BEHAVIOURAL AND PHYSIOLOGICAL ASPECTS OF SWIMMING IN CERCARIAE OF THE DIGENETIC TREMATODE, *PROTEROMETRA MACROSTOMA*

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SUMMARY

1. Cyclical swimming behaviour of the cercariae of the digenetic trematode, *Proterometra macrostoma*, involves a highly regular alternating swim-sink sequence. During periods of swimming the cercariae are propelled upward through the water by alternating lateral contractions of the tail. Following each burst of swimming the cercariae passively drift downward.

2. Suction electrode recordings from the tail during swimming reveal that a single biphasic potential precedes each contraction in a burst.

3. The site of initiation of the rhythmic activity is the transverse band at the junction of the furcae and body of the tail. Sensory feedback does not play a major role in maintenance of the rhythm.

4. A burst of swimming can be initiated by tactile stimulation of the tail. At no time is a cercaria refractory to tactile stimulation.

5. The tail of *P. macrostoma* cercariae is an autonomous locomotor organ specialized for the brief free-living period between emergence from the snail host and infestation of the primary host.

INTRODUCTION

A basic problem encountered by all parasitic flatworms is the necessity of infecting new hosts. However successfully adapted to the internal environment of one host, they must at some time emerge and transfer to another host. At least two hosts are required in the life-cycle of digenetic trematodes (a mollusc and a vertebrate). Development in a molluscan host involves a series of larval stages, such as miracidium, sporocyst, redia and cercaria, which precedes the adult stage. Cercariae leave the molluscan host and, after a brief free-living existence, encyst on or in a second intermediate host or on vegetation. This encysted stage, called a metacercaria, develops into a sexually mature adult after being ingested by an appropriate vertebrate host. Cercariae of several families of digenetic trematodes either penetrate the (vertebrate) host directly (e.g. Schistosomatidae, Spirorchiidae, Sangunicolidae) or are eaten by the host (e.g. Azygiidae, Bivesiculidae) (Schell, 1970). These types of life-cycles lack metacercarial stage and therefore do not require a second intermediate host.

Most cercariae have a long muscular tail which is used for swimming. Swimming is
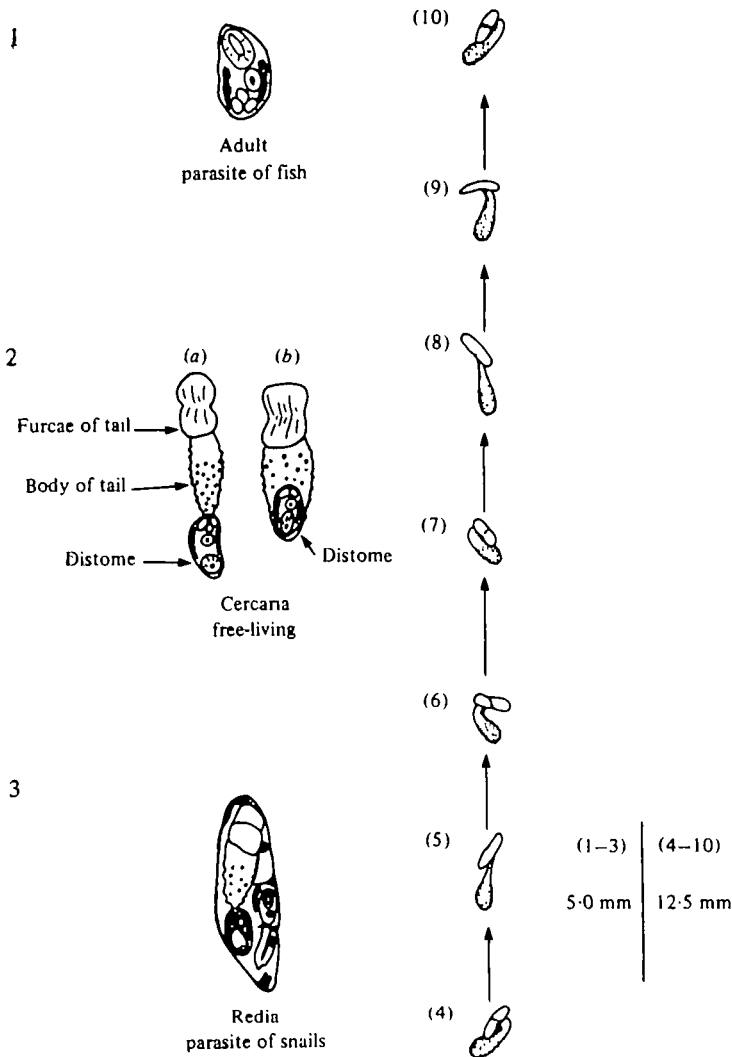
Fig. 1. (1)–(10). *Proterometra macrostoma* forms and swimming. (1) Adult. (2) 'Embryonic' cercaria and naturally shed cercaria with distome withdrawn into tail. (4)–(10) Drawn from selected single frames of films of upward swimming cercariae. This reconstructed sequence represents the positions assumed by a cercaria during a swimming sequence of left contraction (4), extension (5), right contraction (6, 7), extension (8) and left contraction (9, 10). Scale for (1)–(3) 5·0 mm; (4)–(10) (swimming sequence of a cercaria), 12·5 mm.

