges are smaller than the data points. Irradiance data were taken from the California Irrigation Management System website (www.cimis.water.ca.gov) and day length from the United States Naval Observatory website (www.aa.usno.navy.mil).

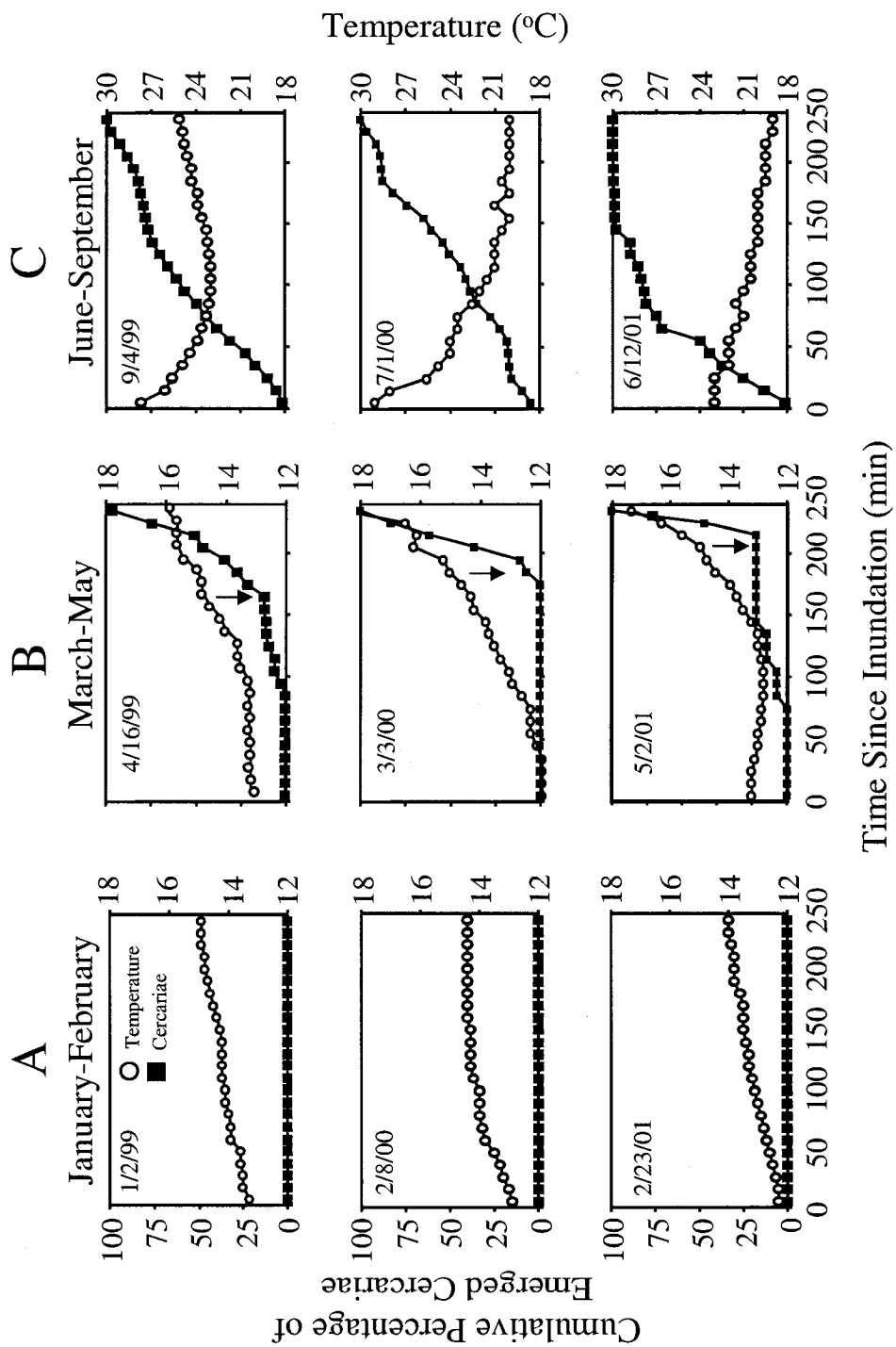
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Figure 1-2. Representative emergence patterns for different parts of the year: (A) January-February, (B) March-May, and (C) June-September. Water temperature (o) and cumulative percentages of emerged cercariae (■) are from samples taken every ten minutes. In (B), arrows correspond to the point in time when water temperature was first $\geq$ 15 °C.

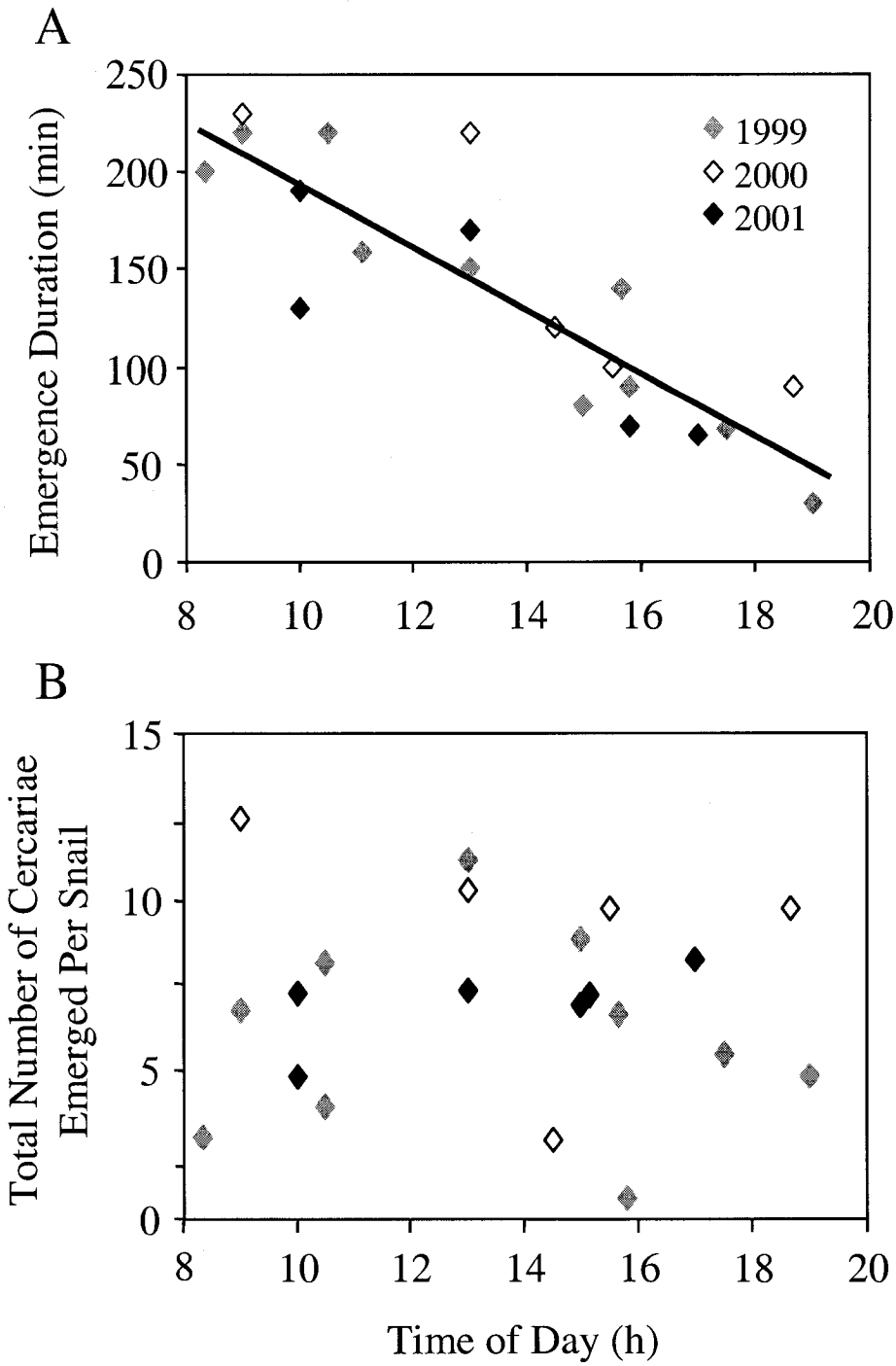
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Figure 1-3. Effect of time of day (TOD) on emergence duration in the field. (A) The amount of time for 95% of all cercariae to emerge during a given event (4 h) as a function of TOD. (B) The total number of cercariae emerged per snail during a given event as a function of TOD.

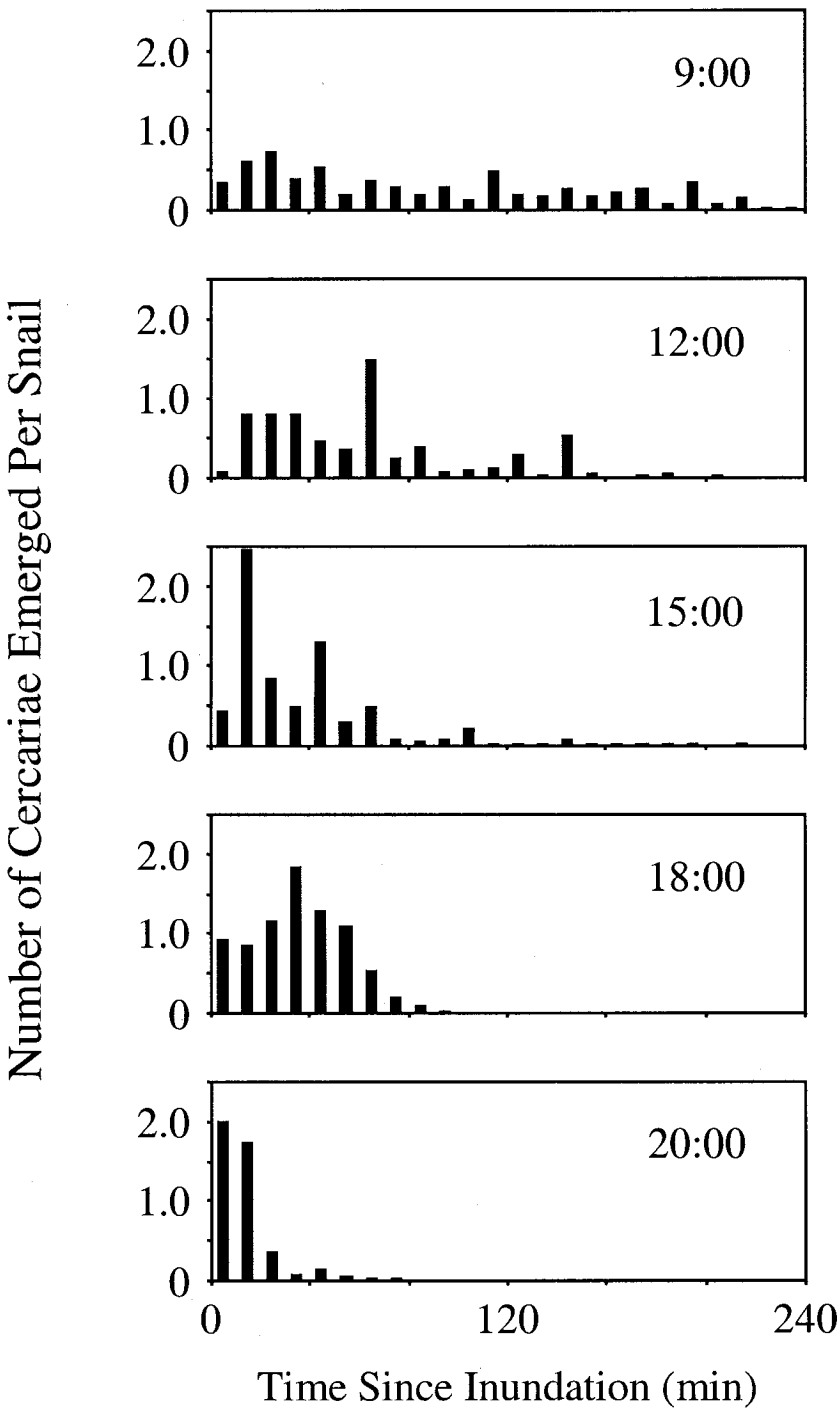
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Figure 1-4. Representative series of histograms throughout the day, showing number of emerged cercariae per snail (10 min samples) as a function of time since tidal inundation. Times indicate when snail hosts were first submerged. Because snail inundation resulted from the natural high tide, each histogram was constructed on a different day.

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Laboratory experiments

Host inundation — Throughout all laboratory studies, emergence occurred only if snails were totally submerged. Average number of cercariae to emerge in paired treatments with submerged (3861 ± 599 SEM) and dry (0 ± 0 SEM) snails showed unequivocally that cercariae would not leave the host unless it was underwater (Student's t-test: $t = 8.497$, $df = 5$, $P < 0.0001$). The muddy channel containing highest snail densities was at a tidal height of ~ 1.3 m MLLW, so hosts spent an average of 16 h/day out of water.

Temperature — Cercariae of five trematode species emerged at three temperature thresholds (Fig. 1-5). Emergence of *H. rhigedana* and *P. acanthus* began in 15 °C water and continued for the maximal temperature (19 °C) tested. *R. buchanani* emerged at an intermediate threshold of 17 °C through 19 °C. The two warm-water species, *E. californiensis* and *microphallid sp.* vacated their hosts only in the warmest waters tested (18-19 °C). Two-way nested ANOVAs were performed for each species separately. Number of emerged cercariae was significantly different among temperatures ($P \leq 0.0001$ for all species) and individual snails ($P \leq 0.04$) for all species except *R. buchanani* ($P = 0.17$). Because the order (ascending, descending) of temperature change had no significant ($P \geq 0.11$) effect on the outcome, Scheffé post-hoc comparisons were done for both series. Either way, there were two significantly ($P \leq 0.005$) different temperature

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groupings for all species, one within their emergence range and the other for the lower temperatures that prohibited emergence (Fig. 1-5).

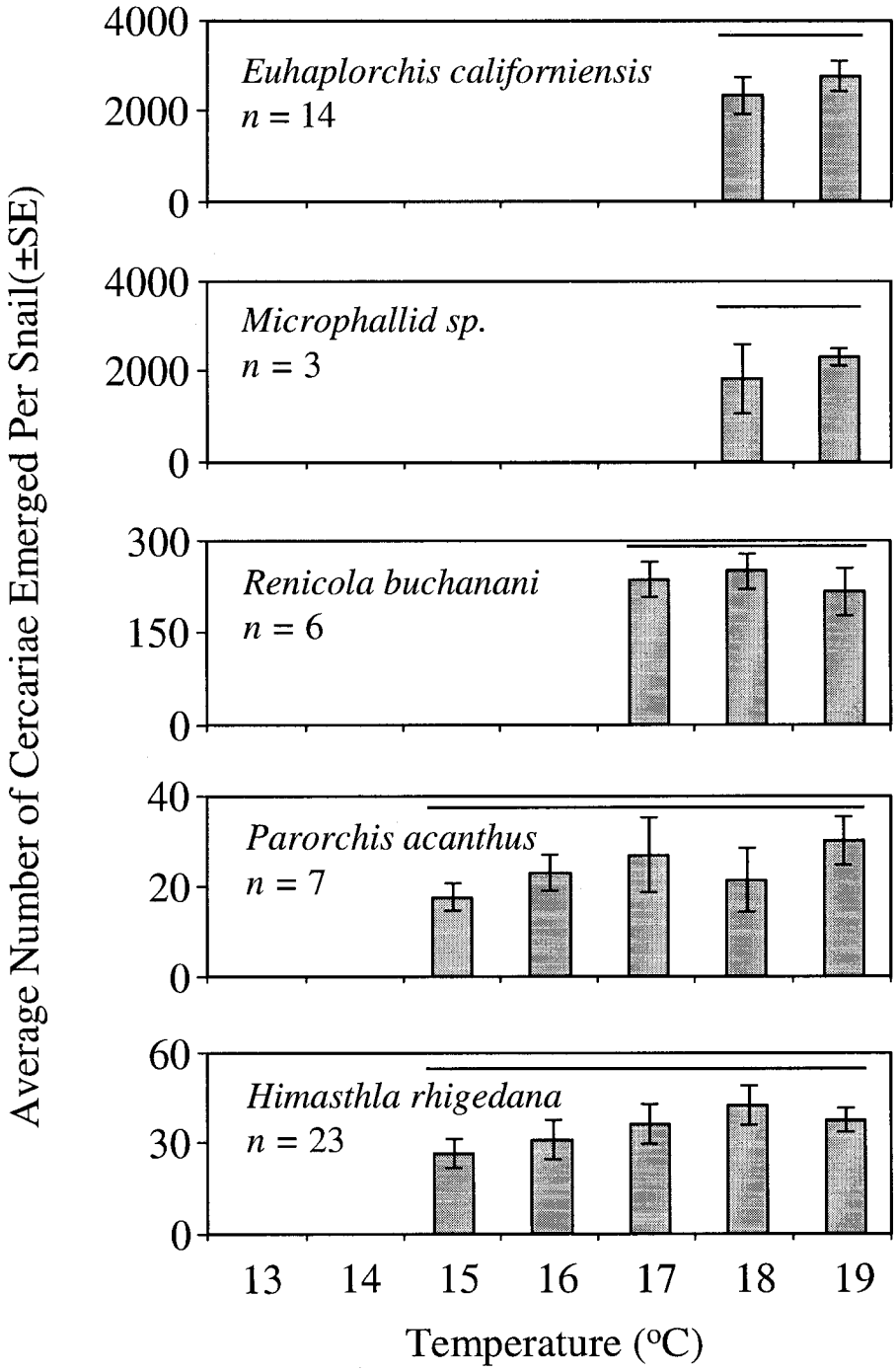
Light — Although daylight is a requirement for emergence (Table 1-2), even low intensities approximating those at dawn/dusk (40 $\mu mol/m^2/s$) were sufficient to trigger emergence under both field and laboratory settings. There was no significant difference, however, in the number of cercariae emerging, nor of the duration of emergence at maximal midday intensity (2000 $\mu mol/m^2/s$) relative to a minimal dawn/dusk intensity (40 $\mu mol/m^2/s$) (Student's t-test: $t = 1.021$, $df = 10$, $P = 0.36$ for duration; $t = 0.475$, $df = 10$, $P = 0.65$ for number emerged)

Time of day — As in the field collections, the duration of emergence decreased over the course of the day (Fig. 1-6A), varying from a modest, protracted stream of cercariae at dawn to a contracted burst at dusk. There was a significant relationship between emergence duration and TOD (Least squares regression: $F = 199.78$, $df = 1/10$, $P < 0.0001$; $r^2 = 0.93$), but not between number of cercariae that emerged and TOD ($P = 0.28$) (Fig. 1-6B). Moreover, there was a noticeable change in the duration of emergence when the host light:dark cycle was shifted forward or back (Fig. 1-7). As predicted, when sunrise was shifted forward, emergence duration decreased. Likewise, when sunrise was shifted back, emergence duration increased. These changes agree qualitatively with the diel pattern observed in both the field and laboratory. Shifting the light:dark cycle had no apparent effect on number of cercariae emerging (data not shown).

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Figure 1-5. Number of cercariae emerged per snail over the temperature range 13-19 °C. Data are shown for five common trematode species. Histograms are means and vertical bars are standard errors. n = number of snail hosts for a given trematode species. Within the temperature range in which a given species emerged, no statistically significant difference in the number of cercariae (ANOVA with Scheffé post-hoc test) was observed.

