

PHOTOCYCLE-DEPENDENT EMERGENCE BY CERCARIAE OF *HALIPEGUS OCCIDUALIS* FROM *HELISOMA ANCEPS*, WITH SPECIAL REFERENCE TO CERCARIAL EMERGENCE PATTERNS AS ADAPTATIONS FOR TRANSMISSION

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ABSTRACT: Emergence by cercariae of *Halipegus occidualis* (Hemiuridae) from naturally infected *Helisoma anceps* (Pulmonata) was evaluated with respect to change in temperature and light. Total cercarial emergence per snail per day increased with temperature in 2 experiments: at constant temperatures of 16, 22, and 28 C, and at temperatures varying within the range 15–30 C. The number of cercariae emerging per snail per day varied extensively among snails and from day to day for individual snails. The proportion of cercariae that emerged during darkness in each 24-hr period on a 12-hr light: 12-hr dark photocycle was consistent for each snail over 3 photocycles, but it varied among snails: a mean of 73% of cercariae emerged during darkness at 16 C, 84% at 22 C, and 89% at 30 C. The ecological consequences of nocturnal emergence by sessile, long-lived cercariae, such as those of *H. occidualis*, are discussed with reference to 3 hypotheses: synchronization with activity of the next host, enhancement of dispersal, and reduction of mortality.

Daily cycles of emergence by cercariae from molluscan hosts are reported widely (Rees, 1948; Macy et al., 1960; Asch, 1972; Betterton, 1979; Théron, 1985, 1989; Lewis et al., 1989). These cycles of emergence correspond to daily changes in ambient light or temperature and often correlate with activity cycles of the next host (Ginetsinskaya, 1968; Cable, 1972; Betterton, 1979; Théron, 1984, 1985; Lewis et al., 1989). An absence of daily cycles has been reported for cercariae that encyst in the external medium following emergence (Kendall and McCullough, 1951; Ginetsinskaya, 1968). Several authors (Ginetsinskaya, 1968; Cable, 1972; Théron, 1984) interpreted these types of contrasting observations as evidence that daily cycles of emergence evolved as adaptations for transmission, by enhancing the ability of active, short-lived cercariae to find hosts rapidly. However, not all digeneans produce such cercariae.

Cercariae of *Halipegus occidualis* are non-motile and long-lived (Shostak and Esch, 1990) and they are ingested by their microcrustacean second intermediate hosts. Macy et al. (1960), in a limited study, reported that cercariae of *H. occidualis* tend to emerge from *Helisoma subcrenatum* during late afternoon and night. This observation seems contrary to the usual hypothesis to explain periodicity of cercarial emergence

(i.e., a necessity to find hosts rapidly). The present study evaluated the emergence of cercariae of *H. occidualis* from *Helisoma anceps*, primarily with respect to photocycle but also with respect to the modifying influence of temperature. Nocturnal emergence by cercariae was confirmed, and alternate hypotheses were developed to explain periodic emergence by cercariae.

MATERIALS AND METHODS

Helisoma anceps was collected from Charlie's Pond, an impoundment in the piedmont area of North Carolina (described in Crews and Esch [1986]), in October 1987 and maintained at 20–24 C under natural lighting in a 50-L aquarium containing pond water and vegetation. Lettuce was provided. After 1–2 mo snails were screened individually for infection with *H. occidualis*, following the methods of Goater et al. (1989).

One experiment evaluated the effect of temperature. At 1030 hr on day 0, 7 naturally infected snails (shell diameter: 9.0–11.3 mm) in individual 55-mm-diameter dishes, containing 30 ml filtered pond water and a 1-cm² piece of lettuce, were placed in a controlled environment chamber at 22 ± 1 C. A 12-hr light: 12-hr dark photocycle (light commencing 0500 hr) was established in the chamber using a 25-watt incandescent light bulb suspended 25 cm above the dishes. Based on the study by Asch (1972), it was assumed that body temperatures of *H. occidualis* changed negligibly when illuminated. At 1030 hr daily until day 24, each snail was transferred to a new dish containing fresh water and lettuce and cercariae in the old dishes were counted (total counts if < 500 cercariae or a 10% aliquot following mixing with a magnetic stirrer if > 500 cercariae). The temperature was changed by 5 C every 3–4 days (Fig. 1). Snails were killed on day 24, and sporocysts and rediae were counted.

A second experiment evaluated the effect of light. At 0700 hr on day 0, 24 infected snails (shell diameter:

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8.4–12.2 mm) were placed in dishes as above, and 8 were assigned randomly to each of 3 controlled environment chambers at 22 C. The lighting in each chamber was as above except that the 12-hr period of illumination commenced at 0700 hr. After 48 hr, snails were transferred to new dishes and cercariae counted. Chamber temperatures were reset to 16, 22, or 28 C. Counts of cercariae were done every 24 hr (at 0700 hr) for 3 days, then every 12 hr (at 1900 and 0700 hr) for 3 days. Snails were killed on day 8, and sporocysts and rediae counted.

