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Research Note

Emergence of cercariae of *Echinostoma caproni* and *Schistosoma mansoni* from *Biomphalaria glabrata* under different laboratory conditions

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Abstract

Release of *Echinostoma caproni* cercariae and *Schistosoma mansoni* from experimentally infected *Biomphalaria glabrata* snails maintained under different laboratory conditions was studied. Infected snails were isolated individually for 1 h in Stender dishes containing 5 ml of artificial spring water and the number of cercariae released during this time was recorded. Of numerous conditions tested, the addition of lettuce, the use of water conditioned by *B. glabrata* snails and a temperature of 35°C significantly increased the release of *E. caproni* cercariae. A significant increase in cercarial release of *S. mansoni* was seen only in cultures fed lettuce. A temperature of 12°C caused a significant decrease in cercarial release of both *E. caproni* and *S. mansoni*. Increased snail activity associated with feeding behaviour was probably responsible for the enhanced cercarial sheds observed in this study.

Schmidt & Fried (1996) examined cercarial release of *Echinostoma trivolvis* from *Helisoma trivolvis* maintained under different conditions and reviewed the salient literature on the emergence of cercariae from snail hosts. Since that study, our laboratory has initiated work on the cercariae of *Echinostoma caproni* and *Schistosoma mansoni* released from experimentally infected *Biomphalaria glabrata* snails. Our studies on these cercariae, e.g. analysis of neutral lipids in the cercariae of *E. caproni* (see Marsit *et al.*, 2000), and effects of exogenous glucose on the cercariae of *S. mansoni* (see Fried *et al.*, 2002) have required a dependable source of newly emerged cercariae. The purpose of this study was to examine various physicochemical factors related to optimal release of *E. caproni* and *S. mansoni* from experimentally infected *B. glabrata* snails.

Biomphalaria glabrata was infected with *S. mansoni* miracidia as described in Fried *et al.* (2001) and with *E. caproni* miracidia as described in Idris & Fried (1996). Snails were maintained 10 to 20 per 1000 ml of artificial spring water (ASW) under 12 h of indirect, overhead fluorescent illumination at 22–23°C. The ASW was prepared as described in Ulmer (1970). For details of the water quality and concentrations of ions in the water see Ulmer (1970). Snails were fed romaine lettuce leaf ad libitum, and water was changed twice weekly. Snails were examined 7 weeks after miracidial exposure, and each snail that released cercariae was marked with nail polish and placed in new cultures, 10 per culture. The cultures were maintained as described above. Infected snails were not used on consecutive days and no attempt was made to determine total cercarial output per snail. The studies were done during daylight hours, between 1000 and 1400 h, to avoid possible factors associated with circadian rhythm.

To determine various physicochemical factors that may influence cercarial release of *E. caproni*, 10 to 39 snails were used in each of 10 trials (see table 1). Except for the data set in trial 7 (large volume), each infected snail was placed in 5 ml of ASW (pH 7.3 ± 0.1) in a 3-cm diameter Stender dish and the number of cercariae release within 1 h was counted. Snails designated as controls (trial 1, 39

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Table 1. Release of *Echinostoma caproni* cercariae during 1 h under various experimental conditions.

Trial no.	Factors tested	No. of infected snails tested	No. (%) of snails that released cercariae	Range of cercariae released	Mean ± SE cercariae released
1	Incandescent light (IL)	39	28 (72)	1–94	27.9 ± 4.9
2	IL, with snails covered	30	25 (83)	2–151	34.2 ± 6.3
3	Fluorescent light (FL)	28	24 (86)	1–248	29.5 ± 10.1
4	FL, with snails covered	20	13 (65)	2–48	12.2 ± 3.1
5	Food in culture	10	9 (90)	2–144	70.9 ± 17.4*
6	Snail-conditioned water	12	9 (75)	25–164	66.2 ± 15.6*
7	Volume of water	10	8 (80)	9–107	50.6 ± 13.7
8	Disturbance of water	10	9 (90)	2–41	18.8 ± 4.4
9	High temperature (35°C)	10	9 (90)	1–214	82.9 ± 21.5*
10	Low temperature (12°C)	10	5 (50)	1–17	2.1 ± 1.7*

*Denotes significant difference compared to the trial 1 controls (Student's t-test, $P < 0.05$).