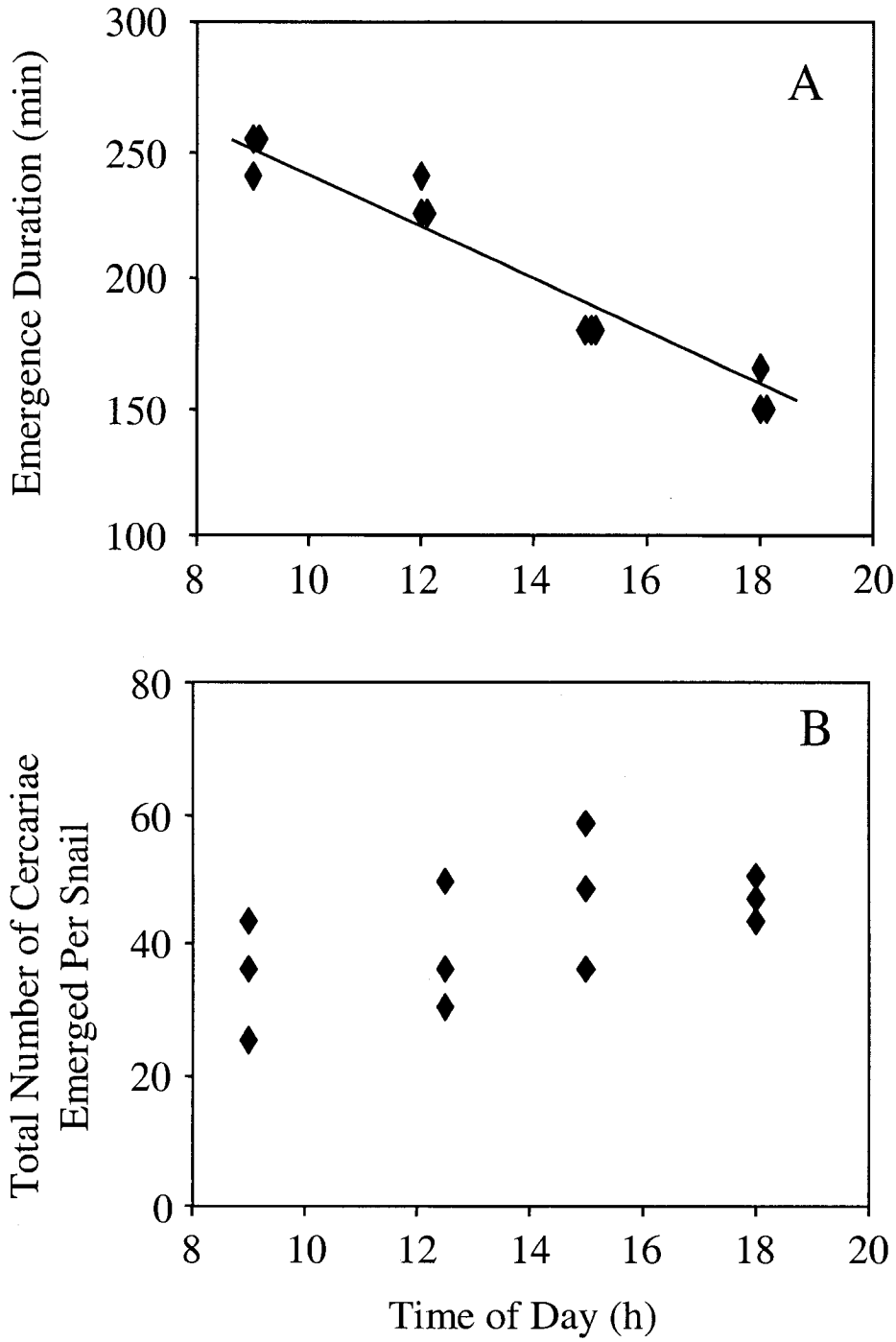
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Figure 1-6. Effect of time of day (TOD) on emergence duration in the laboratory. (A) The amount of time for 95% of all cercariae to emerge during a given event (4 h) as a function of TOD. (B) The total number of cercariae emerged per snail during a given event as a function of TOD.

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Figure 1-7. Effect on duration of cercarial emergence of shifting sunrise forward or backward 8 h, compared to unshifted control groups. Filled symbols indicate control values by year (2000 •, 2001 ■) and open symbols indicate shifted regimes (2000 ○, 2001 □).

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DISCUSSION

Most digenetic trematodes have a free-swimming cercarial stage for transmission from the first intermediate host to the second intermediate or the definitive host. Given the short functional (infective) life-span of cercariae (less than 24 hrs) emergence timing should be optimized to enhance the probability of successful transmission. Herein, larvae emerged over species-specific temperature ranges, exclusively during daylight hours, and only when snails were submerged (Fig. 1-8). The three determinants operate over different time scales: temperature on a monthly scale, light diurnally (24 h period) and water depth controlled by tidal periodicity (12 h period). Each stimulus creates a necessary condition for the next, forming a hierarchy of environmental cues. Duration of cercarial emergence varied over the day, and was under endogenous control. Although many studies have identified two or more factors that affect propagule release, evidence for a cue hierarchy is relatively rare (some exceptions include Bonnewell et al. 1983, Levy et al. 2000, Watson et al. 2000).

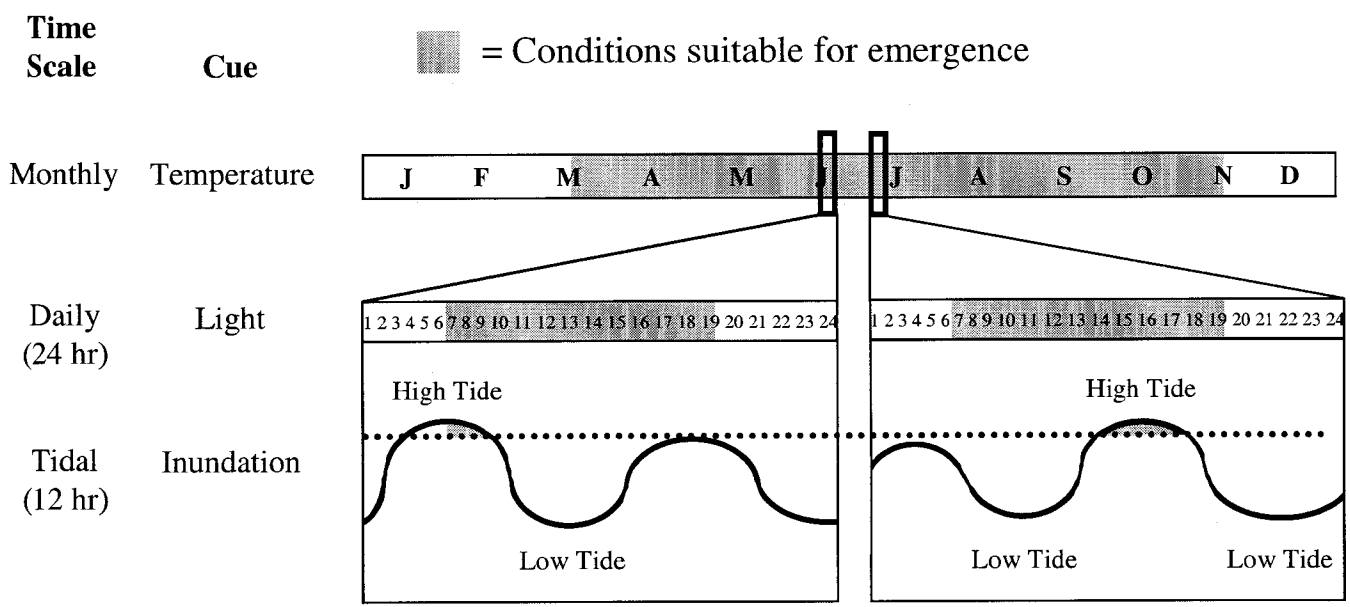
The timing and length of the transmission season was controlled, at least in part, by water temperature. Host snails (*C. californica*) were present on the mud surface of the channel when water was $\geq$ 12 °C. Thus, the narrower temperature ranges for emergence of the five trematode species (compared to those the hosts are exposed to) may be associated with the presence of second intermediate hosts. For

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Figure 1-8. Model of the hierarchy of cues controlling cercarial emergence at monthly (temperature), daily (light) and tidal (host inundation) time scales. Dotted line represents position of an infected snail at tidal height of 1.3 m above MLLW.

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example, cercariae of *H. rhigedana* and *P. acanthus* which use hosts such as *C. californica* and *Pachygrapsus crassipes* (which are both found over a wide temperature range) as second intermediate host emerged over the broadest temperature range ($\geq$ 15 °C). In contrast, *E. californiensis*, which emerges only in $\geq$ 18 °C water, primarily infects *Fundulus parvipinnis* which increases in abundance over the warming summer (Fritz 1975, Brooks 1999, Madon et al. 2001).

Temperature thresholds for cercarial emergence occur in both estuarine and freshwater habitats (Lo and Lee 1996, McCarthy 1999). The number of emerged cercariae also varies with temperature in freshwater species (Shostak and Esch 1990, Lyholt and Buchmann 1996). Temperature-dependent emergence in freshwater species has been ascribed to a trade-off between the number emerged (positively related to temperature) and functional (infective) life span (negatively related to temperature) (McCarthy 1999, Toledo et al 1999a).

A common characteristic of estuarine and freshwater systems is diel variation in cercarial emergence, supporting the first hypothesis (see Introduction). Here, cercariae left submerged snails only during daylight hours. In intertidal regions, tidal currents are a predictable signal that determines both submersion time of the hosts and aquatic transport of the larvae. Thus, emergence of cercariae tracked the daytime flood tide, which changed per diem according to the lunar cycle. The daylight requirement may indicate that light is a critical cue. In

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laboratory flume studies, photo-triggered downward swimming of *H. rhigedana* was effective in the slow flow typical of Carpinteria Salt Marsh (CSM) (see chapter 2). This activity quickly brings larvae to the bed for contact with benthic hosts. Moreover, *E. californiensis* cercariae were observed swimming upwards in response to light (authors' unpubl. data). Such behavior could increase contact with host fish in the water column. Likewise, most freshwater cercariae are shed on the bottom, and light and/or gravity directs swimming up or down, depending on the location of the next host (Combes et al. 1994). Within the CSM estuary, cercarial emergence during flood tides could transport larvae 100-300 m/h. Yet, emerged *H. rhigedana* are dispersed only about 1-2 m before encystment (see chapter 3). Hence, directed swimming by larvae thus may have greatly shortened their transport distances (see chapter 2).

Abbreviated dispersal would retain these asexually reproduced larvae within localized areas of the marsh where their clonal lines could be mixed. Most species in the estuarine trematode guild have benthic second intermediate hosts (snails and crabs) whose populations are interspersed amongst those of their first intermediate host. Turbulent mixing could commingle larval genotypes throughout the marsh, enhancing genetic diversity of the multiple cercariae that encyst on each second intermediate host. Ensuing sexual reproduction between dissimilar genetic parasites within the definitive shorebird host may enhance fitness of the trematode

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population (e.g., Scheltema 1971, Jablonski 1986, Pechenik 1999, for marine invertebrates).

As for digenetic trematodes, larval release in free-living estuarine invertebrates is contingent on tidal flows. For example, estuarine mud-dwelling isopods (e.g., *Paragnathia formica*) only release larvae when high tides reach their burrows, facilitating transport (Tinsley and Reilly 2002). Shore crabs living in high-intertidal refuges limit larval release to nighttime spring tides (Morgan and Christy 1995, Hovel and Morgan 1997). Darkness reduces predation on spawners, whereas large-amplitude tides quickly flush larvae away from diurnal predators. Similarly, in tidally influenced riverine habitats, adult terrestrial crabs, such as *Sesarma haematocheir*, release larvae on a nighttime semi-lunar cycle that minimizes predation on adults while providing optimal conditions for larval survival and dispersal (Saigusa 1982).

The importance of endogenous synchronization of cercarial emergence appears to differ in freshwater verses estuarine systems. For freshwater trematodes, cercarial emergence timing has been shown to be under endogenous control (Theron 1984) and adapted to coincide with host proximity. For example, the vertebrate hosts of *Schistosoma* spp. frequent the waterfront at predictable times of the day. Circadian rhythms of cercarial emergence are tuned to the biological clocks and activity patterns of these vertebrates (Combes 1991, Combes et al. 1994). Cercariae emerge and swim to the surface in the morning, thus infecting the

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animals while they drink at the water's edge (Mouahid et al. 1991, Raymond and Probert 1991). Likewise, afternoon emergence peaks occur in trematode species infecting human hosts. Humans most often wash, play or drink at or after midday (Theron 1984, Pages and Theron 1990). Emergence in non-schistosome species, such as *Proterometra edneyi*, also occurs on a circadian cycle timed to the presence of second intermediate host fish (Lewis et al. 1989). There is no emergence rhythm in *Fasciola hepatica* cercariae, however, because second intermediate host plants are always available (Bouix-Busson et al. 1985).

In the CSM intertidal estuary, duration rather than timing of emergence was apparently under endogenous control, and the tide was the synchronizing agent. There is a finite, tidally determined period of sufficient immersion for larval transport during daylight. Emergence duration decreased throughout the day in response to dwindling daylight. An endogenous rhythm associated with the light:dark cycle may be responsible for the changing emergence rate over the course of a suitable flood tide. Apportioning larvae over the entire lighted submerged interval maximized the dispersal envelope. Contraction of emergence duration throughout the day may optimize use of remaining daylight hours. As the day progresses, larger pulses of larvae must emerge over a shorter interval in order to take advantage of the vestigial light. Such diel adjustments to the emergence period are needless in freshwater systems where water depth is relatively stable. Thus driven by the submergence constraints of their natal habitat, emergence

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strategies of estuarine cercariae involve both exogenous and endogenous factors that optimize transport to tidally accessible hosts.

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Chapter 2

ENVIRONMENTAL CUES, LARVAL TRANSPORT AND MECHANISMS OF PARASITE TRANSMISSION

Transmission of parasites between hosts has long been characterized as a two-step, two-scale process (Salt 1935, Laing 1937). Dispersing larvae first locate host habitat (population scales of meters), and then search for the host (individual scales of centimeters or less). Although transport to the host habitat is requisite for parasite-host interactions, the second step has received markedly greater research attention (Haas 1992, Haas and Haberl 1997, McKerrow and Salter 2002). Moreover, studies of spatial localization in aquatic flukes have been done primarily in still water, mimicking tranquil lentic habitat (Feiler and Haas 1988, Haas et al. 1990, Haas 1994). Different mechanisms may be required for trematodes, in marine and freshwater lotic environments, contending with dynamic flow conditions (Upatham 1974, Bartoli and Combes 1986, de Montaudouin et al. 1998).

Aquatic host-to-host dispersal of cercariae is analogous to planktonic larval transport and settlement of free-living benthic invertebrates. Local (centimeters to meters) variations in supply and settlement can result from interactions between larval behavior and the flow regime (Grosberg 1982, Miron et al. 1991, Mullineaux and Butman 1991). External cues trigger changes in swimming behavior that markedly alter vertical distributions of larvae (Turner et al. 1994, Forward et al.

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1997, Kingsford et al. 2002). Horizontal gradients in velocity and turbulent mixing acting on these vertical distributions then result in differential transport to hospitable habitats (Maldonado and Young 1996, Welch et al. 1999, Garland et al. 2002). Similar research within or mimicking natural flow regimes is lacking for aquatic parasite larvae, but in still water, cercariae rely on external stimuli for host location (Haas et al. 1990, Combes et al. 1994, Loy et al. 2001)

The relative importance of planktonic larval behavior versus passive-transport processes in the maintenance of aquatic parasite infections has never been studied under natural or simulated flow conditions. The present study therefore tested the hypothesis that cercarial swimming behavior of the estuarine trematode *Himasthla rhigedana* favors retention within the benthic habitat of their intermediate hosts (snails and crabs). Within a simulated natural flow regime, I evaluated experimentally whether responses of swimming cercariae to environmental stimuli would facilitate the location of host habitat. This hypothesis could explain the remarkable transmission rate of *H. rhigedana*'s cercarial stage (see next section). Alternatively, cercariae may be transported like passive particles, with high larval production offsetting the need for refined behavior.

The research was conducted in three phases. First, the range of variation in relevant physical factors (light, temperature, salinity and flow) was measured within *H. rhigedana* habitat. Second, effects of light, temperature, and salinity on larval swimming, sinking (anesthetized larvae) and vertical distributions were

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tested in still water. Third, flume experiments were conducted in simulated estuarine flows, using both live and anesthetized larvae to distinguish active from passive movements. Using a new application of laser and digital video imaging technologies, direct measurements were made of larval movements in flow. This technology enabled identification of active versus passive transport processes, with important implications for parasite transmission and larval settlement of free-living invertebrates.

STUDY SPECIES AND FIELD SETTING

The digenetic trematode, *H. rhigedana* (Order Echinostoma), is distributed widely throughout California's estuaries. Sexual reproduction takes place in definitive host shorebirds, from which parasite eggs are released into the marsh. The first intermediate host snails are infected by ingestion of, or penetration by, miracidia larvae that hatch from the eggs. Infected snails cease reproduction while infection persists for the lifetime of the snail (Kuris 1990, Sousa 1990). Asexual reproduction of parthenitae in the snail results in the production of tens to thousands of free-swimming cercariae per snail per day. Cercariae of *H. rhigedana* live for only ~4 h (author's unpub. data) and therefore, once having left their 1st intermediate host snail, must quickly encyst on the hard parts of a second intermediate host snail or crab. Ingestion of this 2nd intermediate host by a shorebird completes the trematode's lifecycle.

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Laboratory conditions were dictated by measurements of light, temperature, salinity and flow in an *H. rhigedana* habitat during the period of greatest cercarial release (May-September, see Chapter 1). Situated in the 93-hectare Carpinteria Salt Marsh Reserve (CSMR), east of Santa Barbara, CA (34° 24' 16" N, 119 31' 30" W), the field site was located within a typical southern California estuary (Ferren 1985, Callaway et al. 1990, Pennings and Callaway 1992). The mudflat used here was situated in a 400-m long by 5-m wide by 0.6 m depth (high tide) channel. This tidal habitat was carpeted with *C. californica* at densities of 460 ± 16 snails/m^2 ($\bar{x}$ ± SE, n = 24 random, bi-monthly quadrats), spanning horizontal distances of 100's of meters (authors' unpubl. data). This species similarly occurs at densities exceeding 500 snails / m2 in other California marshes (McCloy 1979, Race 1981, Sousa 1993). Although only 1-18% of the CSMR snail population was infected by *H. rhigedana* miracidia, up to 100% of same snail populations is infested with *H. rhigedana* metacercariae, indicating highly effective local (within the estuary) dispersal by the cercarial stage (Stevens 1996).

Variations in environmental variables reflect typical estuarine conditions (Table 2-1, Figure 2-1). Temperature rose from 18 °C in May to 24 °C in September, and varied over the tidal cycle. During warmer months (July – September) when cercariae thrived, flood waters were initially heated by the mudflat, but cooled over the first hour to ocean temperature. Daily variations in salinity were 28-34 psu, consistent across seasons. Salinity and temperature were

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relatively constant throughout the water column, indicating well-mixed conditions in the shallow channel. Consistent daily variation in light showed a minimum intensity of ~40 μmol/m^2/s just after sunrise and before sunset, with an afternoon maximum of ~2000 μmol/m^2/s.

Tidal variations in along-channel flow speed and shear velocity (u_*, a measure of turbulent mixing) were consistent through time (month and year) and lunar (spring or neap) phase (two-way ANOVA: $F \leq 0.72$; df = 1/35; $P > 0.40$ for each factor). Along-channel flow speeds were similar between flood and ebb tides (Fig. 2-2A and B), with 1-min averages of 0.6-10.7 cm/s (95% confidence interval, Table 2-1). The predominantly slow flows reached a maximum of only 5 cm/s on 9 of 38 days (Figs. 2-2A and C). There was striking tidal asymmetry in the magnitude of turbulent mixing (Figs. 2-2C and D). Stronger mixing during flood than ebb tides led to significantly higher shear velocities during the flood (one-way ANOVA: $F = 33.04$; df = 1/36; $P < 0.0001$). Usually, u_* was 0.1-0.6 cm/s (95% confidence interval; Table 2-1), with very low values ($\leq$ 0.2 cm/s) characterizing ebb tides and occurring 83% of the time, and values $\geq$ 0.8 cm/s occurring only 1.4 % of the time. Maximal spikes of 1.0-1.5 cm/s were measured on some flood tides during rare storm events.