Data are reported as $\bar{x} \pm SD$ (n) unless indicated otherwise. The magnitude of variability was assessed by the coefficient of variation ($CV = 100 SD/\bar{x}$). All data from snails that died during experiments were excluded. Statistical tests were performed following the methods of Sokal and Rohlf (1981); results were considered statistically significant at $P < 0.05$.

RESULTS

Effect of temperature

Two snails died within 1 day of transfer to 30 C. Individual snails released 4–3,350 cercariae/24-hr period. Cercariae emerged at all temperatures tested, and numbers varied directly with temperature (Fig. 1). The number of cercariae emerging from individual snails usually varied 2–3-fold, and as much as 30-fold, from 1 day to the next. Mean numbers also varied considerably from day to day (Fig. 1), making it difficult to identify any lag phase between temperature changes and number of cercariae. The mean number of cercariae emerging from each snail over 24 days was $17,549 \pm 2,895$ (5). Snails harbored 0–7 sporocysts and 250–1,539 rediae.

Effect of light

Two snails held at 28 C, and 1 at 22 C, died. Individual snails released 0–4,002 cercariae per 24-hr period. The CV for the number of cercariae emerging daily from each snail over 6 days differed markedly among snails: 26–111% at 16 C, 22–84% at 22 C, and 28–139% at 28 C. The number of cercariae emerging per snail over 6 days was $3,265 \pm 1,830$ (8) at 16 C, $4,168 \pm 3,259$ (7) at 22 C, and $6,167 \pm 4,930$ (6) at 28 C. Variability among snails was high, and differences in numbers of cercariae relative to temperature were not significant, whether these were expressed as total cercariae (Kruskal–Wallis test: $P = 0.48$) or mean cercariae per redia (Kruskal–Wallis test; $P = 0.64$).

Cercarial emergence was evaluated over 3 successive light–dark cycles and provided, for each snail, 3 determinations of the proportion of each day's cercariae that emerged during darkness.

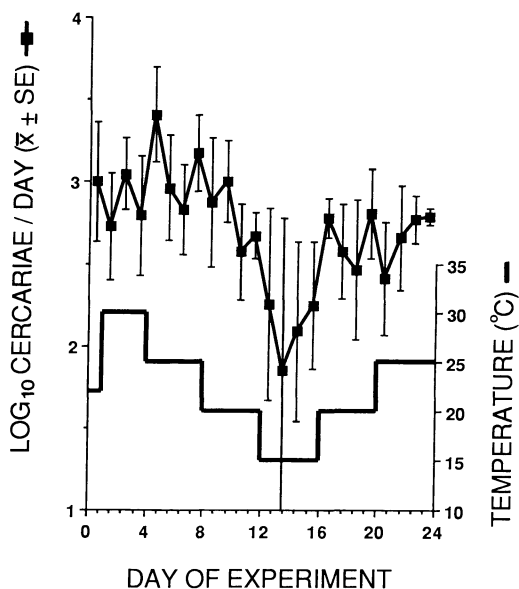


FIGURE 1. Number of cercariae of *Halipegus occidialis* emerging daily from 5 *Helisoma anceps* under a varying temperature regime.

This proportion varied among snails but was consistent for each snail (Kendall's test of concordance: $W = 0.67$, $P = 0.005$). The mean of the 3 proportions from each snail, used as a single metric for the degree of nocturnal emergence present, ranged from 0.47–1.00 among the 21 snails (Fig. 2). Under the null hypothesis that cercarial emergence is independent of the photocycle, 50% of cercariae would be expected to emerge during darkness. This null hypothesis was rejected at all temperatures on the basis of sign tests: at 16 C, 7 of 8 snails released $>50\%$ of cercariae during darkness ($P = 0.035$); at 22 C, 7 of 7 released $>50\%$ ($P = 0.008$); and at 28 C, 6 of 6 released $>50\%$ ($P = 0.016$). The proportion of cercariae emerging during darkness was weakly correlated with the 3-day total numbers of cercariae that emerged ($r^2 = 0.06$) but increased marginally with temperature (Kruskal–Wallis test: $P = 0.070$): 0.73 ± 0.051 (8) at 16 C, 0.84 ± 0.043 (7) at 22 C, and 0.89 ± 0.040 (6) at 28 C. Snails harbored 0–5 sporocysts and 198–998 rediae.

