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The *Hydrobia ulvae–Maritrema subdolum* association: cercarial emergence controlled by host activity

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Abstract

The release of *Maritrema subdolum* cercariae (Digenea: Microphallidae) from the marine mud snail *Hydrobia ulvae* is significantly affected by temperature, salinity, light and exudates from the second intermediate amphipod host. Based on (i) previously published data on temperature–salinity dependent *H. ulvae* activity, (ii) new experimental data on *H. ulvae* activity in light and darkness as well as in the presence and absence of host exudates, and (iii) the cercarial emergence rate from free moving snails and snails prevented from crawling, the present analysis indicates that emergence of *M. subdolum* larvae is regulated mainly by host activity as the ultimate factor for release. The adaptive significance of such an emergence strategy is emphasized.

Introduction

Factors controlling cercarial emergence from their molluscan first intermediate hosts have been studied in a long array of different, mainly freshwater, host–parasite associations. Among well-defined abiotic factors identified as particularly important are light, temperature, pH and salinity. Whereas there seems to exist an optimum pH value for release (usually around neutral; Bauman *et al.*, 1948; Gumbe *et al.*, 1957; Varma, 1961), cercarial emergence generally increases with increasing temperature and (in marine species) salinity within the natural range of values (Kuntz, 1947; Rees, 1948; Sindermann, 1960; Sindermann & Farrin, 1962; Rojo-Vázquez & Simón-Martín, 1985; Shostak & Esch, 1990; Lo & Lee, 1996; Umadevi & Madhavi, 1997). The effect of light, in contrast, depends strongly on the specific host–parasite association under study. Light may release, or darkness may inhibit, cercarial emergence (Givannola, 1936; Asch, 1972; Anderson *et al.*, 1976; Lewis *et al.*, 1989, McCarthy, 1999; Toledo *et al.*, 1999) or, darkness may enhance and light may inhibit emergence (Wagenbach & Alldredge, 1974; Craig, 1975; Lewis *et al.*, 1989; McCarthy, 1999).

The timing of cercarial release is usually recognized as adaptive to the parasite in its attempt to reach the next host in the life cycle (Combes *et al.*, 1994). Whereas this suggests that the parasite is in control, and hence adjusts its emergence according to external factors to optimize transmission success, it is less clear whether or not the regulating factor acts directly upon the larval trematodes. In principle, abiotic factors should be able to affect larval trematodes more or less directly: light levels may reach the parasites through the transparent shell of certain species of snails; changes in temperature by transmission through the tissue of the poikilotherm host; and changes in ambient salinity and pH by changes in the level of osmoregulatory metabolites and the ion-balance in the host's haemolymph. But the same factors may just as well act indirectly through their impact on the activity of the host which in turn may stimulate emergence. Relatively few studies have considered this latter possibility, and the evidence in its favour is largely circumstantial (see Kendall & McCullough, 1951; Gumbe *et al.*, 1957; Asch, 1972; Anderson *et al.*, 1976).

The aim of the present paper was to show that whatever the factor (light, temperature, salinity and host exudates) demonstrated to influence the emergence of the marine cercaria *Maritrema subdolum* (Digenea:

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Microphallidae) from the snail host *Hydrobia ulvae* (Prosobranchia), it is likely to act indirectly by affecting host activity as the ultimate factor regulating cercarial release. The adaptive significance of such an emergence strategy is discussed.

Materials and methods

Snail collection and the experimental protocols have previously been described by Mouritsen (2002). At least two weeks prior to experiments, snails were exposed to constant environmental conditions: a temperature of 21°C, salinity of 27‰, and a 15/9-h natural day/night cycle.

Temperature-salinity dependent cercarial emergence and snail activity

Cercarial emergence rates at different combinations of temperature (15, 20 and 25°C) and salinity (24, 30 and 36‰) were obtained from Mouritsen (2002), whereas data on temperature–salinity dependent activity of *H. ulvae* were obtained from Hylleberg (1975). As a measure of activity, Hylleberg (1975) investigated the influence of temperature and salinity on the snails' egestion rate (μg faeces per mg snail per 24 h) within a range of temperature–salinity combinations that includes those used for cercarial emergence (Mouritsen, 2002). Hylleberg did not apply exactly the same salinity levels though, but by adopting the closest values (20 instead of 24‰, 25 instead of 30‰ and 30 instead of 36‰) an array of temperature–salinity dependent egestion rates was obtained from Hylleberg's data and correlated with the corresponding array of temperature–salinity dependent cercarial emergence rates. In this way the strength of the relationship between emergence rate and snail activity (in terms of egestion rate) can be evaluated.