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Table 2-1. Properties of the physical environment during tidal inundation (4 h interval) at Carpinteria Salt Marsh Reserve for 38 days between May and November (1999 – 2002), the period of cercariae release. The mudflat is exposed to air for ~8 h between each tidal inundation. Maximal water depth was 60 cm during each tide. Using an ORION Model 140 probe, seawater temperature and salinity were recorded 1 cm above the bed every 10 min throughout the tidal cycle. Periodically, data were collected at 1 Hz and three heights above the bottom (1, 5 and 15 cm) to ascertain the presence of temperature or salinity gradients. For these latter measurements, epoxy-encapsulated, copper wire thermistors (YSI model 44000) were used to record temperatures and custom-built, conductivity microelectrodes (based on a modified Wheatstone bridge circuit) to measure salinities. An underwater quantum sensor (LICOR Model 190SA) was flush mounted with the surface of the mudflat before tidal inundation to register light intensity at 1 Hz over the duration of the flood and ebb tides. Flow velocities were measured using an Acoustic Doppler Velocimeter (SONTEK FieldADV), with a sampling volume of ~0.1 cm^3. The probe head of the ADV was mounted on a tripod anchored in the middle of the mudflat and speeds were recorded at 2 cm above bottom. Flows in the along-channel and vertical axes were taken at 1 Hz over each tidal cycle. During the course of the 38 sampling days, the full range of tides, from neap to spring, occurred in approximately equal numbers. Boundary shear velocities (u_*) were calculated using the Reynolds stress method (Schlichting 1979).

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Table 2-1 (cont.)

Variable	Median	Range (percentile)	
		5th	95th
Light (μmol/m^2/s)	1,012	40	2,000
Temperature (°C)	22	18	24
Salinity (psu)	33	28	34
Flow (cm/s)			
Along-channel speed (u)	3.8	0.6	10.7
Fluctuating component † of along-channel speed (u')	0.9	0.1	3.8
Vertical speed (w)	-0.1 §	-1.0 §	0.8
Fluctuating component † of vertical speed (w')	0.2	< 0.1	0.7
Shear velocity (u_*)	0.1	< 0.1	0.6

†Calculated as the standard deviation (root mean square) using repeated measurements (60 ADV recordings) taken over 1-min intervals. §Negative value indicates flow towards the bed.

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Figure 2-1. Representative conditions of (A) water depth, (B) temperature, (C) light intensity and (D) salinity over one complete tidal cycle (June 20, 2000) at the Carpinteria Salt Marsh Reserve. Temperature and salinity determinations were each made using three sensors placed at 1, 5 and 15 cm above the bed. A single temperature trace shows because there was no variation as a function of height above bottom.

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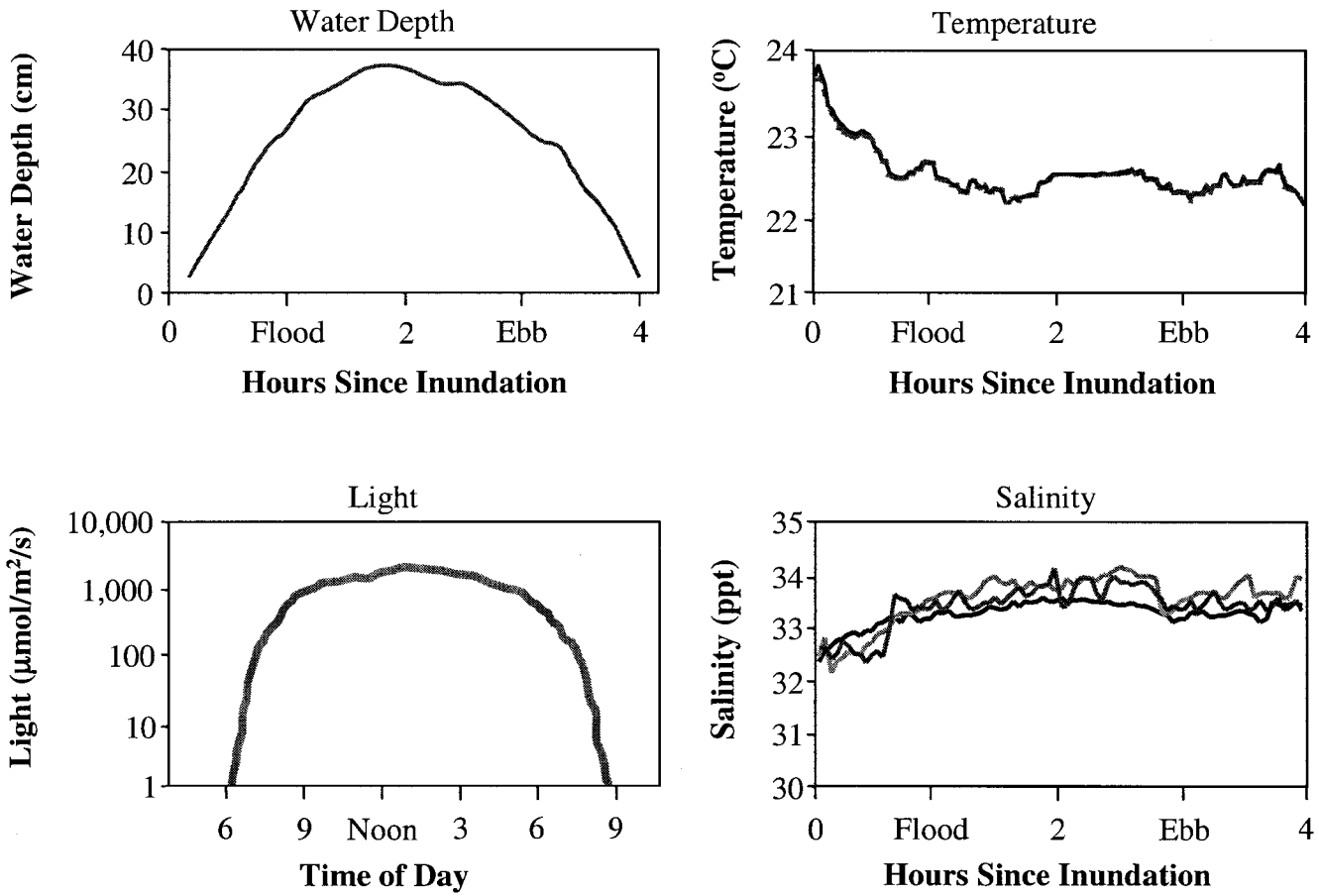
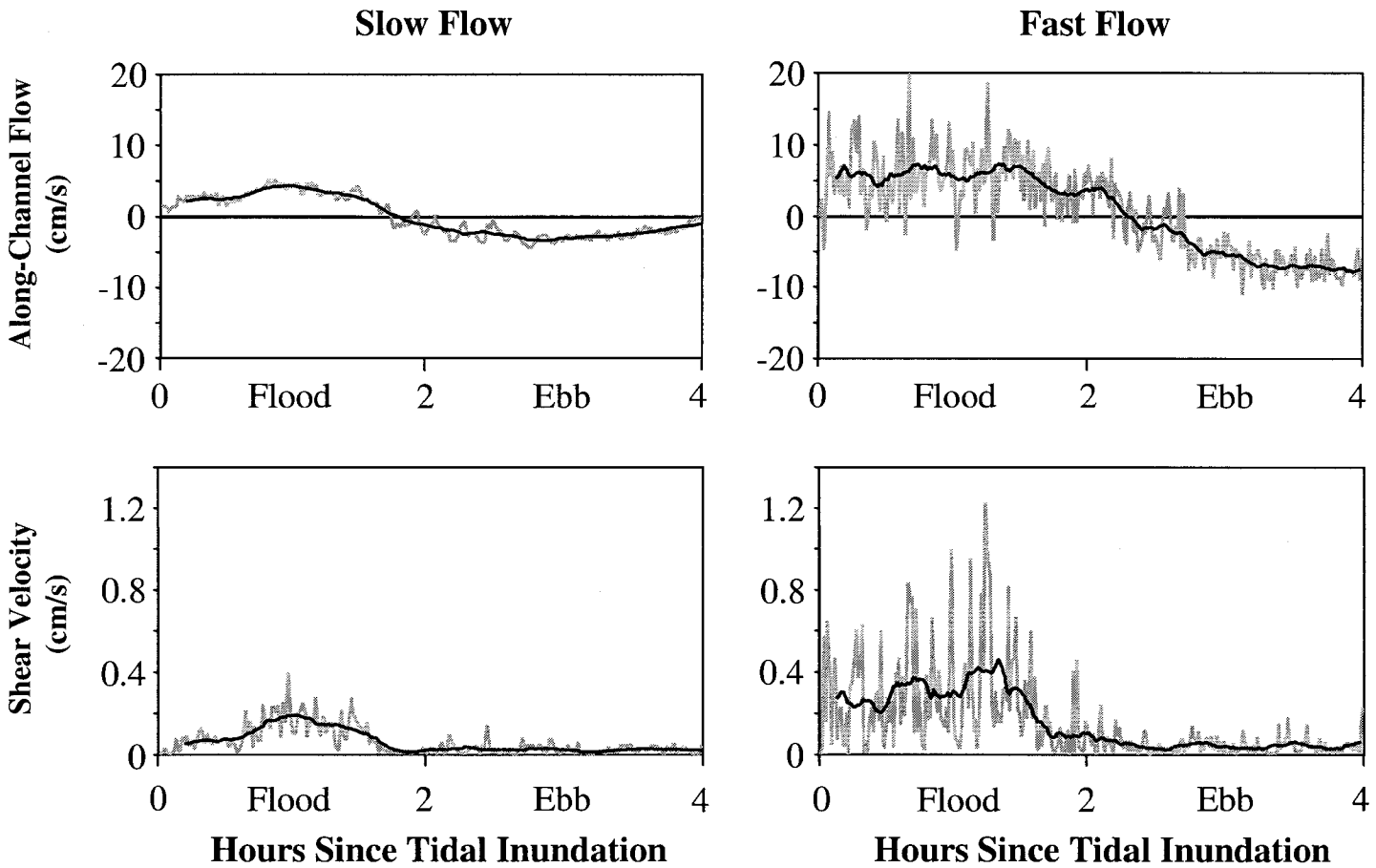


Figure 2-2. Representative tidal flow conditions in the Carpinteria Salt Marsh Reserve. Plotted are two complete tidal cycles, one with typically slow (A) and the other with relatively fast (B) along-channel flow speeds and bottom shear velocities (C, D). The flood tide (positive values in A, B) transports coastal water into the marsh, whereas the ebb tide (negative values in A, B) drains the marsh. In each plot, a 1-min average and 20-min running mean are shown.

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MATERIALS AND METHODS

Quantifying larval swimming and vertical distributions

A new technique was developed to quantify, simultaneously, larval swimming (paths and speeds) and vertical distributions in still and moving water. (1) Larvae were exposed to darkness, or to diffuse, overhead sunlight or incandescent light (GE Daylight Ultra) within a baffled housing. The bulbs reproduced the natural spectral energy and angular distribution of visible sunlight (authors' unpubl. data). Infrared (IR) wavelengths were removed before larval illumination using a low-pass optical filter (< 720 nm). (2) A narrow, focused light sheet was created using an IR (830 nm, 25 mWatt) laser diode (Coherent model NT54-029), equipped with a concave line-generating lens. The thin laser sheet illuminated only larvae within the camera's focal plane (3 mm thick X 10 cm long X 10 cm deep). Behavior and distributions were non-intrusively recorded under IR light that is invisible to cercariae (Rea and Irwin 1992). (3) Larval images were captured using IR-sensitive video cameras (COHU Model 6415-2000, with active heads) oriented 90° to the axis of the laser sheet. A high-pass filter (> 820 nm) on the camera lens ensured detection of only those larvae reflecting the IR light, thereby eliminating errors due to parallax artifacts.

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Computer-Assisted Video Motion Analysis (CAVMA, Motion Analysis Corporation Model VP320 and custom software) was used to digitize images (60 fields/s) and generate paths swum by laser-illuminated larvae (methods of Lopez-de-Victoria et al. 1995). Each larva was 3-4 pixels in length and highly reflective of IR light. To avoid pseudo replication and identify single paths of individuals, one-frame (2 video fields) snapshots were captured at specific times from initiation of a trial. Each path was split into X and Y coordinates thus yielding the exact position and instantaneous swim/transport speed of each larva on a field-by-field basis.

Still-water experiments

Behavioral responses of larvae to environmental variables — Vertical distributions of cercariae were measured over natural ranges of environmental variables: temperatures of 18 and 24 °C, salinities of 28 and 34 psu, and light intensities of 40 and 2000 μmol/m^2/s. To collect cercariae of *H. rhigedana*, large numbers of *C. californica* were transported bi-weekly from CSMR to UCLA. Snails were initially scanned for *H. rhigedana* infections by placing them in individual 27cm^3 chambers filled with water from CSMR. These chambers were placed in light conditions of 40 μmol/m^2/s at 24 °C for 1 hour, after which those snails identified to have shed *H. rhigedana* (Martin 1972) were set aside for further

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use. Groups of 10 infected snails were placed in separate plastic containers (10 cm X 10 cm X 5-cm tall) filled with 0.45 μm-filtered marsh water, replaced daily and aerated constantly. When stimulated by low-intensity incandescent light, snails shed cercariae daily for ~ 2-3 weeks. Larvae were video recorded under controlled environmental conditions in a clear chamber (5 cm long X 5 cm wide X 10 cm tall; 250 ml fluid volume). For each trial, 100 (± 10 range) newly emerged cercariae (< 30 min old) were pipetted into the chamber. A larva was tested only once.

The chamber was placed in a flow-through water bath, with a 10-cm-wide window in front, illuminated from above. After mixing the larvae throughout the water column, video observations began (time = 0 s) when fluid motion ceased. Infrared-sensitive video cameras with macro lenses (SMC Pentax-FA 1:2.8 100 mm) were mounted on an opto-mechanical rail system (Newport Corporation, CA) with the focal area of each camera covering half of the water column. Changes over time of vertical larval distributions were determined from one-frame snapshots captured at 1, 10, 20, 30 and 240 s, and were analyzed by CAVMA. Wall artifacts were avoided by analyzing only larvae that swam ≥ 5 mm from chamber sides (Vogel 1994, Gee and Zimmer-Faust 1997).

Effects on larval swimming behavior of light, temperature and salinity were tested in a modified, factorial-design experiment (Fig. 2-3; 12 treatments, 5-6 replicates per treatment). Because cercariae emerge from hosts only during daylight and live for ~4 h, they are absent from the water column at night (Stevens

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1996, see chapter 1). Thus, trials were conducted in outdoor and indoor light, with dark controls. Outdoor light intensities included full sunlight (~2000 μmol/m^2/s = peak afternoon) and sunlight reduced via a neutral-density filter (~40 μmol/m^2/s = dawn/dusk). To maintain constant light levels between runs, all trials were conducted from 1200 to 1400 h PDT (Pacific Daylight Time) during July and August 2000. Because flume experiments were indoors under artificial light, another set of trials involved full-spectrum lighting (GE Daylight Ultra) at ~40 μmol/m^2/s, mimicking dawn/dusk. Varying the flow of cold water through the bath controlled the temperature (18 or 24 °C) in the chamber. Salinities of 28 or 34 psu were prepared from Forty Fathoms^{tm} artificial seawater. Indoor dark controls involved only IR laser light. A separate set of tests to distinguish between photo- and geo-tactic behaviors was run at 24 °C and 40 μmol/m^2/s by adjusting the relative orientation of the light source and the cercarial chamber to provide up, side, and downwelling light conditions.

Changes in vertical larval distributions were analyzed initially using multi-way contingency tables testing effects of light intensity and source, salinity, temperature and elapsed time of video observations. Raw frequency data from all trials within a treatment were pooled into aggregate cercariae distributions, expressed relative to height above bottom. For each treatment and time, data were combined only after a repeated-measures *G*-test showed no significant difference between larval distributions among replicate trials (*G*-test: $P \geq 0.76$, all

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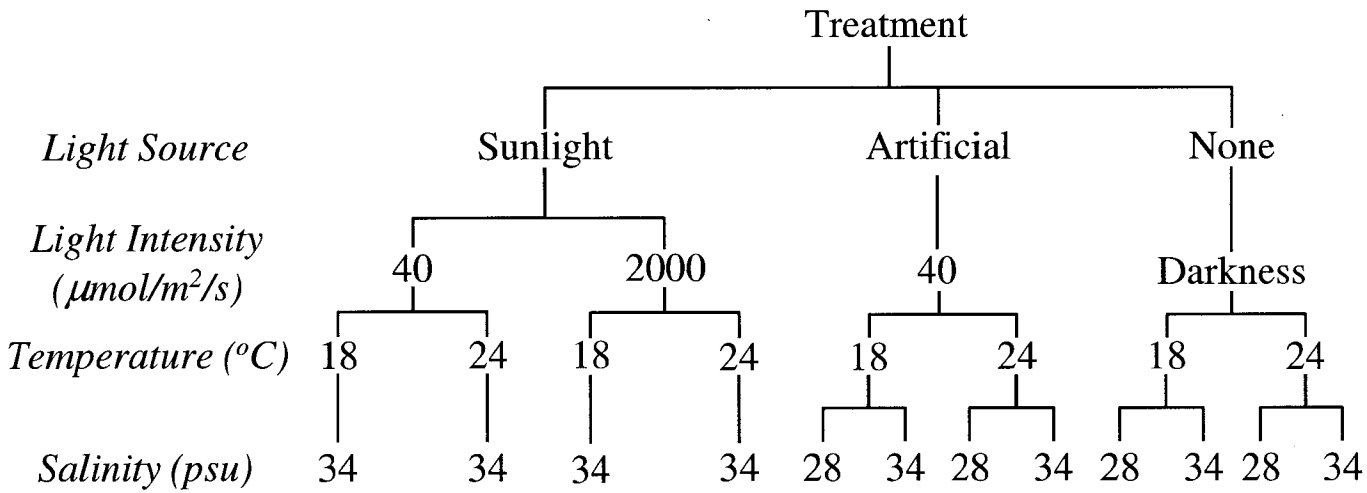
comparisons) (Sokal and Rohlf 1981). Cochran-Mantel-Haenszel (CMH) general association statistics were used to test the overall significance of each main effect in the multi-way tables, holding other factors constant. *G*-tests were then used on two-way tables extracted from the full data matrix.

Gravitational fall velocities of larvae — Passive sinking rates of anesthetized cercariae were measured in a temperature-controlled fall-velocity apparatus (after Hannan 1984). Nested Plexiglas chambers were 15 cm square by 40 cm tall (inner) and 30 cm square by 50 cm tall (outer). The inner chamber was elevated 5 cm above the bottom and below the top of the outer chamber. Both chambers were filled with 1-μm filtered seawater, equilibrated to 18 °C or 24 °C. Larvae narcotized in tricaine methanesulfonate (Finquel), and washed twice in seawater, were added through a snorkel at the top of the inner chamber and video recorded for the middle 20 cm of their descent. These paths were analyzed using CAVMA to obtain terminal sinking velocities.

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Figure 2-3. Schematic diagram showing the 12 experimental treatments in still water, performed as a modified factorial design. Each treatment was replicated in 5-6 trials. Initial tests conducted in artificial light and darkness indicated no significant salinity effects. Thereafter, all trials in sunlight were run at a single salinity (34 psu). Intensity (2000 $\mu mol/m^2/s$) of full sunlight could not be reproduced by the artificial source.

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Flume experiments

Snails infected with *H. rhigedana* were shipped by overnight courier to the Woods Hole Oceanographic Institution (WHOI) for flume studies. Animals were maintained in 0.45-μm-filtered seawater at a salinity (34 psu), temperatures (18 or 24 °C), and light/dark cycle (14 L/10 D at 40 μmol/m^2/s) typical of the CSMR field site. Prior to flume experiments, still-water assays were performed to evaluate larval condition. Gravitational fall velocities and instantaneous vertical swim speeds were virtually identical between cercariae shed at WHOI and at UCLA (Students *t*-test: $t \leq 0.28$; df ≥ 56; $P > 0.75$, all comparisons). Thus, there was no apparent affect of shipping or handling on larval characteristics relevant to active swimming or passive transport.

Experiments were conducted in fully-developed boundary-layer flows in a temperature-controlled flume (17 m length X 0.6 m width X 0.11 m water depth; Butman and Chapman 1989). Seawater was first passed through a 1-μm particle filter, an activated charcoal bed, and an ultraviolet sterilization unit before entering the flume. The working section was located 10-15 m from the channel entrance. After one pass down the channel, cercariae were removed by a 53-μm-mesh net spanning the terminus. Before each run, a Laser Doppler Velocimeter (LDV) measured (4 min each) flow speeds in the working section at 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 7, and 9 cm off the bed. From each LDV profile, shear velocity was calculated

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using a semi-empirical expression for steady, open-channel flow over a smooth bottom (Trowbridge et al. 1989).