snails) were maintained under direct incandescent light (two 50 watt lamps placed about 25 cm from the Stender dish). During the 1-h period, the initial and final temperature of the Stender dish was 21 and 29°C, respectively. The other 10 to 30 snails in each experimental trial were maintained similarly except for the factors being tested (trials 2–10). The factors tested were as follows (see table 1): (1) controls, snails maintained under incandescent light at 25 ± 4°C; (2) darkness, snails maintained under incandescent light at 25 ± 4°C, but covered with black cloth; (3) light, snails maintained at relatively constant temperature of 22 ± 1°C under diffuse overhead fluorescent light; (4) constant temperature in darkness, same condition as in 3, but snails covered with a black cloth; (5) food, a 1-cm^2 romaine lettuce leaf placed in each culture; (6) snail-conditioned water (SCW), snails placed in ASW conditioned by *B. glabrata* snails for 1 day as described in Moné & Fournier (1994); (7) water volume, each snail placed in 50 ml of ASW in a 9-cm diameter finger bowl; (8) turbulence, snails maintained on an orbital platform shaker at 0.75 rpm; (9) high temperature, snails maintained at 35°C; and (10) low temperature, snails maintained at 12°C. In trials 5–8, the temperature was maintained at 25 ± 4°C by use of incandescent lamps as described for trials 1 and 2. Identical experiments were done to examine the release of *S. mansoni* cercariae from experimentally infected *B. glabrata* snails although with different sample sizes (n = 18 snails for trial 1 controls and n = 10 in each of experimental trials 2–10, see table 2).

Results of the *E. caproni* experiments are summarized in table 1. Although all snails were infected and had released cercariae previously, only 50 to 90% of the snails shed cercariae during any given trial. Hence, shedding of *E. caproni* cercariae from experimentally infected *B. glabrata* snails was quite variable. Food, conditioned water, and an elevated temperature of 35°C significantly enhanced the shed of cercariae in these trials (Student's t-test, $P < 0.05$). Likewise, a decreased temperature of 12°C significantly decreased the release of *E. caproni* cercariae.

Results of the *S. mansoni* experiments are summarized in table 2. Shedding of cercariae was more dependable in the *S. mansoni*–*B. glabrata* model than in the *E. caproni*–*B. glabrata* model. Except for trial 4, 100% of the snails released cercariae. Based on the controls (trial 1 for both *E. caproni* and *S. mansoni*), the number of cercariae released in 1 h by *B. glabrata* was about six times greater for *S. mansoni* than for *E. caproni*. The only condition that resulted in enhanced cercarial shed in the *S. mansoni*–*B. glabrata* model was the presence of food (Student's

Table 2. Release of *Schistosoma mansoni* cercariae during 1 h under various experimental conditions.

Trial no.	Factors tested	No. of infected snails tested	No. (%) of snails that released cercariae	Range of cercariae released	Mean ± SE cercariae released
1	Incandescent light (IL)	18	18 (100)	9–364	172 ± 29
2	IL, with snails covered	10	10 (100)	47–248	168 ± 22
3	Fluorescent light (FL)	10	10 (100)	32–240	109 ± 21
4	FL, with snails covered	10	9 (90)	29–480	178 ± 47
5	Food in culture	10	10 (100)	88–617	352 ± 51*
6	Snail-conditioned water	10	10 (100)	7–480	172 ± 47
7	Volume of water	10	10 (100)	7–296	131 ± 29
8	Disturbance of water	10	10 (100)	72–524	251 ± 47
9	High temperature (35°C)	10	10 (100)	104–800	350 ± 82
10	Low temperature (12°C)	10	10 (100)	5–48	21 ± 5*

*Denotes significant difference compared to the trial 1 controls (Student's t-test, $P < 0.05$).

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t-test, $P < 0.05$). As seen with *E. caproni*, the lower temperature (12°C) also resulted in a significant decrease in the number of *S. mansoni* cercariae released.

Conditions for optimal cercarial release depend upon the particular larval trematode–snail relationship examined. *Echinostoma caproni* from the same snail host as *S. mansoni* showed several conditions for optimal release not seen in *S. mansoni* (conditioned water and elevated temperature). Whereas the condition of the water made a difference in the release of cercariae of *E. caproni*, it was not a factor for *S. mansoni*.

Both snail-conditioned water (SCW) and exogenous food, e.g. lettuce, provide nutrient in the ASW, suitable for snail consumption and utilization. Several studies have shown that water conditioned by *B. glabrata* snails (SCW) has significant amounts of neutral lipids (Chaffee *et al.*, 1996), amino acids (Steiner *et al.*, 1998) and sugars (Muller *et al.*, 1999). Increased snail activity associated with feeding behaviour is probably associated with enhanced cercarial shedding of both *E. caproni* and *S. mansoni* from *B. glabrata*. Kendall & McCullough (1951) have demonstrated that increased snail activity is a major factor in enhanced cercarial shedding of *Fasciola hepatica* in *Lymnaea truncatula*.

A significant decrease in cercarial shedding under cold conditions as seen in this study is not unusual. Such results have been reported previously by Schmidt & Fried (1996) for *E. trivolvis* in *H. trivolvis* and by Kendall & McCullough (1951) for *F. hepatica* in *L. truncatula*.

Regardless of the conditions used for the optimal emergence of cercariae of either *E. caproni* or *S. mansoni* from *B. glabrata*, we routinely use the method described in trial 1 to obtain cercariae. This method is more convenient than adding food to the culture or using SCW or subjecting *B. glabrata* to the shock of an elevated temperature of 35°C.

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