Light dependent cercarial emergence and snail activity

The impact of light on the cercarial emergence rate was investigated previously using an experimental design different from the standard experimental protocol described by Mouritsen (2002). In order to keep the influence of light comparable to other results obtained here, an additional light–dark experiment was carried out using the standard experimental design. Fifteen infected snails were placed individually in dishes enclosed in a box excluding the influence of light, and 13 snails were similarly established outside the box under illumination (1050 lux). The salinity of the seawater was 27‰ and the temperature was 22°C during the 8-h experimental period initiated at the beginning of a natural daytime period.

In order to elucidate the influence of light and darkness on the crawling activity of infected snails, two arenas for behavioural observations were established under similar light, temperature and salinity conditions as described above. The arenas were 30 × 22 cm polystyrene boxes with ten numbered concentric circles with diameters increasing by steps of 1 cm drawn on the bottom. The arenas, placed side by side with one of them under a box excluding light penetration, were supplied with 300 ml seawater corresponding to a water depth of 5 mm. An infected snail was then taken from the storage aquarium and placed in the centre of one of the two arenas. After 2.5 min, the position of the snail within the range of the ten concentric circles was recorded, and each snail was assigned a score-value (0–10) as a measure of its crawling activity. The snail was then immediately transferred to the adjacent arena, and the procedure was repeated. Thirty-two paired observations were carried out in this way using a different snail individual in each trial. During the experiments, the water was changed and the bottom of the arenas was brushed and rinsed after every paired observation in order to exclude the possibility that crawling tracks of preceding snails affected the behaviour of subsequent snails. Moreover, snails were alternately placed first in either the illuminated or dark arena, to avoid a systematic change in behaviour during the experiment affecting the final result.

Exudate dependent cercarial emergence and snail activity

Data on the effect of *Corophium volutator* conditioned water on cercarial emergence rate were obtained from Mouritsen (2002). *Corophium volutator* is used as a second intermediate host by *M. subdolum*. In order to elucidate the influence of *Corophium* conditioned water on the crawling activity of *H. ulvae*, an experiment similar to the light–dark experiment described above was carried out, except that: (i) no light-proof box was used; (ii) *Corophium* conditioned water was used in one of the arenas; (iii) the crawling path of the snails was continuously marked on a map showing the concentric circles present also on the bottom of the behavioural arenas; (iv) snails were allowed to crawl until they reached the outer circle or for 4 min, while a stopwatch recorded the elapsed time; and (v) 25 paired observations were carried out. After the experiments, the length of the crawling paths was measured and the crawling velocity was calculated for each snail in both control and *Corophium* conditioned seawater.

The preparation of *Corophium* conditioned seawater followed Mouritsen (2002). Control seawater was prepared in the same way except that amphipods were not added.

Host fixation experiment

To elucidate the direct influence of snail activity on the emergence of cercariae, 21 infected snails were individually placed in dishes supplied with seawater (salinity 27‰) and a silicone-tube (20 mm long, 2 mm inner diameter) resting on the bottom of the dish. In 12 of the dishes, the spire of each snail was pushed into the tube opening enabling the snail to emerge from the shell but not allowing it to reach the bottom of the dish with its foot. This prevented snails from crawling. The experimental set-up was left for 6 h under illumination (88 lux) and at constant temperature (22°C). During the experiment, snails were regularly inspected to ensure that they did not have closed shells.

Data analysis

Statistical tests were performed in SPSS. Although the nature of the data did not always justify the use of mean and standard error as summary statistics, these values are nevertheless given throughout for the sake of simplicity.

Results

Based on ln-transformed egestion rates an almost linear and highly significant positive relationship was found between cercarial emergence and snail egestion rates (fig. 1; $r^2 = 0.86$, n = 9, $P = 0.003$).

In comparison to complete darkness, illumination induced a two-fold higher cercarial emergence rate (Student's t-test with separate variance estimate, $t_{17.1} = 2.66$, $P = 0.016$; fig. 2). The comparable experiment on host activity showed a similar result: the snails' crawling activity was on average two-fold higher in the presence of light than in darkness (Wilcoxon matched-pairs signed-ranks test, $Z = 3.99$, $P = 0.0001$; fig. 2).

Exudates from the second intermediate host reduced the average cercarial emergence rate by more than 50% in comparison to the control, and the mean crawling velocity of infected snails was also significantly lower in *Corophium*-conditioned water than in control water (paired t-test, $t_{24} = 4.18$, $P < 0.001$; fig. 3).