In still water, the influence of salinity on larval behavior was not significant, and the impact of light was not dependent on intensity (see Results). All flume experiments therefore were conducted under a single set of light (~40 $\mu mol/m^2/s$) and salinity (34 psu) conditions. Full-spectrum lights with low-pass optical filters were positioned above the flume and along the complete length of the working section to create nearly uniform illumination. To block ambient light while experiments were underway, the entire set-up was tented under black plastic.

Based on field measurements, flume trials were conducted at two water temperatures (18 and 24 °C) and in two flow regimes ($u_* = 0.2$ and 0.8 cm/s). The low u_* is typical of everyday flows ($u_* \leq 0.2$ cm/s, 83 % of the time in the marsh). In contrast, the high u_* represents the rarer, faster flows that only occur during storm conditions ($u_* \geq 0.8$ cm/s, 1.4 % of the time). Intermediate values are not pertinent because most shear velocities fell below 0.2 cm/s. Separate flume runs were done with live and anesthetized larvae to distinguish passive from active behavioral effects. An average of 8 replicate trials was performed for each of 6 treatments. In vertical profiles of cercariae measured in the field over the season (May-September) of cercarial release, larvae commonly occurred 5 cm above the bottom, and sometimes as high as 15 cm above the bed (see chapter 3). Thus, in each flume trial, an average of 2,150 ($\pm$ 353 range) newly emerged cercariae were

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introduced throughout the water column at the upstream end of the working section. A vertical Lucite tube (3 cm ID), with two 2.5 cm^2 mesh (53 μm Nytex) panels cut into its sides, was inserted just below the water line until the water level equalized. The cercariae were mixed throughout the tube water column, then the cylinder was lifted straight up, and larvae released to drift/swim downstream.

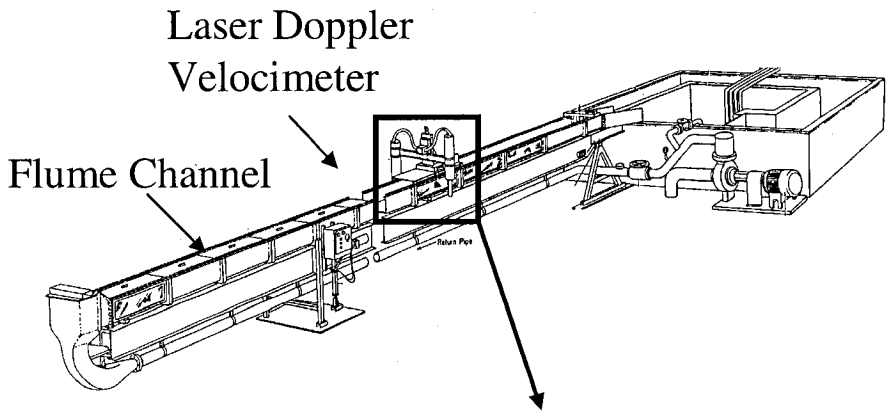
Vertical profiles of cercariae were video recorded 0-5 cm above the bottom. An IR-laser and line-generating lens were positioned above the flume on an opto-mechanical rail and post system downstream of larval introduction. This layout produced a 3-mm thick light sheet, parallel to flow direction, along the flume midline. An IR-sensitive camera, with attached macro-lens, faced inward towards the glass flume wall, orthogonal to the plane of the laser sheet. A cloud of drifting cercariae was video recorded as it passed through the IR-illuminated focal plane (figure 2-4).

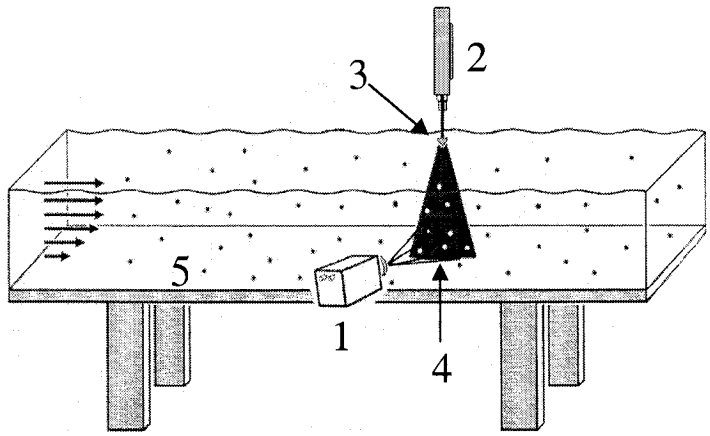
To maintain similarity in behavioral response times between the two flows, I scaled the transport distance from the point of larval addition by first integrating the vertical velocity profiles. Mean speeds of 3.3 cm/s and 15.0 cm/s were then multiplied by the time (30 s) for larval distributions to reach steady state in still water. Thus, cameras were located 1 or 4.5 m downstream from larval addition in slow ($u_* = 0.2$ cm/s) or fast ($u_* = 0.8$ cm/s) flow, respectively.

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Figure 2-4. Diagram of the flume experimental set up. (Upper panel) View from the upstream end of the flume. The large apparatus in the middle, with instrument cases on either side of the flume, is the Laser Doppler Velocimeter (LDV) used for detailed vertical profiles of velocity. (Lower panel) View of flume water column in the working section. Particles (anesthetized or live cercariae) are shown in white when illuminated by an infrared (IR)-laser sheet. Images are recorded by an IR-sensitive video camera positioned to the side. The laser and line-generating lens are mounted above on a rail system.

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1. Camera, macro lens and filter
2. Infra-red laser diode
3. Sheet-generating optics
4. Video field
5. Flume bed

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RESULTS

Still-water experiments

Behavioral responses of larvae to light — Stimulation by light caused a directional swimming response in cercariae. Upon immediate exposure ($\leq$ 1 s), and regardless of light intensity (40-2000 μmol/m^2/s), source (real or artificial), or orientation (up, side or downwelling), cercariae swam straight toward the bottom (Fig. 2-5 and Table 2-2). Significant bottom-skewed distributions were observed by 10 s (Fig. 2-6, and *G*-test comparing data collected at 1 and 10 s: $G \geq 6.92$; df = 1; $P < 0.01$, all comparisons), and by 20 s, > 80% of all larvae were within the lower half of the chamber. There was no significant difference between vertical larval profiles at 30 or 240 s (Fig. 2-6, and *G*-Test: $G \leq 0.26$; df = 1; $P > 0.50$, all comparisons), and thus, distributions reached equilibrium without observed settlement or encystment. Once contacting the bottom, most larvae swam either up-and-down or back-and-forth within ~1.5 cm of the bed. Horizontal swimming was infrequent compared to vertically directed movements and the bottom acted as a reflective boundary, causing cercariae to swim upward briefly before swimming downward again. As a result, direction and speed of swimming varied predictably as a function of larval height above the bed (Fig. 2-5B and D, and Table 2-3). In

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dark control trials, cercariae swam upward, and significant top-skewed distributions developed over time (Fig. 2-6 and Table 2-2).

Behavioral responses of larvae to temperature and salinity, and gravitational fall velocities — To eliminate interactions between cercariae and the bottom, horizontal and vertical swim speeds were measured for larvae swimming in the top half of the chamber during the initial 10 s of each trial. Light triggered downward movements, but had no effect at any intensity on swim speeds (Table 2-4). Varying salinity had no impact on swim speeds or directions. In contrast, a 33% increase in temperature (18 to 24 °C), elevated swim speeds by 71% (2.1 ± 0.1 mm/s to 3.6 ± 0.2 mm/s) (Table 2-5), and effects were constant over the entire life span (~4 h) of a cercaria (Fig. 2-7B). A gradual decrease in temperature from 24 to 18 °C over 1 h, mimicking a typical flood tide in the field (Fig. 2-7A), caused cercaria swimming to slow down accordingly (Fig. 7C; Model I linear regression, F-test: $F = 141.65$; df = 1/175; $P < 0.0001$). Not surprisingly, gravitational fall velocities of anesthetized cercariae were essentially identical for all treatments (Table 2-5; ANOVA: $P \geq 0.66$, all treatments and interactions). Moreover, downward swimming minus passive sinking yielded typical horizontal swim speeds (Table 5).

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Table 2-2. Summary of multiway contingency table analysis using Cochran-Mantel-Haenszel general association statistics to evaluate vertical larval distributions in still-water trials. Effects of light/dark, light intensity (40 and 2000 μmol/m^2/s), light source (natural and artificial), salinity (28 and 34 psu), temperature (18 and 24 °C), and elapsed time (1, 10, 20, 30 and 240 s) of video observations were considered. Raw frequency data were categorized (0-9, 10-19, 20-29, 30-29, 40-49, ≥ 50 mm) according to larval heights above the bottom of a 100-mm tall test chamber. Observations above 50-mm height were pooled to produce expected frequencies of ≥ 5 larvae in each height class.

Variable	CMH statistic	df	*P*
Illumination			
Light/dark	1,547.24	80	< 0.0001
Light source	38.70	40	0.53
Light intensity	29.99	40	0.88
Salinity	87.02	80	0.34
Temperature	168.81	120	0.003
Elapsed time	1,076.76	144	< 0.0001

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Table 2-3. Regression equations describing the relationship between instantaneous vertical swim/transport speed (x-values, mm/s) and larval height above the bottom (y-values, mm) in laboratory still-water and flume experiments. In each trial, data were captured as one frame (two video fields) snapshots at 30 s elapsed time, and at 34 psu (salinity) and 40 µmol/m^2/s (light intensity). The y-intercept specifies the height at which the mean speed of the larval population equals zero; a negative slope indicates that larvae move faster towards the bed with increasing height.

Flow condition	Temperature (°C)	Larval activity state	Regression. equation	df	F-value	P
Still water	18	Live	$y = 19.3 - 4.3x$	1, 104	5.08	0.021
	24	Live	$y = 13.0 - 4.6x$	1, 108	21.42	< 0.001
Flume, slow	18	Live	$y = 18.9 - 5.2x$	1, 31	4.58	0.032
	24	Live	$y = 14.2 - 4.1x$	1, 28	7.71	< 0.001
	18	Anesth.	$y = 23.9 + 0.5x$	1, 44	0.10	0.916
	24	Anesth.	$y = 27.0 + 22.5x$	1, 69	0.93	0.378
Flume, fast	18	Live	$y = 25.2 + 0.4x$	1, 45	1.48	0.481
	24	Live	$y = 24.5 + 0.2x$	1, 50	0.51	0.837
	18	Anesth.	$y = 24.8 + 0.3x$	1, 53	0.62	0.732
	24	Anesth.	$y = 45.1 + 0.5x$	1, 44	0.10	0.914

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Table 2-4. Results of two-way ANOVAs testing effects of light source (natural and artificial), light intensity (40 and 2000 μmol/m^2/s), temperature (18 and 24 °C) and salinity (28 and 34 psu) on larval swim speeds.

Source of variation	df	SS	MS	*F*-value	*P*
A.					
Light source	1	0.52	0.52	1.09	0.30
Temperature	1	86.92	86.92	182.77	< 0.0001
Source X temp	1	0.04	0.04	0.08	0.77
Error	168	79.90	0.48		
Total	171	167.38			
B.					
Light intensity	1	< 0.01	< 0.01	0.01	0.92
Temperature	1	85.20	85.20	178.95	< 0.0001
Intensity X temp	1	0.46	0.46	0.96	0.33
Error	168	79.99	0.48		
Total	171	165.65			
C.					
Salinity	1	0.11	0.11	0.08	0.78
Temperature	1	119.56	119.56	81.02	< 0.0001
Salinity X temp	1	< 0.01	< 0.01	< 0.01	0.95
Error	132	194.78	1.48		
Total	135	314.45			

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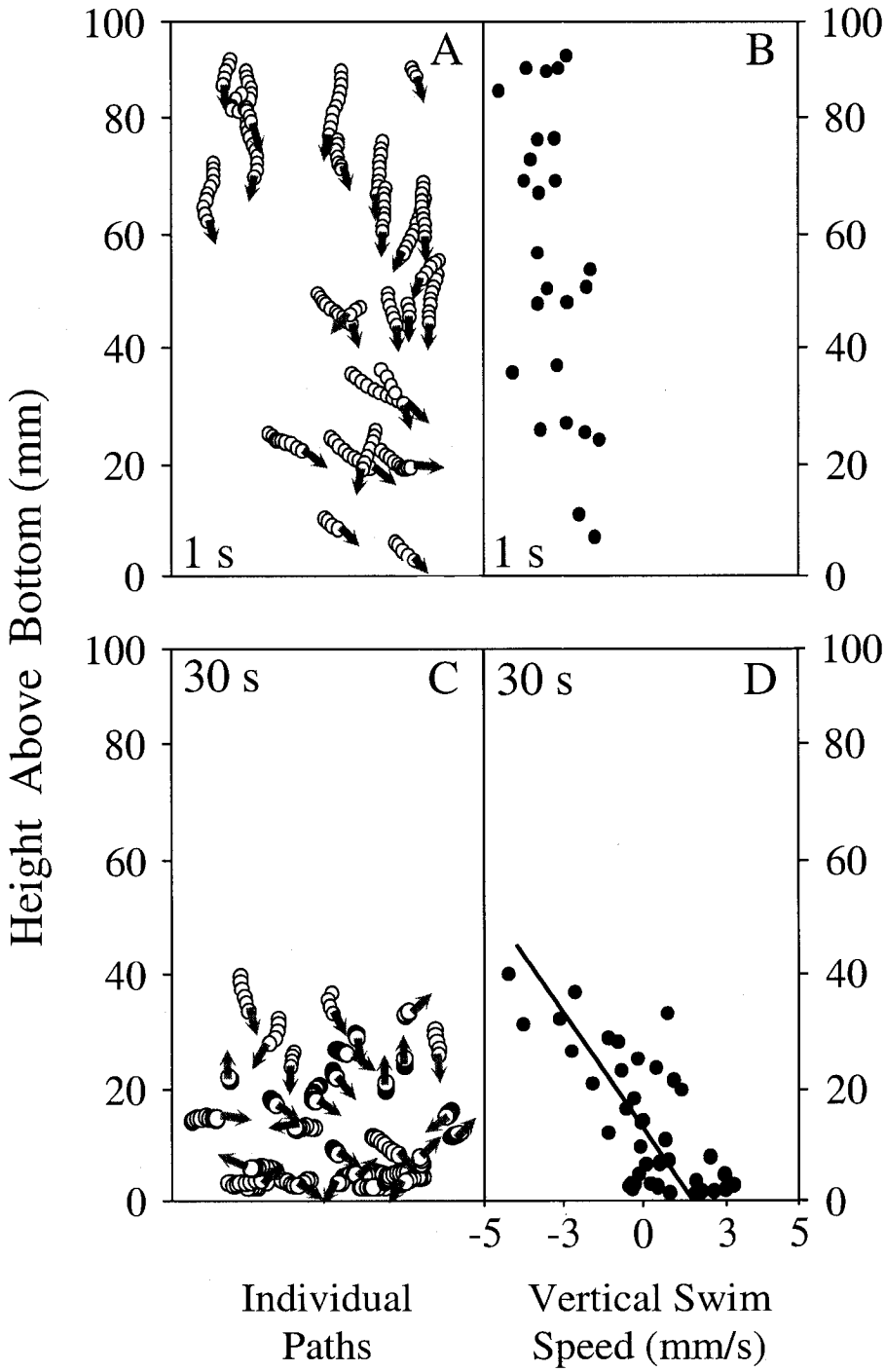
Table 2-5. Effects of temperature on cercaria gravitational fall velocities and swim speeds in still water. Observations are pooled for different light intensities and salinities (see text and Table 4).

Type and direction of movement	Temperature (°C)	Mean speed (± SEM) (mm/s)	Number of larvae observed
Swimming			
Horizontal			
	18	2.1 (± 0.1)	152
	24	3.6 (± 0.1)	168
Vertical downward			
	18	4.2 (± 0.2)	157
	24	5.9 (± 0.2)	165
Gravitational fall			
Vertical downward			
	18	1.9 (± 0.1)	28
	24	1.9 (± 0.1)	32

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Figure 2-5. Real-time paths and instantaneous vertical swim speeds of cercariae in one representative still-water trial (artificial light at 40 μmol/m^2/s, 34 psu and 24 ^{o}C). Elapsed times were 1 s (top) and 30 s (bottom) after initiation of video observations. Each path of dots in (A) and (C) indicates larval position at consecutive one-frame intervals (video rate of 10 frames/s). Instantaneous vertical swim speeds in (B) and (D) are based on one-frame snapshots (two video fields); + is upward and - is downward swimming. In one case (D), the linear regression of swim speed on height was significant ($y = 12.38 - 4.7x$; F-test: $F = 29.99$; df = 1/37; $P < 0.001$).

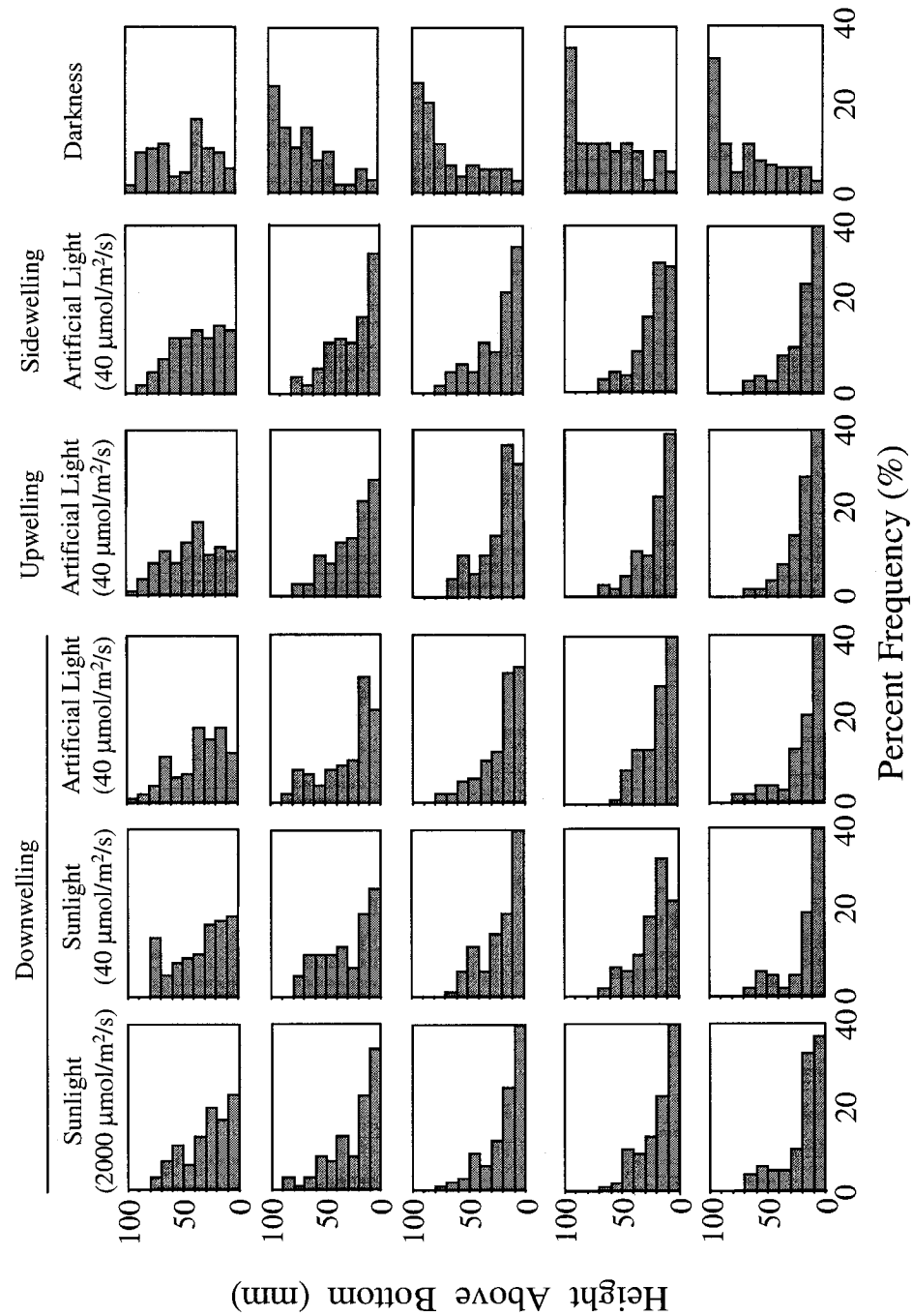
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Figure 2-6. Effects of light source, light intensity, light orientation, and darkness on the evolution (1, 10, 20, 30, 240 s) of vertical larval distributions in still water. Height-frequency histograms were constructed from data pooled over replicate trials, such that $N \geq 138$ larvae for each treatment. These histograms summarize results for experiments run at 24 °C and 34 psu only. At 18 °C, vertical distributions evolved more slowly to the same end (i.e., as at 24 °C). Salinity had no significant effect on the evolution of larval distributions (see Table 2).