DISCUSSION

The null hypothesis that emergence of cercariae of *H. occidialis* is random with respect to time of day was rejected in favor of the alternative that emergence is nocturnal, confirming

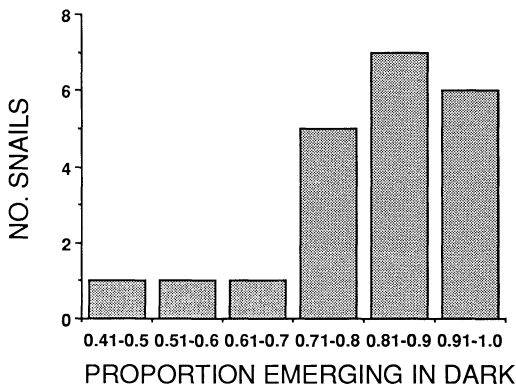


FIGURE 2. Proportion of cercariae of *Halipegus occidualis* emerging from *Helisoma anceps* during darkness when under a 12-hr light : 12-hr dark photocycle. Proportions for each snail were calculated as the unweighted mean of proportions determined for 3 successive photocycles.

the observation of Macy et al. (1960). The present study established that nocturnal emergence was maintained across successive photocycles and at temperatures nearly spanning the range between the lowest temperature (12 C) at which cercariae emerge and the maximum temperature (30 C) naturally encountered (Crews and Esch, 1986). Moreover, each snail exhibited a characteristic pattern of cercarial emergence, varying from the occasional individual from which similar numbers of cercariae emerged during light and darkness to the more typical case where most cercariae emerged during darkness.

Variation with temperature in the mean number of *H. occidualis* emerging from *H. anceps* was relatively small compared to studies on other species (Rees, 1948; Mao et al., 1949; Pflüger, 1980; Rojo-Vázquez and Simón-Martín, 1985), and temperature effects on *H. occidualis* were obscured by large variability among snails. Daily variation in cercarial emergence associated with the photocycle was unaffected by temperature, confirming other studies (Rees, 1948; Mao et al., 1949; Théron, 1989).

Superimposed on the pattern of periodic emergence of *H. occidualis* was variation in the numbers of cercariae emerging daily. This may reflect variation in host size (Betterton, 1979), age and composition of the parasite population within (Sluiter et al., 1980; Théron, 1985), or genetic variation of host and parasite (Mouahid and Théron, 1987; Cohen and Eveland, 1988). Heterogeneity (size, numbers of sporocysts and rediae, and unknown time course of infection) of

the naturally infected snails in the present study was probably a major influence on nonperiodic variability in cercarial emergence.

Light seems to be the most important proximate environmental cue for cercarial emergence (Rees, 1948; Asch, 1972; Théron, 1985; Lewis et al., 1989). However, emergence patterns of some species respond rapidly to change in temperature (Kuntz, 1947), salinity (Rees, 1948), pH (Mao et al., 1949), and water level (Mao et al., 1949; Kendall and McCullough, 1951). In our study, ambient temperatures were held constant and food and water were changed at the start of each light or dark period. Light intensity appeared to be the only environmental factor that changed on a periodic basis.

Transmission of digenean cercariae in aquatic habitats involves dispersal patterns that reflect host movements and mobility of cercariae (Bartoli and Combes, 1986). The dispersal pattern characteristic of a species will influence whether cercariae are most susceptible to endogenous factors (death resulting from depletion of energy reserves) or exogenous factors (lethal environmental conditions, predation, entrance into unsuitable host). Cercariae that swim or crawl generally have active roles in leaving the mollusc, dispersing to the habitat of the next host and contacting that host. Activity enables habitat choice, predator avoidance, and host choice, but it rapidly depletes energy reserves. Nonmotile cercariae are transmitted passively; there is little ability to change habitats, avoid predators, or choose hosts, but energy reserves enable lengthy survival relative to active cercariae (Shostak and Esch, 1990). The effect on mortality of exogenous, relative to endogenous, factors presumably is greater for nonmotile cercariae than for active cercariae.

Nocturnal emergence by *H. occidualis* raises questions regarding its adaptive significance. There is no a priori reason to suspect that daily cycles of cercarial emergence must enhance parasite transmission. Although emergence rhythms of some digeneans are heritable (Théron, 1989), differ among host populations (Théron, 1985), and may be subject to local selection pressures (Théron and Combes, 1988), an appropriate null hypothesis (H_0) is that transmission is independent of when cercariae emerge (i.e., cyclic emergence has no adaptive significance). A general alternative (H_a) is that transmission success varies with time of cercarial emergence. This may take several forms (H_{a1} , H_{a2} , etc.), depending

upon the type of periodicity (emergence during light vs. darkness) or the aspect of transmission (escape from host, dispersal, host-finding) of interest.