The above indication that the cercarial emergence rate is related to snail activity was directly demonstrated by the significantly and two-fold higher emergence rate among free-crawling snails (mean no. cercariae per snail ± SE : 583.9 ± 115.6, n = 9) than in snails prevented from crawling (234.9 ± 78.4, n = 12; Student's t-test, $t_{19} = 2.59$, $P = 0.018$).

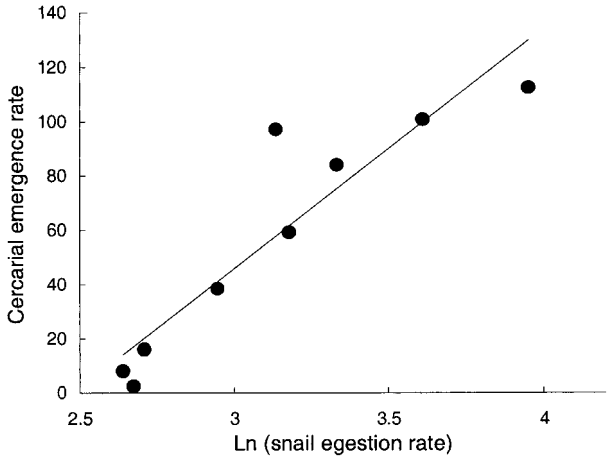

Fig. 1. The relationship between emergence rates of *Maritrema subdolum* cercariae from *Hydrobia ulvae* (mean no. cercariae per snail per 12 h) and the egestion rates of *H. ulvae* (mean μg faeces per mg snail per 24 h) across nine corresponding temperature–salinity combinations. Data on emergence and egestion rates were obtained from Mouritsen (2002) and Hylleberg (1975), respectively.

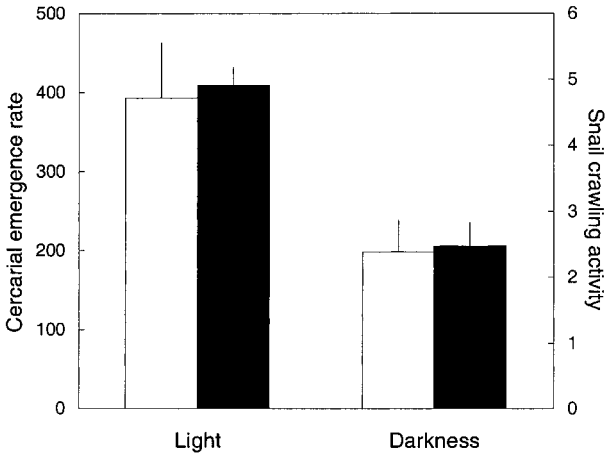

Fig. 2. Emergence rates (□) of *Maritrema subdolum* cercariae from *Hydrobia ulvae* (mean no. cercariae per snail per 8 h ± SE) and the snails' crawling activity (■) (mean score ± SE, n = 32) in light and darkness. For emergence data, n = 13 in light and n = 15 in darkness.

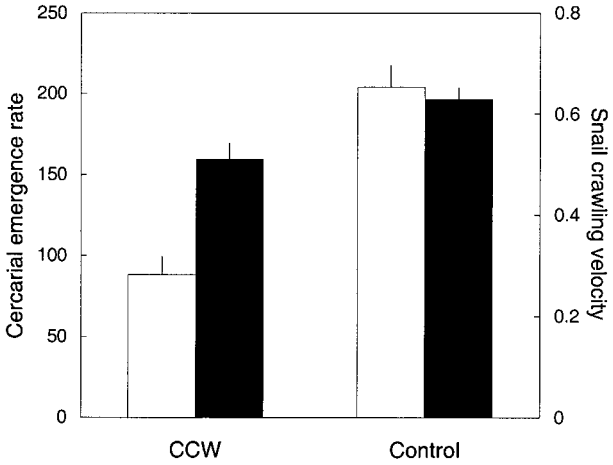

Fig. 3. Emergence rates (□) of *Maritrema subdolum* cercariae from *Hydrobia ulvae* (mean no. cercariae per snail per 6 h ± SE) in *Corophium*-conditioned water (CCW) (n = 13) and in control water (n = 18), and the mean crawling velocity (■) (mm s^{-1}) ± SE of *H. ulvae* in CCW and control water (n = 25). Emergence data were obtained from Mouritsen (2002).