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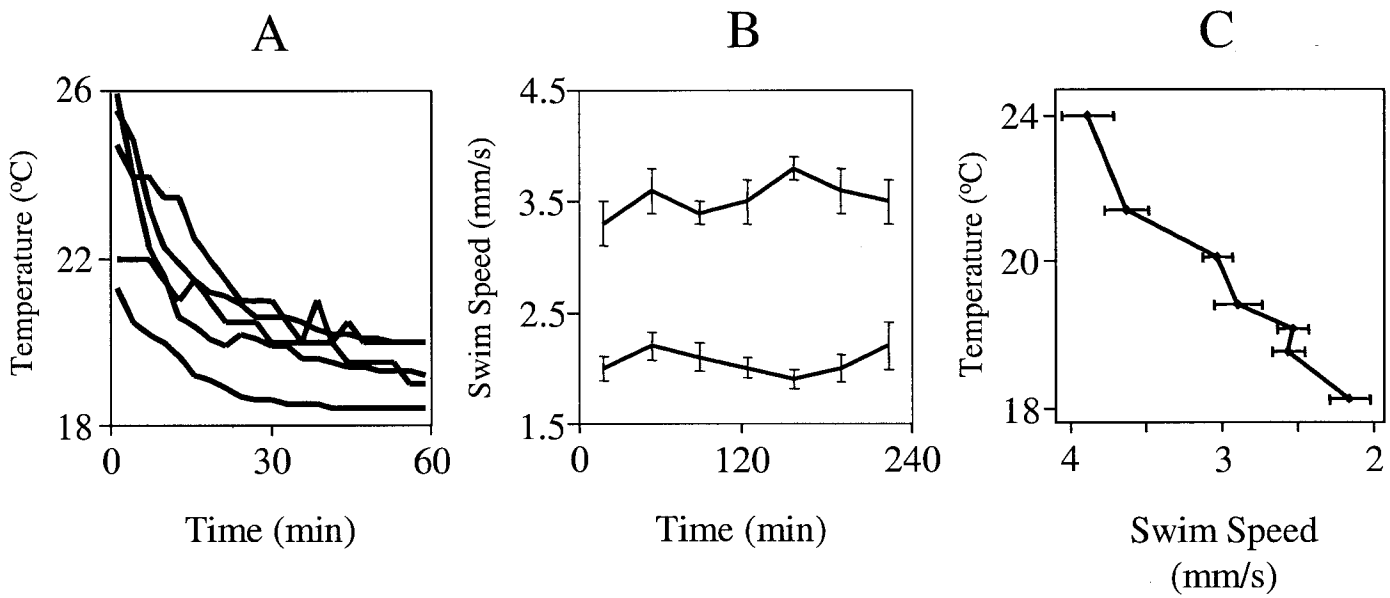


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Figure 2-7. Effect of temperature on horizontal swim speed. (A) Five representative temperature time series recorded over the first hour of tidal inundation (see Table 1 for methods). (B) Mean (± SE) larval swim speeds at constant high (24 °C) and low (18 °C) temperatures (34 psu and 40 $\mu mol/m^2/s$) over the course of the cercaria life span (~4 h). (C) Swim speeds of cercariae placed in a temperature regime approximating tidal inundation in the field: temperature decrease from 24 to 18 °C over 1 h (see A). For both (B) and (C), $N \geq 30$ larvae at each data point.

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Flume experiments

During flume experiments, video "snapshots" were taken of cercariae clouds passing by downstream measurement sites. Because suspended larvae were subjected to instantaneous fluctuations in flow speed (Table 2-6), their exact swimming rates could not be determined. Nevertheless, using CAVMA the relative contributions of behavior and hydrodynamics to larval distributions and transport/swim speeds along the vertical axis can be clearly established. In fast flow, turbulent mixing overwhelmed behavioral effects and distributed cercariae, like passive particles, throughout the water column (Fig. 2-8 and Table 2-3). Vertical distributions varied little with depth; there was no temperature effect; and, these patterns did not differ significantly for live and anesthetized cercariae (Fig. 2-9; *G*-Test: $G \leq 6.92$; df = 4; $P \geq 0.16$, both comparisons).

In contrast, larval behavior prevailed over turbulent mixing under slow-flow conditions and the evolution of vertical profiles was remarkably similar to those in still water. Relative to anesthetized larvae, significant bottom-skewed distributions of live cercariae developed at both temperatures in slow flow (Fig. 2-9, and *G*-Test: $G \geq 9.62$; df = 4; $P \leq 0.042$, both comparisons). This pattern was more pronounced at 24 °C, denoting faster downward swimming in warmer water. Under still water and slow flow conditions, regression equations describing vertical transport/swim speeds as a function of height above bottom were almost identical

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for live larvae (Fig. 2-9 and Table 2-3; ANCOVAs: $P \geq 0.75$, all comparisons). The flume bottom thus acted as a reflective boundary to downward swimming larvae; live cercariae did not behave like passive particles (anesthetized larvae) either nearby or away from the bed.

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Table 2-6. Mean (± SD) shear velocities and flow speeds used in flume experiments.

Flow treatment	Shear velocity (cm/s) (u_*)	Along-channel speed (cm/s) ($\overline{u} \pm u'$)	Vertical speed[†] (cm/s) ($\overline{w} \pm w'$)
Slow	0.22 (± 0.01)	3.26 (± 0.40)	-0.02 (± 0.23)
Fast	0.83 (± 0.02)	14.95 (± 1.44)	-0.12 (± 0.95)

[†]Negative value indicates flow towards the bed.

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Figure 2-8. Instantaneous speeds of vertical movement (swimming or sinking) for live and anesthetized cercariae as a function of height off the bottom. Experiments were run at 40 μmol/m^2/s, 24 °C, and 34 psu in still water (A), slow flow (B, C), and fast flow (D, E). Each dot indicates the speed of vertical movement (+ is upward and - is downward) for a single larva, based on one-frame snapshots (two video fields) captured over 30 s. Data were pooled over replicate trials; $N \geq 30$ larvae for each treatment. In two cases (A, B), the linear regression of swim speed on height was significant (*F*-tests: $P < 0.05$; see Table 3).

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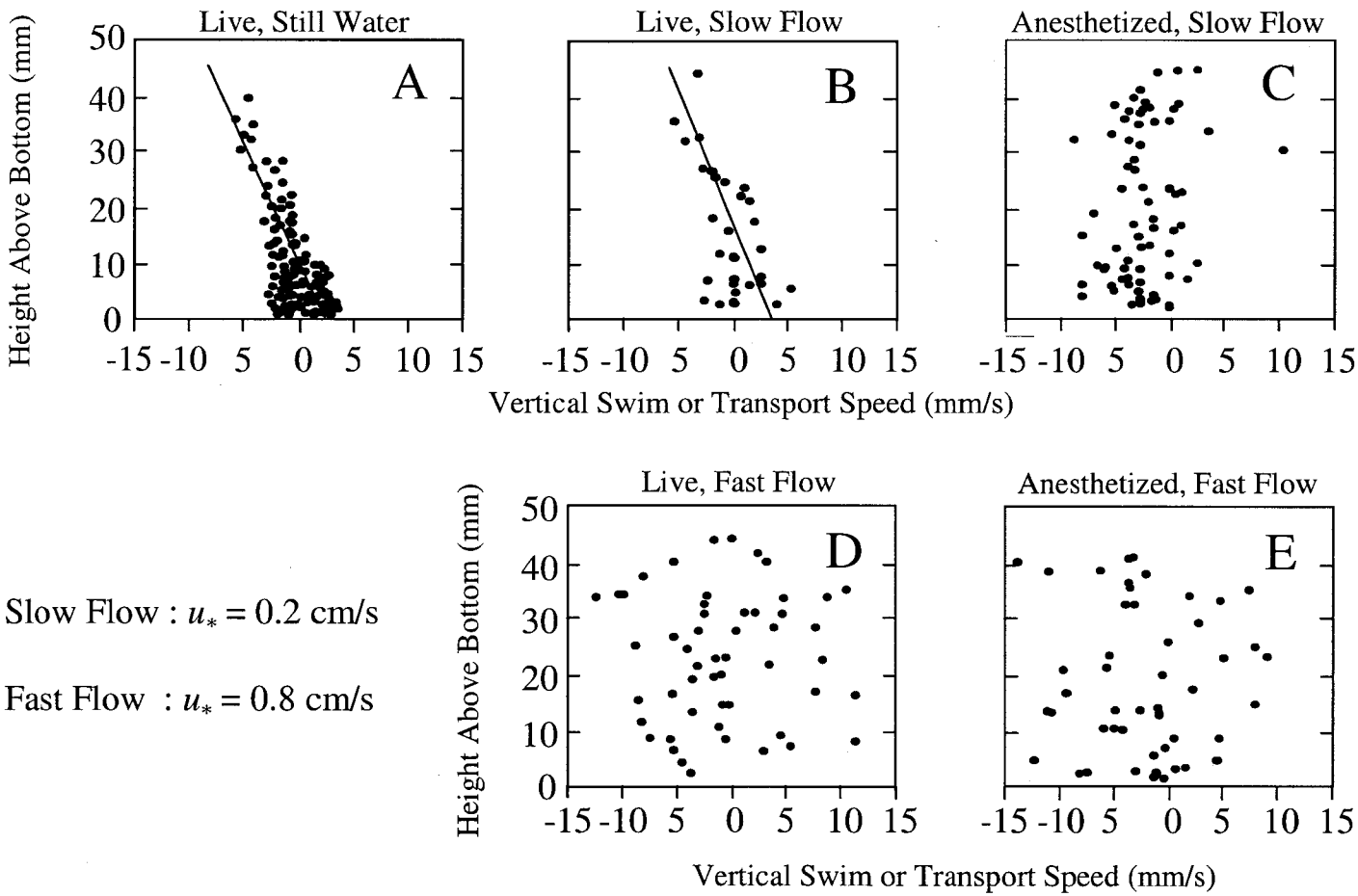
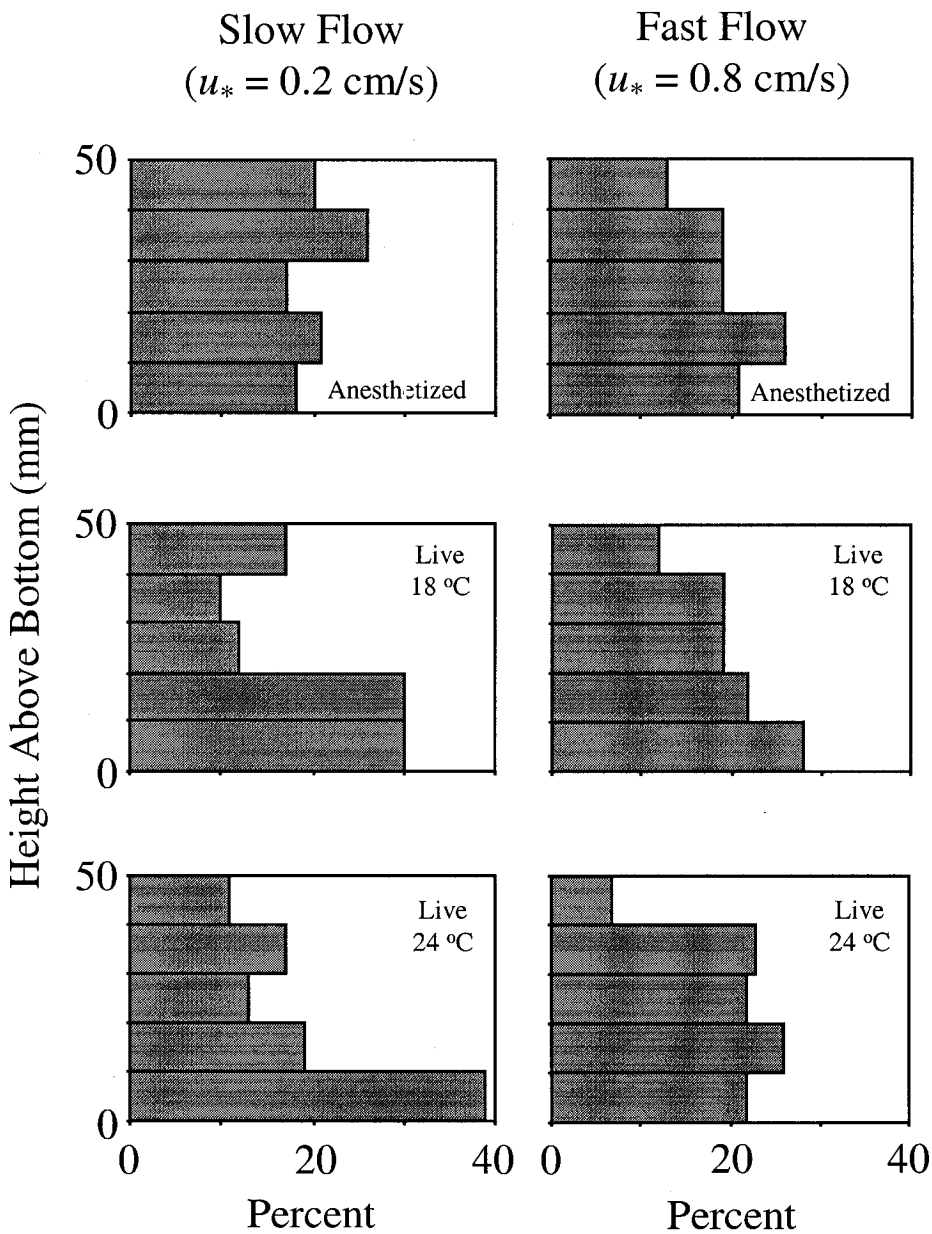


Figure 2-9. Effects of temperature and flow on the evolution of vertical larval profiles. Height-frequency histograms were constructed from data pooled over replicate trials, such that $N \geq 42$ live or anesthetized cercariae for each treatment. These histograms summarize results for experiments run at 40 $\mu mol/m^2/s$ and 34 psu, with measurements recorded over 30 seconds. The vertical distributions of anesthetized larvae at 18 and 24 ^{o}C were non-significantly different (G-test: G = 0.36; df = 4; P = 0.91) and therefore combined.

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DISCUSSION

Aquatic dispersal stages of parasites respond to environmental signals that would facilitate navigation toward host habitat (Ulmer 1971, Haas 1994). This study is the first, however, to demonstrate that cercarial swimming behavior is effective within the simulated hydrodynamic regime of their benthic hosts. Photo-triggered downward swimming resulted in rapidly developing bottom-skewed larval distributions. The consistent movement of larvae towards the bed, regardless of orientation, identified the behavior as positive geotaxis rather than phototaxis. Downward swimming would deliver larvae to the bed three times faster than would gravitational sinking alone. Moreover, present flume experiments show that vertical swimming would be effective within well-mixed estuaries having relatively slow ($u_* \leq 0.2$ cm/s) currents – the physical regime in which hosts of *H. rhigedana* typically reside. Such flows would transport cercariae less than one meter horizontally before they touch down, distances well within field distributions of the snail hosts (authors' unpubl. data). In the much rarer, faster flows, turbulent mixing resulted in relatively uniform vertical profiles of larvae, preventing or considerably increasing the time for cercariae to reach the bed, increasing the chance they could be transported away from snail habitat.

Sources of guidance information used by planktonic parasite (Combes et al. 1994, Haas 1994, Haas et al. 1995) and free-living invertebrate (Thorson 1964,

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Forward 1988, Young 1995) larvae navigating to a particular destination include physical (light, temperature, gravity) and chemical (salinity, adsorbed or dissolved organics) stimuli. Refined larval behaviors in response to environmental factors are common for larvae with short-term dispersal (Doyle 1975, 1976, Pechenik 1999). Cercariae of *H. rhigedana* have but a few hours to locate a second intermediate snail or crab host. Venturing too far from intermediate host habitat may yield high mortality costs due to predation or lack of available hosts. Traits that would enhance retention within the natal habitat are therefore predictably shared among short-lived parasitic and free-living larvae. Undergoing a brief planktonic period, non-feeding (lecithotrophic) larvae often disperse tens of meters or less (Grosberg 1987, Prince et al. 1987, Sammarco and Andrews 1989). Like short-lived cercariae, lecithotrophs rely on light, gravity, and dissolved chemicals for guidance to appropriate settlement habitats (Hadfield and Scheuer 1985, Butman et al. 1988, Krug and Zimmer 2000). Long-lived feeding (planktotrophic) larvae may also use bottom-directed cues, but not until they become competent to metamorphose (Thorson 1964, Miller and Hadfield 1986, Wendt and Woollacott 1999).

In shallow, southern California estuaries, temperature and flow vary over the tidal cycle, but temperature and salinity are typically well mixed throughout the water column. Light intensity is vertically stratified and varies throughout the day (Barron et al. 2000, current study). Consequently, light was the only measured

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variable that induced directional swimming (positive geotaxis) in *H. rhigedana* cercariae. Whereas swim speeds did not vary over the seasonal range in salinity, a small elevation in temperature caused a large increase in swim speed. Similar temperature effects on swim speed have been reported for other cercariae (Meyrowitsch et al. 1991, Rea and Irwin 1992).

Temperature-regulated swimming of *H. rhigedana* cercariae has compelling ecological implications. In southern California salt marshes, flood waters initially warm appreciably as they inundate the mudflat, and then gradually cool. Highest rates of encystment are predicted at the onset of flood tide. From low to high water, swim speeds should decrease, resulting in more uniform vertical larval profiles. Therefore, the dispersal distances should increase whereas the probability of cercariae reaching their benthic hosts should decrease. Likewise, as seawater temperature rises from May to September, the typical season of cercariae transmission, faster-swimming larvae should become more concentrated near the bed, potentially boosting the spread of infestation to benthic hosts.

Swimming behavior of cercariae was similar in still water and slow ($u_* =$ 0.2 cm/s) flume flow. In slow flow, downward-swimming larvae were concentrated near the bottom, increasing the probability of contact with their benthic hosts. The magnitude of the mean vertical swim speed (0.59 cm/s at 24 °C) was about three times the vertical fluctuations ($w' = 0.23$ cm/s) in flow. Larval swimming apparently overpowered these weak eddy velocities, but presumably at a

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modest energetic cost. Flow measurements in two other southern California marshes (Mission Bay, and Newport Bay) with large parasite and host populations, also had weak flows ($u_* \leq 0.2$ cm/s ~80 % of the time). In such low-energy regimes, environmentally triggered swimming behavior should greatly facilitate local dispersal of *H. rhigedana* cercariae.

In contrast, downward swimming behavior either was not expressed or was futile in the relatively fast flow ($u_* = 0.8$ cm/s) that characterizes rare storm events, where vertical profiles of larvae were only moderately bottom-skewed (18 °C) or essentially uniform (24 °C). Here, w' was 0.95 cm/s, almost twice the mean vertical swim speed. Eddy mixing could easily overwhelm active larval movements. In fact, near-bed turbulence was observed to rotate or tumble the larvae, rendering directional swimming behavior ineffectual. Such shear-induced torque may have spun the animals, causing them to swim haphazardly rather than towards the bed, as reported for clam larvae (Jonsson et al. 1991) and phytoplankton (Karp-Boss and Jumars 1998). In other higher-energy environments, such as open coasts, invertebrate larvae with swimming capabilities similar to those of *H. rhigedana* may be unable to control vertical position and settle (Butman 1986, Pawlik and Butman 1993).