facilitated by a variety of structural modifications that aid the cercaria in moving either forward or backward through the water. Some species can swim in either direction depending upon the poise of the body and tail, and the position of the caudal forks of the tail. At the other extreme are the microcercous and tail-less cercariae which are unable to swim. Other modifications enable cercariae to float, unite in clusters, and retract the body into the tail (Cable, 1965).

Cercariae of *Proterometra macrostoma* and other Azygiids are among the most

striking of the larval trematodes because of their large size, pigmentation, and regular swimming movements. The body is attached to the anterior end of the large tail and is usually drawn back into the tail at the time of emergence (Fig. 1). Papillae and spines are scattered in a regular pattern over the surface of the tail. The cercaria is pulled through the water by alternating movements of a pair of flat furcae at the posterior end of the tail. The cercariae swim to the surface of the water and then sink slowly to the bottom before movement is again initiated. Following ingestion of the cercaria by a sunfish (which actively prey upon them), the body of the cercaria detaches from the tail and develops into the adult form in the oesophagus or pharyngeal cavity of the sunfish (Dickerman, 1934).

There has been no thorough study of the neural elements involved in the swimming behaviour of *P. macrostoma* cercariae. In this report we describe the nervous system of the tail and the electrical activity associated with the rhythmic swimming of *P. macrostoma*.

METHODS

Cercariae of *P. macrostoma* were obtained from infected snails (*Goniobasis livescens*). Snails were collected from local spring-fed streams and placed in pans of natural spring water. Cercariae emerged from the snails after several hours in the laboratory. Other cercariae were obtained by cracking snails in spring water and testing specimens from the tissues. Behavioural and physiological observations were made on cercariae bathed in either natural spring water or artificial spring water (ASW; 0·5 mM-NaCl, 0·05 mM-KCl and 0·4 mM-CaCl$_2$) at 25 °C. Cinematographic analysis of swimming was done by photographing the cercariae as they swam in a 100 ml graduated cylinder (15–20 cercariae/cylinder). The films were made at 16 frames/s with a Bolex H-16 movie camera (with a Vario-Switar 86 OE Zoom Lens) and analysed with a stop action projector (L-W Photo Optical Data Analyzer, Model 224A MK IV). Drawings and measurements were made directly from the films. The electrophysiological recordings were made by applying thin plastic tubing suction electrodes to various locations on the tails of cercariae that had been pinned to the bottom of a recording chamber. The signals were amplified and recorded with a Grass P79 polygraph or kymograph camera.

RESULTS

The day before cercariae were used, infected snails were collected and placed in shallow pans containing natural spring water. In that naturally shed cercariae usually emerge at night, some of the cercariae were aged 12 h or more before electrical recordings were made. 'Embryonic' cercariae obtained by dissecting snails were used immediately. The only apparent morphological difference between embryonic and naturally emerged cercariae was the position of the distome (Fig. 1). The distome in the embryonic forms is rarely found withdrawn into the tail although inversion and, less frequently, eversion of the distome was seen. In naturally shed cercariae the distome was usually found withdrawn into the tail. Cercariae obtained by either means gave identical experimental results.

Behavioural observations

P. macrostoma cercariae displayed highly regular behaviour consisting of alternating periods of swimming and sinking. Swimming consisted of rapid tail contractions that propelled the cercariae tail-first to the surface of the water following which they sank slowly in a parachute-like fashion until swimming was again initiated. Sinking cercariae were inactive until they touched the bottom of the container or made contact with other swimming cercariae. Since any contact triggered the next period of swimming, the duration of the sink period was quite variable. There were no observable differences between the behaviour of naturally emerged cercariae and those obtained from dissected snails. Furthermore, the swimming pattern was the same in tails that had been separated from distomes.