Discussion

The present results demonstrate that whatever the factor studied, its influence on the emergence of *M. subdolum* cercariae from *H. ulvae* could merely be indirect, by affecting snail activity as the ultimate factor for release. Those factors that stimulate emergence also stimulate snail activity, and the factor that depresses cercarial emergence (secondary host exudates) also reduces the crawling activity of the snails. Insofar that experiment-induced stress (forced fixation) can be excluded as the sole explanation for the result, the significantly higher emergence rate from free moving than from fixed snails strongly supports this interpretation.

Few studies have attempted to clarify whether an investigated factor directly triggers emergence through its impact on the parasite itself or indirectly through its influence on the host. Among those studies, some (Bauman *et al.*, 1948; Kendall & McCullough, 1951; Gumbe *et al.*, 1957; Anderson *et al.*, 1976) have emphasized the possible positive relationship between host activity and cercarial output, but do not present direct experimental evidence. Other studies have also proposed merely an indirect effect of the releasing factor. Asch (1972) showed that the positive effect of light on emergence of *Schistosoma mansoni* was similar in albino (light penetrates the shell) and pigmented (light does not penetrate) *Biomphalaria glabrata* snails, and Mitchell *et al.* (1983) showed that the shedding pattern of *Gorgoderina vitelliloba* was partly controlled by stimuli originating from the bivalve hosts (*Pisidium* spp.) by comparing the release of cercariae from isolated *in vivo* sporocysts and intact hosts.

As the only contribution besides the present one that directly tests the influence of host activity, Williams & Gilbertson (1983), in contrast, found no relationship between host activity and cercarial emergence in the *B. glabrata–S. mansoni* association. In addition, several papers covering different host–parasite systems, but all studying one host species infected by two different species (or strains) of trematodes, show in concert that the emergence pattern from the host differs depending on the parasite species in question (Cort, 1922; Pitchford *et al.*, 1969; Craig, 1975; Combes & Théron, 1977 in Smyth & Halton, 1983; Théron, 1984; Lewis *et al.*, 1989; Lo & Lee, 1996; McCarthy, 1999). This suggests that snail activity *per se* is without influence in those associations. Furthermore, West (1961) has shown that *Philophthalmus gralli* rediae isolated from the host produced many times more cercariae in darkness than in light, demonstrating that the releasing factor (light) can affect the parasite directly. In addition, Théron & Combes (1988) and Théron (1989) have convincingly demonstrated that the timing of emergence of *Schistosoma* cercariae from *B. glabrata* is under the genetic control of the parasite, which suggests little evidence for a direct influence of the host.

Nevertheless, more cases of host-activity induced cercarial emission will probably emerge when future studies are designed with the explicit purpose of unravelling such a relationship. Until then, the *Hydrobia–Maritrema* example seems to be an exception to the rule representing a case of cercarial emission largely controlled by host activity.

The adaptive significance of such a strategy should be considered in light of the parasites' overall transmission window. As argued elsewhere (Mouritsen, 2002), it appears beneficial to the *Maritrema* cercariae to emerge in intertidal pools during daytime at low tides. In order to achieve this, it will be insufficient to react upon only one of the three factors (light, high temperature and high salinity at higher temperatures) shown to stimulate cercarial emergence (Mouritsen, 2002) as: (i) high tide occurs also during daytime; (ii) water temperature varies not only according to tidal height but also with season; and (iii) water salinity can fluctuate significantly during high water in coastal areas (particularly in estuaries) due to the combined effect of freshwater outlets and on- or off-shore winds. Hence, in order to emerge under presumably optimal conditions, all three factors have to be taken into account by the parasite, which necessitates specific adaptations (stimuli receptors) for each one of them. A simpler and hence evolutionary more plausible solution would be to adjust the timing of cercarial emergence according to only one factor, namely host activity. By doing so, the same transmission window is achieved. Beside those mentioned elsewhere (see Mouritsen, 2002), additional benefits may be attached to a cercarial emergence pattern largely controlled by host activity. In most coastal areas, the targets of cercariae are infaunal and mainly sedentary amphipods that acquire them through their ventilation current (see Mouritsen & Jensen, 1997). Emerging while the snail host is moving will tend to distribute the larval trematodes across several second intermediate host individuals. This is likely to increase the probability that a metacercaria from a given clone is transmitted to a shorebird final host preying on amphipods, and also reduce the risk of undesirable host mortality due to high parasite loads. At the same time, it reduces the likelihood that several cercariae from the same *Maritrema*-clone infect the same amphipod individual. Following transmission, this may minimize the risk of inbreeding among adult parasites, as emphasized also by Combes *et al.* (1994).

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