To be effective, environmental stimuli should instigate cercarial movements towards the habitat of the intended hosts. Previously, such behavior has been studied primarily in still water or inferred from field distributions (Pike 1990, Rea

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and Irwin 1992, Haas 1994, Kimura *et al.* 1994). I have shown that still-water behavior of *H. rhigedana* cercariae is effective in the slow flows ($u_* \leq$ 0.2 cm/s) that dominate their natural habitat. During ebb tide, offshore transport is minimized because larval vertical profiles are highly bottom-skewed. Downward swimming would be less effective, however, during the faster flood-tide flows, but because the current is directed landward, cercariae are retained anyway. *H. rhigedana* cercariae therefore seem remarkably well suited for finding and encysting on benthic hosts within their local, natal habitat.

Digenetic trematodes like *H. rhigedana* have three avenues for dispersing infection – via two free-living larval stages, and adults infecting definitive host birds. Following defecation by birds, the free-swimming miracidia are analogous to founding larvae of free-living invertebrates (Toonen and Pawlik 1994, Krug 2001). Once established within a first intermediate host, trematode infection can persist during the remaining life of the snail, with each parasite asexually proliferating 10^4–10^5 cercariae. Large founding populations of miracidia are thus unnecessary to cultivate substantial parasite populations within an estuary because cercariae perform this amplification function. Cercarial encystment on up to 100% of snail and crab hosts (Stevens 1996) greatly enhances the probability of transmission, through trophic transmission, to definitive host birds. In fact, completion of the parasite lifecycle and subsequent dissemination of the infection

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over long distances by birds may be predicated on the highly effective, but localized dispersal of the cercarial stage.

Within the context of its natal environment, I have identified a dominant mechanism by which cercariae of a digenetic trematode locate the habitat of their second intermediate hosts. Unlike schistosome and other trematodes inhabiting pools of tranquil freshwater, marine cercariae must contend with dynamic flow regimes. Environmentally-triggered vertical swimming in typical marsh flows identifies larval behavior, not passive transport, as the principal dispersal mechanism. Thus, whether adaptive or fortuitous, parasite transmission is largely controlled by the cercariae and not by the vagaries of flow.

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Chapter 3

CLOSING THE LARVAL LOOP: CONSEQUENCES OF BEHAVIOR IN SUPPLY-SIDE ECOLOGY

Complex life cycles of many aquatic animals involve a sedentary adult stage preceded by a pelagic larval phase. Despite decades of research, there is little accord regarding the role of dispersing larvae in population dynamics (Ólafsson et al. 1994, Booth and Brosnan 1995, Caley et al. 1996, Underwood and Keough 2001). The extent to which pre-settlement processes determine distributions and abundances of organisms depends, in part, on connectivity among phases within the larval loop (release, dispersal and settlement) and with recruitment. A significant relationship between source (larval release) and sink (larval settlement) terms, for example, would allow predictions of settlement, irrespective of dispersal dynamics (Hurlbut 1992). Yet, quantifying transition probabilities among phases has posed daunting challenges. Even for closed populations, studies have measured either release and dispersal (Hurlbut 1987, Hughes et al. 2000), or dispersal and settlement (Olson 1985, Grosberg 1987, Raimondi and Morse 2000, Bingham and Young 1991), but not all three phases at once. The validity of predicting settlement from release is unknown even for these relatively self-contained populations.

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Digenetic trematodes (flukes) are particularly tractable systems for studying recruitment phenomena. Like free-living invertebrates, flukes have mobile dissemination phases. For many aquatic species (e.g., echinostomes and schistosomes), both the origin of larval release (first intermediate host) and the ultimate settlement destination (second intermediate or primary host) are discrete and clearly identifiable (Martin 1972, Haas 1994). Furthermore, larval release, dispersal, and settlement are often confined within a relatively small body of water (lake, pond, estuary or lagoon). Notwithstanding these advantages as a field system, larval parasites (miracidia, cercariae) have been studied almost exclusively in the laboratory (Combes et al. 1994, Haas 1994, Rea and Irwin 1994, Haas and Haberl 1997; exceptions include Upatham 1974, Theron et al. 1977, Kimura 1994).

This field study involved *Himasthla rhigedana*, one of the most prevalent and competitively dominant flukes in southern California estuaries (Kuris 1990, Sousa 1990). Sexual reproduction occurs in primary host shorebirds, which defecate eggs into marsh habitat. Eggs hatch into miracidia that infect first intermediate host snails *(Cerithidea californica)*, causing castration and other sub-lethal effects (Sousa 1983, Sousa and Gleason 1989). Continuous asexual reproduction ensues, liberating free-swimming, short-lived (4 h) cercariae from snail hosts (Kuris 1990, Sousa 1990). On a daily basis, as many as 50-100 cercariae emerge from an infected snail. Cercariae encyst primarily on *C. californica* and the lined shore crab (*Pachygrapsus crassipes)* as second

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intermediate hosts, and metamorphose into the metacercarial resting stage. Bird ingestion of the snails and crabs completes the parasite life cycle.

Our previous laboratory studies of emergence and swimming by *H. rhigedana* cercariae revealed larval behaviors that could greatly facilitate transmission. Cercariae emerged over species-specific temperature ranges, exclusively during daylight hours, and only when host snails were submerged (Fingerut et al. 2003b). Moreover, in flume experiments simulating typical tidal flows of southern California marshes, light-triggered downward swimming overwhelmed turbulent mixing, quickly bringing larvae to the bed (Fingerut et al. 2003a). Such behaviors should promote contact with and retention within benthic intermediate host habitat.

In this one-year field program, the *H. rhigedana* cercarial loop was closed by simultaneously measuring productivity, supply, and settlement. Motivated by previous findings for this species (Fingerut et al. 2003a,b), we tested the hypothesis that the three larval phases would be tightly coupled within the loop, resulting in a closed population. Mechanisms proposed for observed relationships among phases were predicated on known emergence, swimming and settlement behaviors, relative to passive transport processes. The cercerial stage of *H. rhigedana* was chosen because of its exceptional transmission rate. Whereas only 5-15% of snail populations are parasitized by miracidia, cercariae encyst on up to 100% of the possible second intermediate hosts (Stevens 1996). Magnification of infection

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through cercarial dispersal presumably amplifies the chances of trophic transmission back to primary host shorebirds (Choisy et al. 2003). Thus, the cercarial stage is pivotal in the life cycle of this species. Our quantitative transit around the entire cercarial loop of *H. rhigendana* revealed the behavioral basis for maintenance of a closed population within an oscillatory tidal flow; for modest larval mixing coincident with localized retention; and for escalating infection prior to dissemination by shorebirds.

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MATERIALS AND METHODS

A series of three, progressive field studies were conducted to quantify connectivity among the three phases within the cercarial loop of *H. rhigedana*. In total, release (number of cercariae released per unit area), supply (number of dispersing cercariae per unit volume), and settlement (number of metacercariae per unit area) were measured concurrently on 47 d over 12 mo (2/01/02-1/03/03). Instrumentation, tailored specifically for collecting these larval phases in an intertidal estuarine environment, is described below, followed by the rationale for, and description of, the field program.

Instrumentation and techniques

Larval release — Cercariae are produced in the area previously filled by the snail gonad. When cued, larvae crawl along the snail hindgut to emerge from rectal tissues. A specially designed instrument was used to collect, non-intrusively, cercariae emerging from a designated group of snail hosts. The main body of the collector is a flat, clear Plexiglass chamber (50-cm wide by 50-cm deep by 2.5-cm tall) with 53 μm mesh side panels and a solid bottom. Penetrating the top of the chamber is a 4 by 4 array of 8-cm-diameter holes (covering 32% of the surface area), each fitted with an inverted funnel (i.e., small end pointing away from the

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chamber) intake. Contents of all funnels are united in a single 12.5-mm (ID) tube that feeds into a peristaltic pump (Masterflex IP variable speed). The pump continuously filtered water from the chamber over 53 μm mesh, flushing the volume of the sampler at least once per 10-min sampling period. During each sampling day, 200 snails were haphazardly selected and placed in the collector. As the tide began to flood on the mudflat, the chamber and the free-ranging snail population were gradually inundated with seawater at the same time. Oxygenated water at ambient temperature was supplied to the chamber. The mesh panels eliminated emigration or immigration from the chamber, and thus, cercariae collected on the filters could be ascribed solely to the enclosed host population. The mesh captured the large (1 mm) *H. rhigedana* cercariae, but allowed virtually all other species of trematode larvae to pass through.

The pump self-primed when the water level reached the bottom of the funnels (5 cm depth), usually 5-10 min after the water first rose to the level of the mudflat. Every 10 min the filter was removed (and replaced), dipped in 90% ethyl alcohol, and placed in a sealed Petri dish for later staining in 0.1% Lugols solution. Only the red-stained *H. rhigedana* cercariae were counted. Cercariae of this species are easily distinguished by their large size and central eyespot (Adams and Martin 1963, Martin 1972).

Efficiency of the sampler was determined in the laboratory by adding 500 larvae to a large tub (70 liter volume; seawater at 22 °C, 33 psu) containing the

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chamber. Over 4 replicate, 10-min trials, mean sampling efficiency was 81 ± 6% (range).

Larval dispersal — Cercarial abundance in the water column was measured using three vertically stacked intakes. The sampling array, based on the design of Shimeta and Sisson (1999), continuously filtered a defined volume of water from which *H. rhigedana* cercariae were quantified. Each of the three intakes is a flat, triangular, Plexiglas (1.6-mm thick) funnel 15-cm wide by 0.5-cm high by 40-cm long. The leading edges of the funnels are beveled at a 20° angle to minimize disturbances to the oncoming flow, and thus escape responses of the larvae. The funnels are attached at four points to vertical rods at heights of 0.5, 5 and 15 cm from the bottom. These rods are then suspended from a larger four-point support structure that allows the funnels to be rotated into the predominant flow direction as the tide changes from flood to ebb. The entire sampler was anchored on a large (120 cm in diameter by 4-mm thick), sharply beveled, aluminum base plate, which created a smooth flow of water to the bottom intake while minimizing sediment entrainment. Water was collected through tubing (8 mm ID) that ran from each funnel to a Masterflex peristaltic variable-speed pump.

Sampling was essentially isokinetic. Pumping rates were adjusted hourly to match the intake speed of the funnels with the average along-channel flow speed, measured by an acoustic Doppler velocimeter (Sontek Field ADV), with a

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sampling volume of ~1 cm^3. Water from each funnel was filtered separately and cercariae were stained, identified, and counted as with the emergence sampler.

Larval settlement — Preferred encystment sites for *H. rhigedana* in nature are the opercula of *C. californica,* and the thorax and abdomen of *P. crassipes* and *Hemigrapsus oregenenis*. To select a surface that similarly attracts high numbers of encysting cercariae, four types of Plexiglas plates (white/smooth, grey/smooth, black/smooth and black/rough) were tested at the CSMR site. Plate dimensions were 20-cm along-channel by 10-cm cross-channel by 0.3-cm thick. Each of the three cross-channel arrays contained all four plate types, arranged contiguously and in random order. Arrays were located 100-m apart in the along-channel direction. On each of three days, the arrays were deployed at slack low water. Plates were retrieved 4 h after inundation, and the number of metacercariae counted.

Because the black/smooth treatment received the highest encystment (see Results), we next conducted a laboratory settlement experiment testing this surface relative to a preferred encystment substrate in the field – the opercula of *C. californica*. Two small chambers (8 cm by 8 cm by 3-cm deep) were lined with mud from CSMR. The chambers contained arrays of either 16 black Plexiglas plugs (5-mm diameter) or 16 opercula (similar in size) resting on the same type of plug (a control for color), flush with the mud surface. Twenty-four newly emerged (<30 min) cercariae were added to each chamber. After all cercariae had either

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encysted or disappeared from the water column (within 4-6 h), the number of metacercariae was counted. Metacercariae of *H. rhigedana* were easily enumerated because they are white and elevated relative to the smooth, dark surface. This experiment was replicated five times for each treatment, using fresh cercariae, plugs and opercula each time

Field experiments involved two sizes of smooth/black Plexiglas surfaces (see Results). In the spatial-variability experiment (see (3), next section), strips (4.8-m along-channel by 0.1-m cross-channel by 0.003-m thick) were anchored along their margins by galvanized nails. For coupled measurements of release, dispersal and settlement (see (1), (2) and (3) next section), smaller, rectangular plates (20-cm along-channel by 10-cm cross-channel by 0.3-cm thick) were secured in the mud via a small central nail.

Environmental variables — Because previous laboratory studies showed that affected cercarial emergence (Fingerut et al. 2003b) and swimming behavior (Fingerut et al. 2003a) were a strong function of temperature, light and tidal flow, these variables and salinity were measured during all field studies. Temperature and salinity (Orion model 140 probe) at 1 cm above the bed, and light levels (LICOR Model 190SA underwater quantum sensor) at the mudflat surface were recorded every 10 min. Flow velocities (along-channel and vertical) were measured using an ADV. Instantaneous (1 Hz) speeds were recorded 2 cm above

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the bottom. Boundary shear velocities were calculated using the Reynolds stress method (Schlichting 1979).

The Field Program

The field program consisted of three, successive field studies – building blocks that together established the degree of coupling between the phases of the cercarial loop. While complementary, each study approached these relationships from a different perspective. The study site (intertidal channel) was located in the Carpinteria Salt Marsh Reserve (CSMR), southeast of Santa Barbara, California (detailed physical description in Fingerut et al. 2003b). Measurements and experiments took place along the centerline of a 400-m long by 5-m wide by 0.6-m deep (high tide) channel. From February – November, a centrally located 3-m-wide mudflat was carpeted ($460 \pm 16/m^2$ SEM) with *C. californica* for 100's of meters in the along-channel direction. The mudflat is located 1.3 m above MLLW, inundating the snails for an average of 4 h per high tide. Environmental conditions during this field study were typical of those measured in previous years at CSMR: ranges were 16.9-25.9 °C for temperature, 31.5-33.2 psu for salinity, 1.63-7.84 cm/s for mean horizontal flow speed (U), 0.03-0.30 cm/s for shear velocity (a measure of turbulent mixing) (u_*), and 0.07-0.26 cm/s for the mean vertical fluctuating component of velocity (w').

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In all three field studies, the three larval samplers and instruments measuring environmental variables were located within a 40-m (along-channel) region of the mudflat. All instruments were placed 10-m apart (along channel), and their order randomized during each deployment. Dye studies indicated that 10 m was sufficient for disturbances from an upstream sampler to dissipate before the flow reached the next instrument downstream.

(1) Short-term temporal variability — Duration of cercarial emergence is known to decrease over the course of the day, varying from a modest protracted stream at dawn to a contracted burst at dusk (Fingerut et al. 2003b). This behavior would maximize the use of available light, as a cue for downward swimming, thus facilitating settlement before the tide changed direction. Field measurements were designed to assess whether dispersal and settlement tracked this striking and repeatable hourly pattern of emergence.

During June and September, 2002, all three larval stages were sampled concurrently during flood tides with different start times (N = 3 per start time). For release and dispersal, counts from 6 contiguous 10-min intervals were pooled to obtain numbers released per hour over the 4-h sampling period. Because the overwhelming majority of planktonic larvae were collected in the bottom-most funnel (see Results), samples from all three heights (0.5, 5, and 15 cm) were pooled. Triplicate rectangular (20 by 10 by 0.3-cm thick) settlement plates were centrally located in the mudflat, with the long axis oriented in the along-channel

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direction. Plates were separated by 0.5 m cross stream, and were exchanged hourly over the 4-h immersion interval.

(2) Long-term temporal variability — An annual cycle of emergence for *H. rhigedana* cercariae coincided with seasonal variations in water temperature (Fingerut et al. 2003b). Cercariae first emerged in March (15 °C), with maximal release occurring between June and October (>19 °C). This predictable monthly variation in emergence again served as a template for comparison to dispersal and settlement. In the field study, concurrent sampling of all three larval phases occurred monthly, commencing at 17:00 (± 30 min) each day. This time was chosen because it ensured two successive days per month of appropriate flood tides throughout the year, 2002. Methods were identical to those described in (1) above.

(3) Spatial variability — As transport and settlement distances from a source decrease, the degree of coupling should increase among all three phases of the larval loop. Transport and settlement may be minimized passively, by the flow regime (slow advection or vertical mixing), or behaviorally (e.g., by downward swimming or substrate affinity at settlement). Our final field study quantified cercarial encystment on experimental strips as a function of distance from a source snail population during a unidirectional portion of the tidal flow (flood tide). In addition, settlement rate was quantified on a second array of surfaces separated from the strips by 200 m in the along-channel direction. During each of the

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settlement experiments, cercarial release and dispersal also were measured, but under bounded conditions dictated from the previous two field studies.

Using a field set-up identical to that used in (1) and (2), settlement strips were placed along the channel axis, on a 3-m-wide mud shelf located 200-m seaward of the other samplers. Contiguous 1 m by 1 m plots (natural mud surface) were marked at the upstream edges of the strips. To one plot, natural densities (496 $\pm$ 23/m^2 SEM) of snails were relocated, whereas the other plot was cleared of all snails as a control. The surrounding mud shelf was also cleared of snails for 6 m downstream and 4 m upstream. A group of three strips (10-cm apart) was centered on the downstream edge of each plot. During each collecting interval, the strips were deployed before the tide inundated the shelf (~1.1 m above MLLW) and were retrieved 2 h later, before the tide turned, ensuring unidirectional flow. Recovered strips were transported to the laboratory wrapped in felt cloth to prevent cyst dislodgement. Distributions of metacercariae on plates were quantified visually. After 2 h, the location of each metacercaria was recorded (2-cm intervals) as a function of distance from the strips' leading edge. Settlement strips were deployed 10 times, and plates during 7 of these replicate runs. We carefully selected only those flood tides that began between 12:00 and 14:00.

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RESULTS

Short-term temporal variability

Vertical distribution of cercariae — Vertical distributions of cercariae were significantly bottom skewed (Fig. 3-1A, Table 3-1). Averaged over all 10-min samples, 85% of the cercariae were found in the bottom intake, with 14 % and 1 % collected by intakes 5 and 15 cm above the bed, respectively (Fig. 3-1A). There was no significant effect of temperature on vertical distributions of cercariae, at least over the range occurring at the field site (Table 3-1).

Identifying a suitable settlement surface — Of the plate surfaces tested in the field, there was significantly greater settlement on the black/smooth texture than on the white/smooth or grey/smooth surfaces (one-way ANOVA with Scheffe post-hoc test, $P = 0.002$ for both surfaces) (Fig. 3-2). Settlement also was consistently high on the black/rough plates, supporting previous qualitative observations, but values were not significantly ($P = 0.07$) different from those on the black/smooth plates. In laboratory experiments, average percent settlement was similar between the black/smooth Plexiglas plugs and natural snail opercula ($t = 0.884$, df = 8, $P = 0.41$) (Fig. 3-2).

Productivity, dispersal and settlement — Hourly values of productivity, dispersal and settlement were normalized to the 4-h totals to allow comparisons

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over time, within a flood tide. Collections by all three larval samplers were not significantly different within 1-h sampling periods, nor among tides starting at different hours of the day (Fig. 3-3; three-way ANOVA: non-significant sample type and time of initial immersion effects, Table 3-2). There was, however, significant variation among 1-h intervals within a given 4-h flood tide (Fig. 3-3), as revealed by the significant elapsed time effect and the immersion by elapsed time interaction (Table 3-2). Time of initial immersion was not significant because of the high variances (Fig. 3-3). The interaction indicates that the hourly changes over each 4-h interval vary among tides, but it explains only a small amount of the variance ($F_{12, 120} = 10.88$) relative to the elapsed time effect ($F_{3, 120} = 332.05$). Except at 10:00, most cercariae were released during the first hour of the tide, during which snails were initially inundated. The total duration of a daily emergence event decreased, however, for flood tides starting progressively later in the day (Fig. 3-3). In the morning (10:00), cercariae vacated snails in a protracted stream, which became a contracted burst toward dusk (18:00).

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Table 3-1. Results of a two-way ANOVA testing effects of temperature (18 ±1 °C vs. 24 ±1 °C) and height (0 and 5cm) on number of larvae captured (see Fig. 3-1).