Fig. 1 (4)–(10) is a series of drawings made from selected single frames (not in actual sequence) of a film that included numerous swimming cercariae. This series was assembled to illustrate the various positions of a cercaria during a small portion of a swim sequence.

During a burst of swimming activity the tail flexes laterally with alternating strokes of the right and left furcae (Fig. 1, (4)–(10)). Each lateral contraction is followed respectively by extension of the tail and contraction of the opposite side. Since each lateral contraction places the long axis of the tail approximately 45° from vertical, the tail is moved through a 90° arc during a contraction–extension–contraction cycle. The mean frequency of contractions (total left and right strokes) in freely moving cercariae was $13 \cdot 1 \pm 1 \cdot 20$ contractions/s ($N = 13$ animals; range $11 \cdot 2$–$14 \cdot 6$) while the mean velocity of swimming was $0 \cdot 7 \pm 0 \cdot 11$ cm/s ($N = 29$; range $0 \cdot 5$–$1 \cdot 0$). Although there was some variation in certain aspects of swimming among cercariae (frequency range of $11 \cdot 2$–$14 \cdot 6$ contractions/s), the pattern of an individual cercaria was regular throughout an experiment (see Fig. 2). Some cercariae tended to swim in a somewhat spiral path but this also varied among individuals.

Physiological observations

In order to study the electrical events underlying the swimming activity, cercariae were pinned to the bottom of a recording chamber filled with artificial spring water (ASW, see Methods and Materials). Electrical events were recorded with plastic suction electrodes applied in various arrangements to the surface of the tail (see Fig. 4). One of the most striking aspects of the recorded activity was the regularity of the pattern (Fig. 2). The rhythm that was maintained under recording conditions closely resembled that of free swimming cercariae (Table 1).

Fig. 3 illustrates simultaneous recordings of electrical activity and tail movements during swimming (a similar method has been used with adult *S. mansoni* by Fetterer, Pax & Bennett, 1977). The lower trace is a typical sequence of potentials recorded with a suction electrode from the central portion of the tail. The upper trace is a record of contractions of one furca of the tail. The tail was pinned down with one side to the bottom of the recording chamber so that only movements of one furca were recorded, hence the contractions are associated with every other impulse. There are slight deflexions in the recording of the last three movements due to contractions on the opposite (down) side (Fig. 3A). The potentials preceded the contractions in each
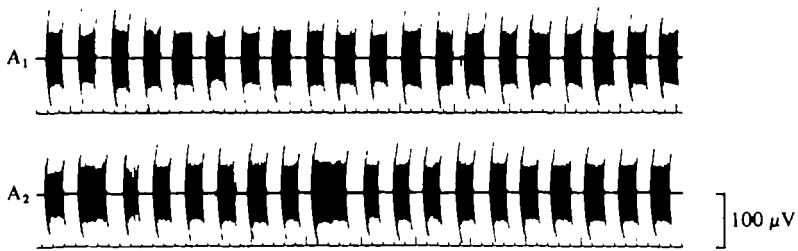
Fig. 2. Continuous suction electrode recording of electrical activity from the tail of a restrained cercaria (A_1, A_2). Note the regularity of the swim pattern. A summary of the characteristics of the swim pattern are in Table 1. Calibration scale is 100 μV; the time-scale is 1 s per time marker division.
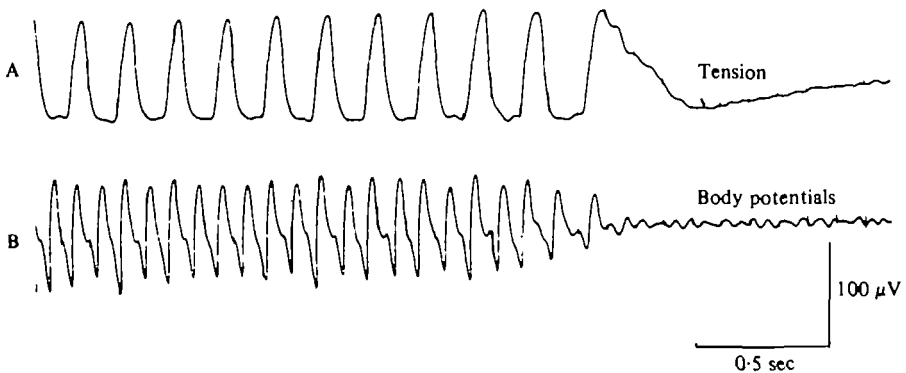

Fig. 3. Simultaneous recordings of tail movement (A) and tail body wall potentials (B) from a restrained cercaria. Note that only the upward (from the bottom of the recording chamber) contractions of the tail are recorded. Alternate potentials precede each contraction of the tail. Slight movements due to the contractions of the opposite wall of the tail were recorded between the last three major deflections (A). Time-scale is 0·5 s; amplitude scale for (B) is 100 μV.