One alternate hypothesis (H_{a1}) that dominates the literature is that periodicity increases the chance of finding a host. This hypothesis assumes that endogenous mortality factors predominate, rapid host finding is a key component of transmission, and the next host has a daily activity cycle. Any advantage to emergence at a particular time during the daily activity cycle of the next host rapidly diminishes as the number of cycles that can be survived by the parasite increases. Therefore, H_{a1} predicts periodic emergence, coincident with activity cycles of the next host, for species with active but relatively short-lived cercariae. Many digeneans produce such cercariae, and many studies (Ginetsinskaya, 1968; Cable, 1972; Betterton, 1979; Théron, 1984, 1985; Lewis et al., 1989) support the prediction even though the assumption that rapid host finding is important has not been tested critically.

A second alternative (H_{a2}) is that periodicity enhances dispersal from the molluscan host. This hypothesis assumes that the location of the molluscan host, relative to some aspect of the physical environment, exhibits daily variation and that this relative location when cercariae emerge is a key component of transmission. Relative location may be influenced by events such as movement of the mollusc or by environmental factors, such as tidal flooding or water currents generated by patterns of insolation. This hypothesis predicts periodic emergence of cercariae for species that depend extensively on the host for dispersal of cercariae, and it may apply to species that alter the behavior of their molluscan host or to species that produce sessile or weakly mobile cercariae. Curtis (1987) reported that *Ilyanassa obsoleta* infected with *Gynaecotyla adunca* strand high on beaches, in proximity to the next host. This host behavior coordinated with tidal cycles, but it also had a diurnal component.

A third alternative (H_{a3}) is that periodicity reduces mortality. This hypothesis assumes that exogenous mortality factors predominate and that the probability of death of cercariae varies over the course of a day. Death may result from predation or unfavorable physical conditions. This hypothesis predicts periodic emergence of cercariae for species that have frequent contact with, but poor ability to escape from, predators that

have regular feeding cycles. Predators may be avoided if cercariae emerge when predators are not feeding or if, by emerging synchronously, they swamp a predator's ability to handle prey. In addition to mortality that occurs after cercariae leave the host, the commensal oligochaete *Chaetogaster limnaei* may prey on cercariae while they are emerging from freshwater molluscs (Sankurathri and Holmes, 1976). The prevalence of *C. limnaei* varies seasonally to over 90% (Sankurathri and Holmes, 1976), with mean intensities up to 40 (Gruffydd, 1965; Sankurathri and Holmes, 1976).

With respect to *H. occidualis*, H_{a1} seems inadequate. These cercariae are sessile and live for weeks to months (Shostak and Esch, 1990). The next host is a benthic crustacean, and regardless of the daily feeding cycle of that host, *H. occidualis* emerging at any time of day or night will be available over many feeding cycles. Hypothesis H_{a2} seems appropriate for *H. occidualis*, which relies solely on the host for dispersal in space. Horizontal movements by individual *H. anceps* are not extensive, even over long time spans (Goater et al., 1989), but nocturnal movement of infected snails toward the surface of the water would enhance the ability of water currents to disperse cercariae. Because no published study exists on *H. anceps* to test the assumption that the host has predictable daily movement patterns, we did a laboratory experiment on movements of uninfected and infected *H. anceps*. No daily or infection-related variation in movements was observed, but we consider these results preliminary and inconclusive in the absence of observations in the field. Cercariae of *H. occidualis* sink rapidly in vitro and presumably would be exposed to visual predators in the water column only briefly, but H_{a3} may be applicable because cercariae are subject to predation by *C. limnaei*. Although *C. limnaei* was found only occasionally in the snails used in the present study, we have observed more than 20 cercariae of *H. occidualis* in *C. limnaei* removed from field-collected *H. anceps*. Predation by *C. limnaei* may be a significant factor in the transmission of *H. occidualis*. Given the average daily number of cercariae (about 500–1,000, depending on temperature), then 20–40 oligochaetes/snail, each ingesting 20 cercariae/day, could remove a substantial proportion. Nocturnal emergence probably does not enhance transmission by means of predator avoidance because *C. limnaei* ingests cercariae of *H. occidualis* during light and dark-

ness (J. Fernandez, 1990, pers. comm.), but nocturnal emergence may swamp this predator's ability to handle prey items.

The 3 alternate hypotheses discussed above, although not exhaustive, present a broader perspective than adopted previously to address the transmission consequences of daily periodicity of cercarial emergence. Insufficient information is available at present to resolve the null and alternate hypotheses with respect to nocturnal emergence by *H. occidua*lis. Recognition that many alternatives exist suggests that critical tests will be required in future studies on *H. occidua*lis, or other digeneans, if conclusions are to be drawn about the adaptive significance of cercarial emergence patterns. Care in the design of those tests will be necessary because these alternatives are not mutually exclusive and may involve trade-offs. Nocturnal emergence may, for example, simultaneously reduce predation by visually oriented predators, aid dispersal from a host with a daily vertical migration, and increase the probability of encountering a nocturnally active next host, whereas emergence during light to coincide with diurnal activity patterns of the next host also increases the risk of predation by visually oriented predators.

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