Source of variation	df	SS	MS	*F*	*P*
Temperature	1	0.29	0.29	1.24	0.28
Position	2	14.22	7.11	29.92	<0.001
Temperature x Position	2	0.45	0.23	0.95	0.39
Error	108	25.66	0.24		

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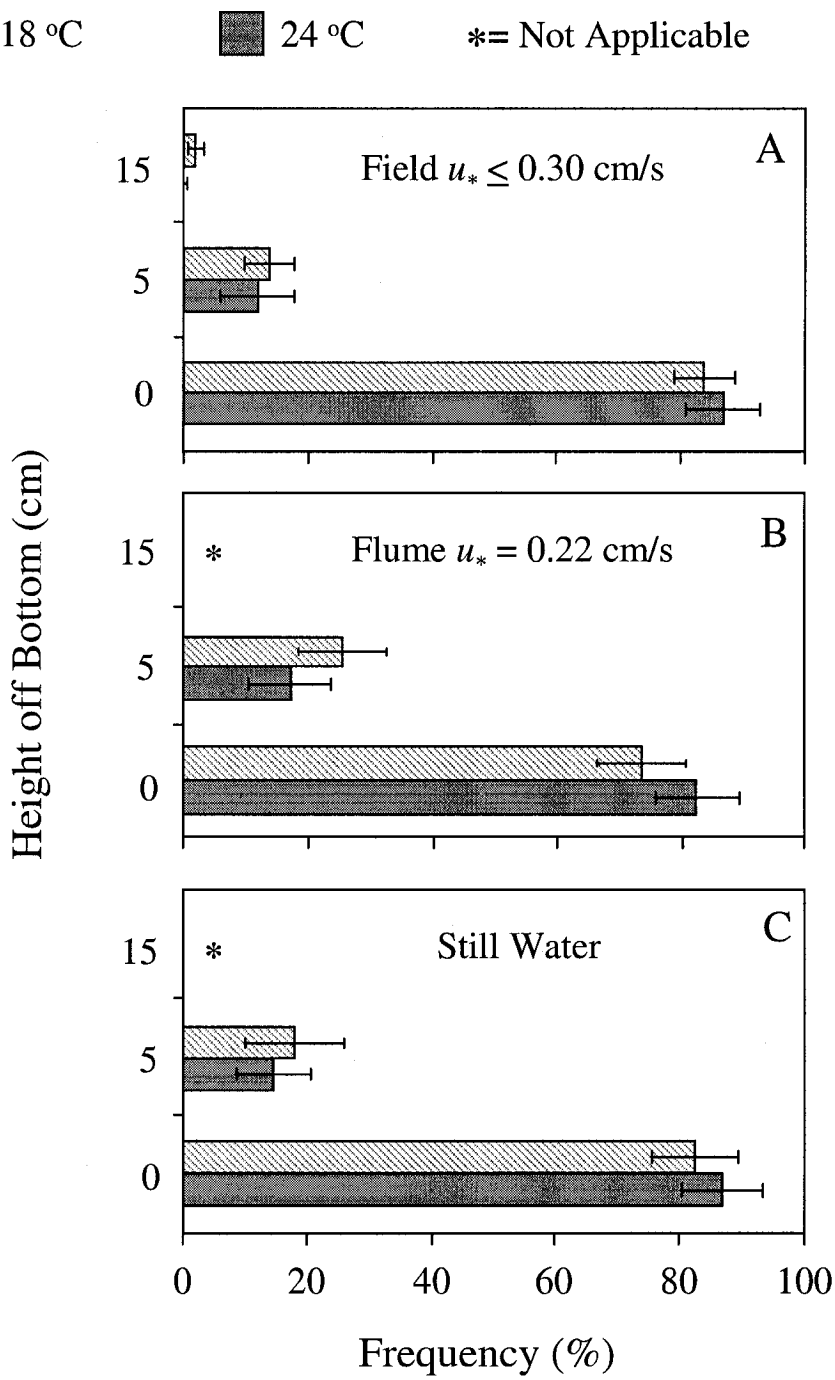
Table 3-2. Results of a three-way ANOVA testing effects of time of initial immersion, elapsed time (h), and sample type (productivity, dispersal and settlement) on larval distributions (see Fig. 3-3). Data were normalized (to 4-h total) and arcsine transformed prior to analysis.

Source of variation	df	SS	MS	*F*	*P*
Time of initial immersion	4	257.18	64.29	0.70	0.60
Elapsed time	3	91,755.12	30,585.04	332.05	< 0.0001
Sample type	2	55.08	27.54	0.30	0.74
Immersion X elapsed time	12	12,022.64	1,001.89	10.88	< 0.0001
Immersion X sampler type	8	85.82	10.73	0.12	0.99
Elapsed time X sample type	6	1,046.06	174.34	1.89	0.09
Immersion X elapsed time X sample type	24	1,832.50	76.35	0.83	0.69
Error	120	11,053.07	92.11		
Total	179	118,107.47			

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Figure 3-1. Height-frequency histograms of cercariae collected in (A) the field, (B) a laboratory flume (Fingerut et al. 2003a), and (C) still water (Fingerut et al. 2003a). Plotted are relative (to total in water column) mean percentages ± SEM of larvae collected at a given height. * height not sampled. (A) Thirty-eight field collections (10 min each) were separated into two temperature intervals: 18 ± 1 °C (range) (N = 23) and 24 ± 1 °C (range) (N = 15); data shown in Figs. 2 and 3. (B) For each flume temperature (18 and 24°C), data were drawn from replicate runs, with N ≥ 8 total trials in each case. (C) For each still-water temperature (18 and 24°C), data were drawn from replicate runs, with N ≥ 5 total trials in each case.

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Figure 3-2. Cercarial settlement (encysted metacercariae) on various substrate surfaces in (A) field and (B) laboratory experiments. (A) Settlement (metacercariae per unit substrate area) during a 4-h period on four types of surfaces. Bars are means ± SEM. Means differ significantly when highlighted by a different letter (one-way ANOVA with post hoc Scheffé test: $P < 0.05$). (B) Percent (of total cercariae added) encysted on natural *C. californica* operculum or smooth-black plugs of equal size, tested separately.

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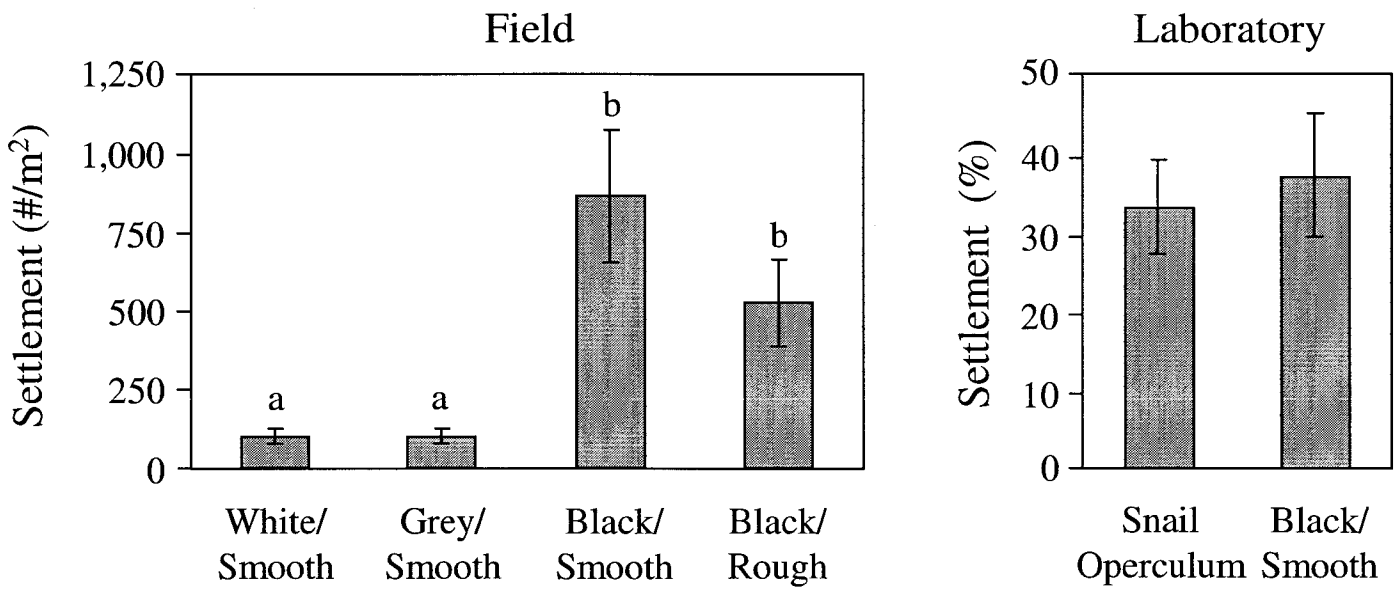
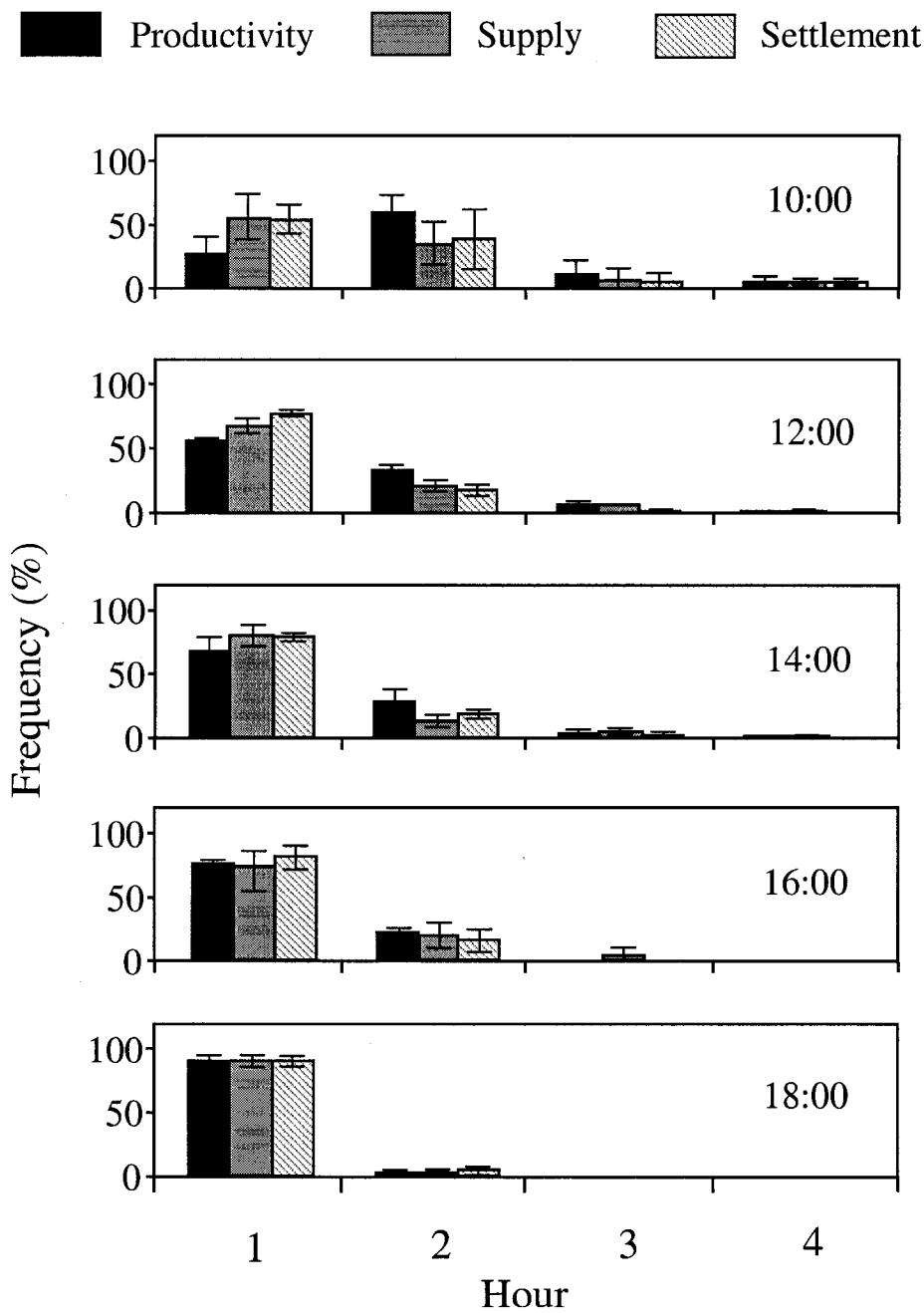


Figure 3-3. Relative (to 4-h total) percentage of cercariae collected hourly, at different times (± 1 h) of initial immersion during the flood tide. There were N = 3 collections per start time, all during June and September. Data are plotted separately for productivity, supply and settlement. Bars indicate means and vertical lines depict ranges.

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Long-term temporal variability

The major season of *H. rhigedana* emergence ran from May through September, with modest encystment continuing through October (Fig. 3-4). During the 6-mo period of cercarial emergence, normalized productivity, dispersal and settlement were not significantly different, nor were collections among months (Fig. 3-4; non-significant sample type and month effects, Table 3-3). As in the short-term field study, there was significant variation among 1-h intervals within a flood tide (Fig. 3-4), as revealed by the significant elapsed time effect and the month by elapsed time interaction (Table 3-3). The interaction indicates that the hourly pattern within each 4-h interval varied among monthly tides, but the amount of variation explained by this effect is small ($F_{15, 60} = 4.70$) relative to the elapsed time effect ($F_{3, 60} = 1{,}865.35$).

Spatial variability

Settlement distances from a source snail population — The magnitude of encystment varied among sampling dates (Table 3-4). Within any given date, cercarial settlement along "snail" strips (i.e., having a source snail population upstream) was highest within the first 20-60 cm of the leading edge, usually decreasing to background densities by the end of the strips (480 cm downstream)

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(Fig. 3-5). Across all days, a factor of 5.4 (± 0.8 SEM) more cercariae settled on snail versus control strips. Settlement along the snail strips was consistent with advective/diffusive transport producing a three-dimensional Gaussian distribution. Regression analysis revealed best fit to an exponential model in all ten cases ($P <$ 0.0001) (Table 3-4). In contrast, control strips (no upstream snails) received substantially fewer larvae and showed no systematic variation in the along-channel direction (Fig. 3-5). Although regression analysis showed best fit to a linear model in 4/10 experiments ($P \leq 0.04$), regression lines were nearly horizontal as indicated by the very shallow slopes and small correlation coefficients (Table 3-4).

To further assess the relationship between settlement distributions and aspects of the flow regime, we calculated extinction coefficients (λ) for each experiment. Lambda indicates the rate of decay of settlement density as a function of distance from (snail) source, and is equivalent to the coefficient (m) of the exponential regression function ($y = b\, e^{mx}$) (Table 3-4). Whereas λ and u_* were not significantly correlated (Fig. 3-6B; $r^2 = 0.0003$: $F = 0.09$; df = 1/8; $P = 0.76$), the correlation between λ and U was highly significant (Fig. 3-6A; $r^2 = 0.86$ Model I linear regression, F-test: $F = 51.37$; df = 1/8; $P < 0.0001$). Assuming transport by U, we calculated distances at which half of the cercariae had settled: median = 1.95 m, 10^{th} and 90^{th} percentiles = 0.72 and 3.85 m, respectively.

Coupling between release, dispersal and settlement— Simultaneous measurements of productivity, supply and settlement successfully completed the

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larval loop. All correlations between the three cercarial phases were statistically significant (Fig. 3-7). The magnitude of settlement on plates subject to cercarial release from the ambient snails was highly correlated with those on snail strips (r^2 = 0.80; df = 5, P = 0.002). These plates thus serve as an internal control for the snail strip experimental manipulation. Moreover, productivity, supply and settlement were all correlated significantly with seawater temperature ($r^2 \geq 0.70$; df = 5, P = 0.009, all comparisons), but they did not covary as a function of salinity or light intensity.

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Table 3-3. Results of a three-way ANOVA testing effects of month, elapsed time (h) after initial immersion, and sample type (productivity, dispersal and settlement) on larval abundance during the period of larval emergence (May-October) (see Fig. 3-4). Data were normalized (to 4-h total) and arcsine transformed prior to analysis.

Source of variation	df	SS	MS	*F*	*P*
Time of initial immersion	5	2.78	0.56	0.02	0.99
Elapsed time	3	154,621.75	51,540.59	1,865.35	<0.0001
Sample type	2	0.71	0.35	0.01	0.99
Immersion X elapsed time	15	1,948.30	129.89	4.70	<0.0001
Immersion X sampler type	10	7.66	0.74	0.03	0.99
Elapsed time X sample type	6	146.81	24.47	0.89	0.51
Immersion X elapsed time X sample type	30	1,034.20	34.27	1.25	0.23
Error	60	1,657.83	27.63		
Total	131	159,419.74			

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Table 3-3. Regression equations describing the number of settlers (y) versus distance downstream (x) in cm, using both exponential [$y=ae^{\lambda x}$] and linear models [y=ax + b]. Each distribution was tested against both models with the best fit (largest *F*-value) shown here.

Trial	Snails	Best fit	Regression equation	*F*-value	df	*P*	r^2	Total number of settlers
1	Present	Exp.	$y = 14.906e^{-0.0079x}$	496.32	1/239	<0.0001	0.68	981
	Absent	Linear	$y = 0.0003x + 0.4377$	1.16	1/239	0.28	0.01	121
2	Present	Exp.	$y = 20.192e^{-0.0062x}$	592.28	1/239	<0.0001	0.69	1,745
	Absent	Linear	$y = -0.0011x + 1.4412$	4.18	1/239	0.04	0.02	283
3	Present	Exp.	$y = 4.3984e^{-0.0025x}$	114.79	1/239	<0.0001	0.25	603
	Absent	Linear	$y = 0.0073x - 0.7361$	7.59	1/239	0.01	0.02	240
4	Present	Exp.	$y = 3.9194e^{-0.0022x}$	103.64	1/239	<0.0001	0.20	596
	Absent	Linear	$y = 0.0142x - 0.6537$	2.82	1/239	0.09	0.01	332
5	Present	Exp.	$y = 11.558e^{-0.0095x}$	454.62	1/239	<0.0001	0.58	1,145
	Absent	Linear	$y = 0.001x + 0.1465$	2.55	1/239	0.11	0.01	324

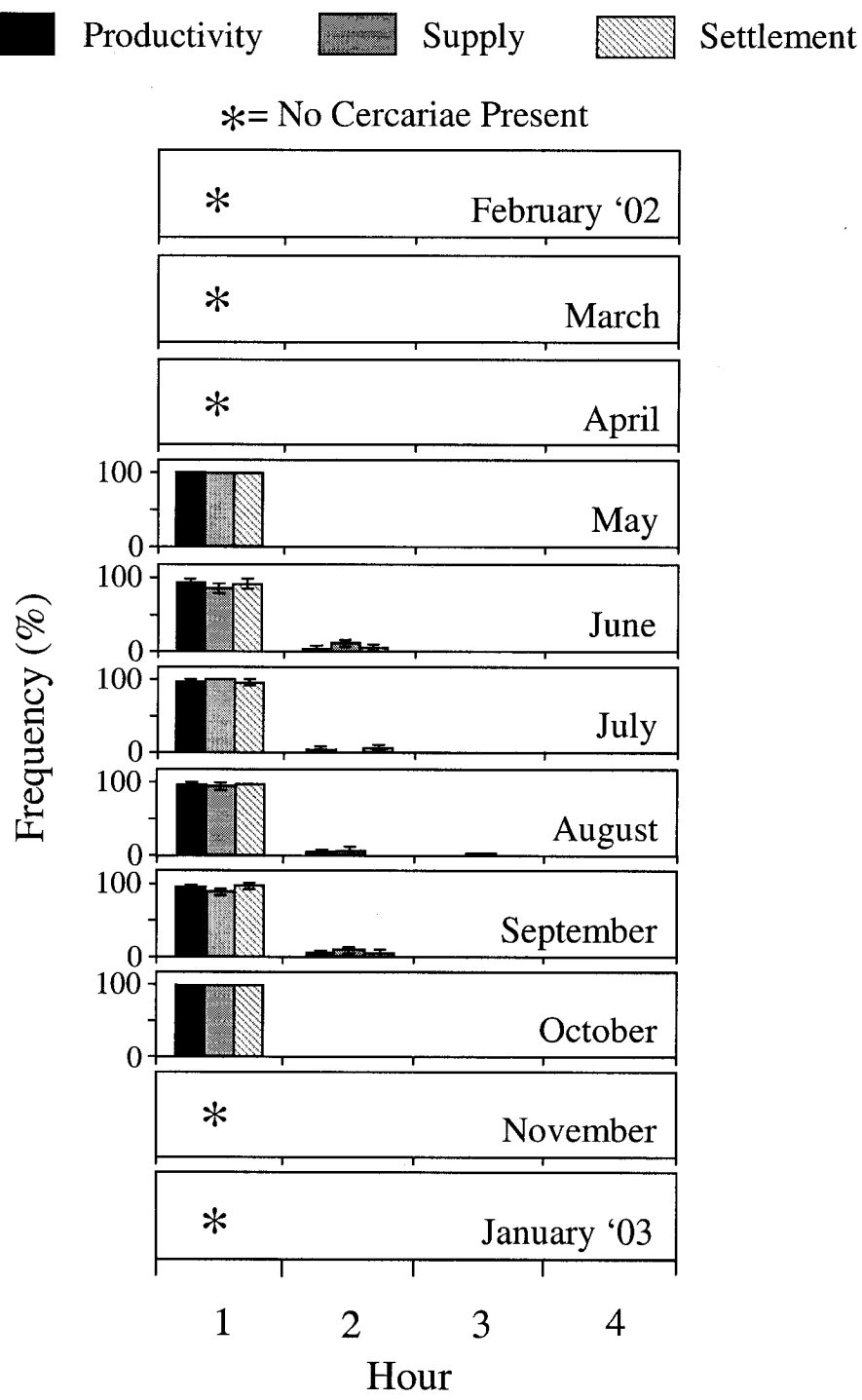
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TABLE 3-3 (cont.)