Table 1

Interburst interval s ± s.d. (range)	Impulse frequency, impulses/s ± s.d. (range)	Impulses/ burst ± s.d. (range)	Burst duration, s ± s.d. (range)
	(A) Normal swimming from Fig. 2		
3·0 ± 0·4 (2·6 − 4·8)	14·3 ± 1·2 (11·3 − 16·0)	22·6 ± 3·9 (18·0 − 42·0)	1·6 ± 0·3 (1·3 − 3·2)
	(B) Swimming before and after tactile stimulus (from Fig. 5)		
9·3 ± 0·9 (10·0 − 8·2)	11·8 ± 0·2 (11·3 − 12·2)	44·1 ± 3·9 (52·0 − 40·0)	3·8 ± 0·4 (4·4 − 3·4)

case and seemed to be generated by the lateral musculature causing the swimming contractions.

The direction and the conduction velocity of the large potentials were determined by recording simultaneously at two sites (1·0 mm apart) on the tail of a cercaria (Fig. 4). The conduction velocity was found to be 7·1 cm/s ± 0·03 and the potentials
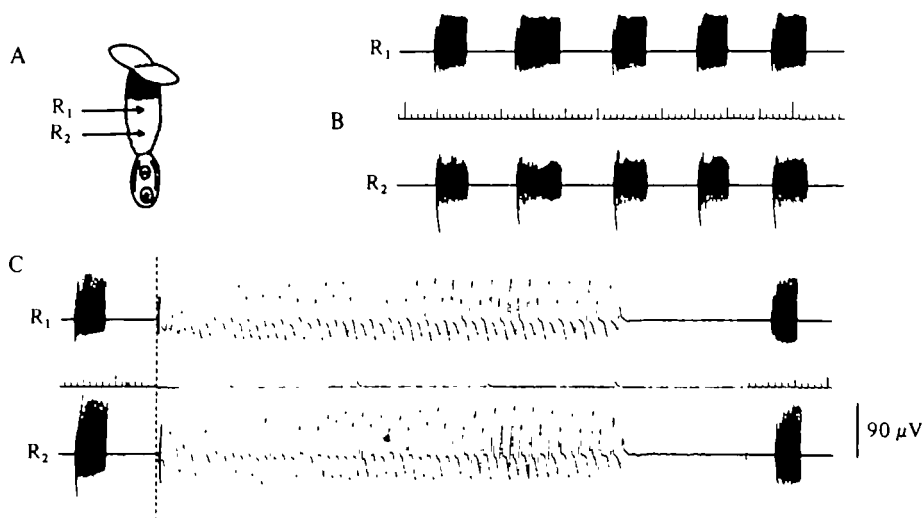
Fig. 4. (A) illustrates the suction electrode arrangement on the tail of a cercaria for simultaneous recordings, a sample of which is seen in (B). In (C), the recordings are expanded to determine direction of conduction. Each potential is recorded initially at R_1 and secondly, with a constant delay, at R_2. Hence the potentials are conducted from the transverse band toward the anterior (distome) end. Time-scales in (B) and (C): one time marker division equals 1 s; amplitude scale is 90 μV for both (A) and (B).

were conducted from posterior to anterior, that is from the furcae to the distome. Thus, the site of initiation of the rhythmic potentials associated with the swimming pattern seems to be the transverse band (a histochemically distinct region; unpublished observations) at the junction of the furcae and body of the tail. Further attempts to localize the site of pattern generation involved recording the burst pattern following various cuts or entire removal of portions of the tail. Neither diagonal cuts of the tail nor complete removal of the furcae altered the bursting pattern. Only removal of the transverse band between the furcae and body of the tail caused complete cessation of bursting activity.