6	Present	Exp.	$y = 29.458e\text{-}0.0115x$	630.37	1/239	<0.0001	0.74	2,553
	Absent	Linear	$y = -0.0007x + 2.0165$	0.31	1/239	0.57	0.01	465
7	Present	Exp.	$y = 1.0819e\text{-}0.0004x$	12.94	1/239	<0.0001	0.05	128
	Absent	Linear	$y = -0.0003x + 0.2243$	15.14	1/239	0.0001	0.07	45
8	Present	Exp.	$y = 12.9444e\text{-}0.0096x$	472.15	1/239	<0.0001	0.58	1,211
	Absent	Linear	$y = -0.0142x + 1.7222$	0.25	1/239	0.87	0.01	404
9	Present	Exp.	$y = 6.7426e\text{-}0.0020x$	240.21	1/239	<0.0001	0.27	939
	Absent	Linear	$y = -0.0019x + 0.9862$	16.51	1/239	<0.0001	0.06	138
10	Present	Exp.	$y = 1.9524e\text{-}0.0018x$	29.43	1/239	<0.0001	0.08	338
	Absent	Linear	$y = -0.0002x + 0.2131$	0.51	1/239	0.48	0.01	45

Figure 3-4. Relative (to 4-h total) percentage of cercariae collected hourly, during the flood tide. Normalized data are plotted separately for productivity, supply and settlement. Sampling commenced at 17:00 (± 30 min) each day. Bars indicate means and vertical lines depict ranges. Only one collection during both May and September yielded cercariae, whereas N = 2 for all other months.

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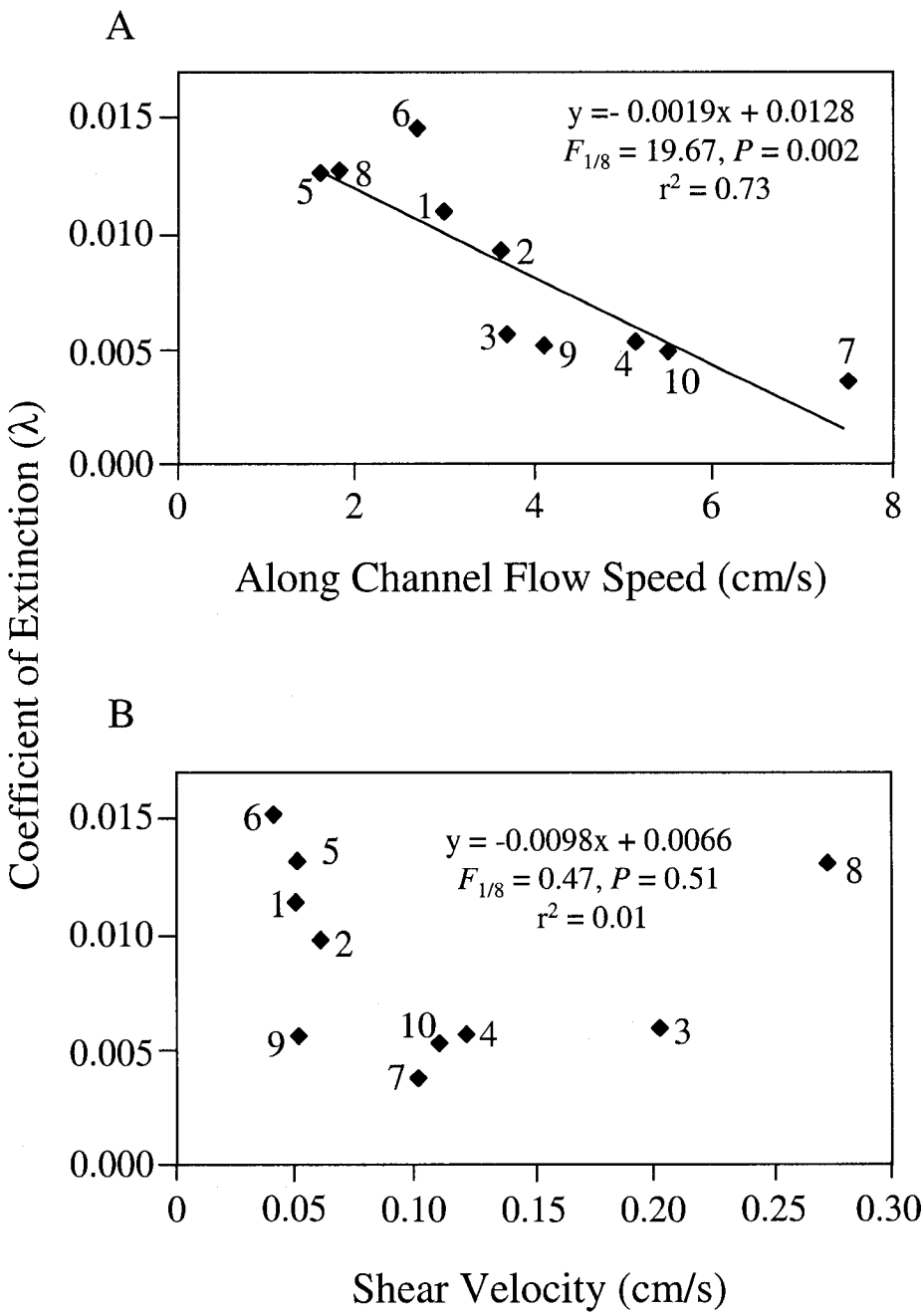
Figure 3-5. Spatial variation in cercarial settlement along Plexiglas strips deployed in the field, with snails (at natural densities) or no snails located upstream. Encystment distributions were not significantly different among triplicate strips within a treatment for all sampling dates (pairwise two-sample Kolmolgorov-Smirnov test, $P > 0.05$ for all comparisons within sampling days). Thus, data were pooled into one settlement distribution per treatment per date. Plotted are settlement distributions from three days that span the range of densities collected during all experiments. Vertically paired panels are from a single deployment. For each graph, bars are the pooled number of metacercariae on 2-cm-long sections of 3 replicate strips.

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Figure 3-6. Coefficients of extinction for cercarial settlement on experimental strips as a function of (A) along-channel flow speed and (B) shear velocity. Numbers refer to trials listed in Table 3-4. The linear regression equation and statistics are shown for each graph.

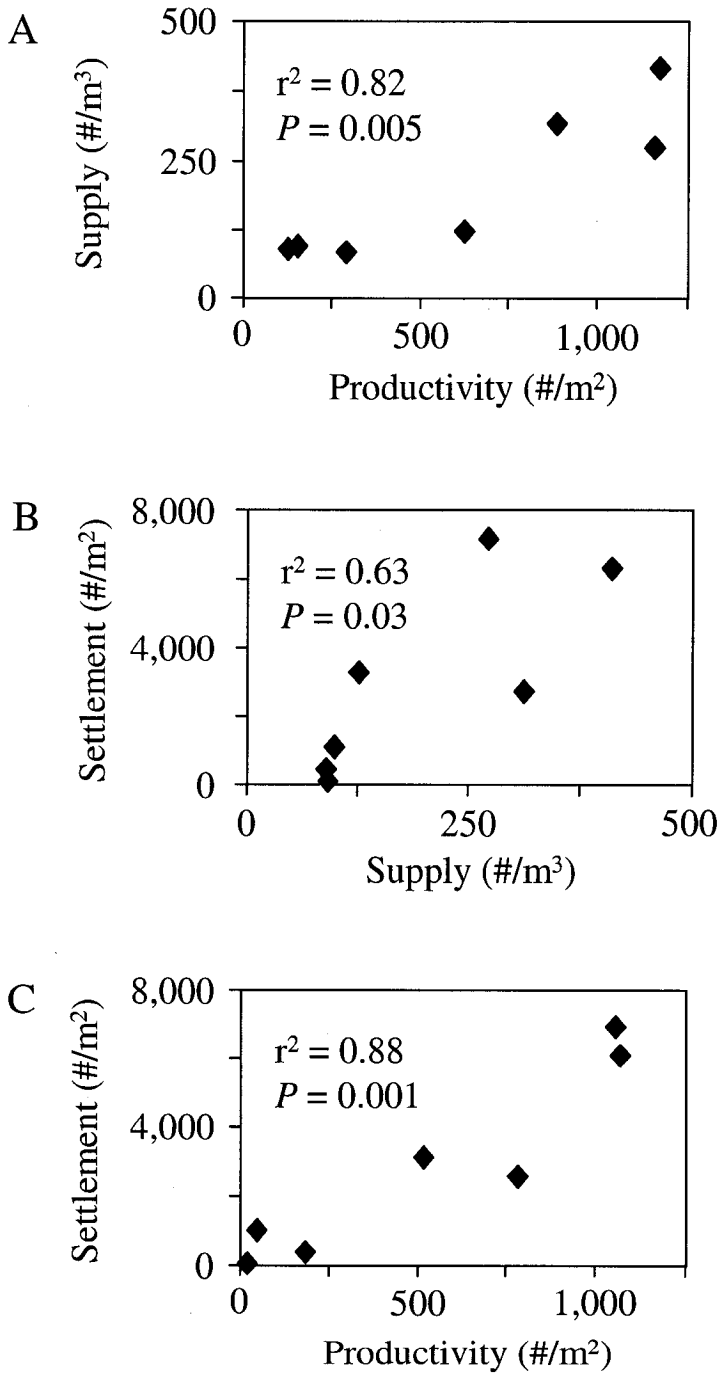
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Figure 3-7. Pairwise comparisons between the three types of cercarial collections, over 1-h sampling intervals: (A) supply and productivity, (B) settlement and supply, and (C) settlement and productivity. Measurements were made during seven of the strip experiments (Figs. 3-5 and 3-6), with settlement plates (N= 3) and other collectors located >200 m downstream of the strips. Correlation coefficients and significance of the correlation are shown on each graph.

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DISCUSSION

Short-lived cercariae of *Himasthla rhigedana* must emerge from first intermediate host snails and colonize their second intermediate hosts (snails or crabs) during a single flood tide. In this field study, cercarial cohorts that were tracked from release to settlement showed greatly foreshortened transport distances. Release, dispersal and settlement dynamics involving strategic larval behaviors are consistent with the quantitative results. Hydrodynamics ostensibly serves a vital function as well, providing time-dependent transport and mixing of cercariae within the local marsh habitat. Even this modest mingling of genotypes is likely to limit inbreeding depression during sexual reproduction inside primary host shorebirds. These behavioral and physical mechanisms explain the remarkable efficacy of the cercarial stage in transmitting infection within its natal milieu.

The Larval Loop is Behaviorally Controlled

For closed populations with a very short-lived larva, dispersal and settlement should quickly follow release. This tight larval loop would be greatly facilitated by a repertoire of behaviors that enhance supply to suitable habitat (Grosberg 1982, Bingham and Young 1991, Miron et al. 1991). For example, interactions between species-specific behaviors and the local flow regime at small

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scales (of order 1 m) can result in vertical larval distributions that determine transport (Turner et al. 1994, Maldonado and Young 1996, Welch et al. 1999, Raimondi and Morse 2000) and supply to the bed. Likewise, external cues (e.g., light, temperature, gravity) can trigger changes in vertical swimming, ultimately concentrating larvae near the bottom (Sulkin 1984, Young 1995, Tamburri et al. 1996, Forward and Tankersley 2001, Fingerut et al., 2003a), sometimes with profound effects on settlement (Eckman 1994, Kingsford et al. 2002).

In this field study, cercarial release, dispersal and settlement were closely and consistently linked at time scales from hours to months. Significant correlations (r^2) in all paired comparisons – supply versus productivity (0.82) or settlement (0.63), and settlement versus productivity (0.88) – indicated that the three processes are dependent. Emerging larvae were quickly concentrated near the bottom in the field (Fig. 3-1A), as in laboratory flume flows simulating the CSMR regime (Fig. 3-1B) and still water (Fig. 3-1C) (Fingerut et al., 2003a). Median larval transport distance was 1.95 m (0.72 and 3.85 m for 10^{th} and 90^{th} percentiles, respectively) in the snail strip experiments. Moreover, settlement was 8-times higher, on average, than supply in the field, and cercariae only encysted opercula and mimics in the laboratory. Therefore, suitable substrate (snails and crabs) may be limiting in the marsh. Temperature (9 °C range over cercarial season) was positively correlated (r^2) with emergence (0.80) and settlement (0.70), thus modulating dynamics within the larval loop.

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Larval behaviors identified in laboratory experiments (Fingerut et al. 2003a,b) provide a mechanistic understanding of the field results. Timing emergence to exploit the first hour of the incoming tide would maximize larval retention within the marsh. Short-lived cercariae would be settled or dead before the tide turned. Photo-induced downward swimming and temperature-regulated swim speeds principally controlled subsequent dispersal. In fact, despite their 4-h life span, larvae were suspended < 1 min, on average, from release to settlement on suitable substrate. Passive horizontal advection by marsh flows (median = 3.75 cm/s; 1.90 and 5.60 cm/s for 10^{th} and 90^{th} percentiles, respectively) would have been 270.0 m (median; 136.8 and 403.2 m for 10^{th} and 90^{th} percentiles, respectively) over 2 h ($\leq$ 2 cm above bottom). Thus, larval swimming decreased the advective length scale by over two orders of magnitude. In addition to retaining larvae within the marsh, such short transit times may also limit losses due to piscine predation (Forrester 1994, Mcintosh et al. 2002), which has led to similarly short dispersal distances for lotic insect larvae (Elliott 2002, Holomuzki and Van loan 2002). To summarize, biological, not physical, processes foreshortened cercarial dispersal, confining encystment to the ample snail substrate available within the local marsh environment.

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Tracking larval cohorts from release through dispersal to settlement has been persistently challenging (Hedgecock 1982, Levin 1990, Gaines and Bertness 1993). Even for small numbers of short-lived larvae, techniques are still evolving. The greatest elaboration of methodologies has been for source/sink studies, involving histocompatiblity assays (Grosberg 1987), and tagging with rare earth metals (Levin et al. 1993), molecular probes (Meideiros-Bergen 1995, Bell and Grassle 1997, 1998, Andre et al. 1999) and heavy metals (Zacherl et al. 2003). However, because the larval supply phase is not sampled, there is no mass balance. Direct tracking of individuals (Olson 1985, Bingham and Young 1991, Carlon and Olson 1993) addresses all three larval phases, but lacks a population perspective. The present study, involved newly developed technology for direct, time-series sampling of the entire larval loop, thus integrating the positive aspects of source/sink and direct-tracking approaches.

Maintenance of free-living invertebrates within localized patches has been attributed to spatially limited recruitment of short-lived larvae (Olson 1985, Grosberg 1987, Graham and Sebens 1996, Smith and Witman 1999). Length of larval life balances the risks of mortality in the plankton with benefits of growth (Vance 1973, Christiansen and Fenchel 1979) or dispersal (Cohen 1967, Strathmann 1974, Palmer and Strathmann 1980). A dispersing larval stage

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potentially de-couples larval source and sink populations (Caley et al. 1996), ultimately enhancing fitness over the species' range (Scheltema 1971, Jablonski 1986, Pechenik 1999). In contrast, non-dispersing larvae must re-colonize their natal milieu, with potentially severe population-genetic consequences (Ibarra et al. 1995, Bierne et al. 1998).

Intermediate between these options is abbreviated dispersal, as illustrated by *H. rhigedana*, whose very short-lived cercariae emerge from and settle onto the same host population. In this case, asexually reproduced cercariae released at high tide are mixed within marsh waters, commingling larval genotypes. Several cercariae (genotypes) encyst a given host, which is subsequently consumed by a shorebird. Sexual reproduction within the primary bird host may enhance fitness of the trematode population (Scheltema 1971, Jablonski 1986, Pechenik 1999). Risk of inbreeding depression due to limited cercarial transport within the marsh conceivably is offset by long-distance migrations of the shorebirds. Moreover, transmission characteristics of this trematode parasite may be co-adapted with the ecology of its intermediate snail host. *C. californica* is typically distributed in dense patches within semi-protected, tidally inundated marshes and estuaries (Race 1981, Sousa 1983, Byers 2000), habitat characteristics that facilitate transport of *H. rhigedana* from host to host.

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Supply-Side Ecology and Trematode Parasites

"Supply-side ecology" purports that near-shore currents, or other physical processes, entirely control larval flux to the bed, and thus, settlement rate (Lewin 1986, for Roughgarden et al. 1987; but see also Young 1987). In contrast to earlier formulations of this idea, supply-side ecology was not proposed as a physical null hypothesis against which biological factors could be tested (Pratt 1953, Eckman 1983, Hannan 1984, Butman 1987). There was no explicit role for larval behavior. Over time "supply-side" and "pre-settlement" processes have become synonymous, both phrases referring to a suite of factors – no longer restricted to physical – acting prior to settlement and ultimately controlling recruitment dynamics (Miron et al. 1995, Fonseca and Hart 1996, Kennedy and Guégan 1996, Connell and Green 2000, Hughes et al. 2000). Behavior can play a significant role in determining larval distributions both in the water column and on the bed. It is within the context of this broader, more contemporary definition that we interpret the role of larval supply in the transmission of infection via the cercarial stage.

For *H. rhigedana,* which completes it entire life cycle on tidally inundated mudflats having relatively slow flows, behaviorally mediated larval supply determined settlement. But, behavior also controlled emergence timing and duration, as well as encystment sites. In fact, supply was no more important than any other larval phase in the transmission of infection by the cercarial stage.

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Likewise, behavior was comparatively important within the entire larval loop. Physics controlled vertical mixing and larval transport only over a narrow range of rare, high-flow conditions. Thus, concurrent quantification of productivity, supply and settlement in the field, together with mechanistic studies under flume and still-water conditions in the laboratory, have shown that behavior supersedes physical processes in explaining pattern and dynamics throughout the larval loop.

Ecologically, digenetic flatworms control host population dynamics via debilitation, castration and death. Nearly one billion humans worldwide are affected by parasites, with fluke schistosomes infecting more than 200 million humans and a diversity of agriculturally important animals (World Health Organization 2001). The complex fluke life cycle, involving multiple hosts, is seemingly intractable unless a key stage has a disproportionate effect on transmission. Here, we have shown how behaviors of the pivotal, cercarial stage can magnify local prevalence, ultimately enhancing the large-scale spread of the infection.

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