In order to determine if the burst pattern was initiated by an endogenous oscillator as opposed to being reflexly controlled, we recorded from cercariae that had been completely restrained by securing them to the bottom of the recording chamber with numerous pins. There were no observable differences between the activity recorded from this type of preparation and that recorded from the standard preparation in which the furcae were free to move. This suggests that sensory feedback does not play a major role in the timing of the rhythmic pattern.

The complex nature of the oscillator system is illustrated by the observation that, after cutting the furcae longitudinally, the rhythms of the halves were not co-ordinated. Each segment of a single furca had varying rhythms. A single oscillator mechanism would be expected to drive each portion synchronously. The oscillator system was further examined by applying tactile stimuli (touching the tail with a glass probe) to a cercaria during an ongoing swim sequence (Figs. 5, 6). Behavioural observations had indicated that tactile stimulation of a cercaria would initiate a swim sequence at any time during the sink period. During a burst of swimming, tactile stimulation caused
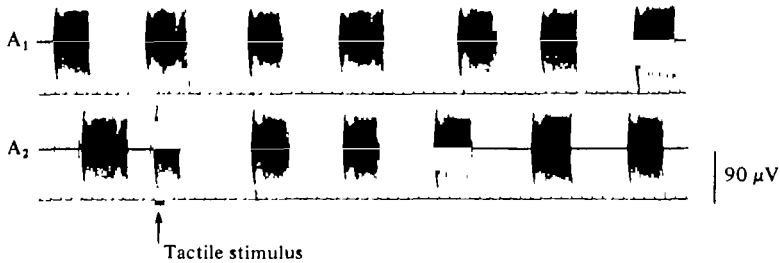
Fig. 5. Continuous suction electrode recording of swimming activity in a restrained cercaria (A_1, A_2). A tactile stimulus was applied at the marker on the time-scale trace. An extra (based on the preceding rhythm) burst was elicited. Note the regularity of the number of impulses/burst, burst duration and interburst interval preceding the stimulated burst (see Table 1 B). The stimulated burst was composed of fewer potentials, but at the same frequency as the other bursts. Note that the phase of the burst pattern was reset by the stimulation of an extra burst. There was no indication of a return to the original phase. Time-scale is 1 s per time marker division; amplitude scale is 90 μV.
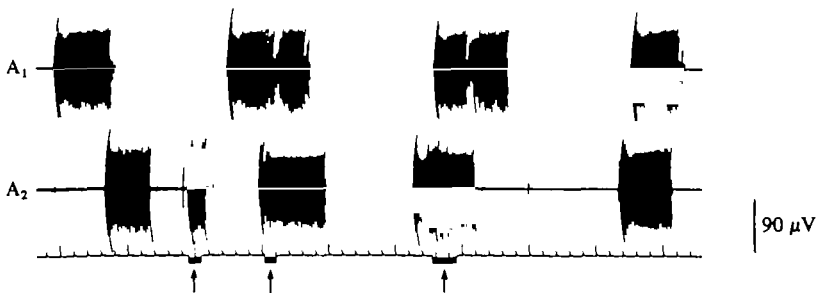

Fig. 6. Continuous suction electrode recording from the tail of a restrained cercaria during swimming activity. At the first marker on the time marker trace, a tactile stimulus was applied to the tail of the cercaria (with a glass capillary tube). The stimulus elicited an extra burst that was out of phase with the ongoing rhythm. Note the shorter duration of the stimulus elicited burst. At the second marker a tactile stimulus was applied at the time of the next expected burst. Note that this burst is the regular duration (5·0–6·5 s). Tactile stimulation was applied during a swim burst at the third marker. Note that the burst duration is not greatly altered. Time-scale is 1 s per time marker division; amplitude scale is 90 μV.

an increase in the duration of that burst followed by an increased interburst interval for one cycle. When a tactile stimulus was applied during the interburst interval (the sink period) an early but normal swim burst was generated (Fig. 5). The timing of the subsequent burst pattern was altered, the bursts remaining out of phase with those preceding the stimulation. The rhythm of cyclical swimming can be reset by tactile stimulation.

The only indication of refractoriness following a burst was the reduced duration of bursts that were elicited in the interburst interval (see Figs. 5, 6). The increased delay following a naturally occurring long burst or one activated by tactile stimulation correlates with the behavioural observation of a variable length sink period. The characteristics of the swimming rhythm are quite rigid but the initiation process is flexible.

DISCUSSION

The most striking aspect of the swimming behaviour of *P. macrostoma* cercariae is the regularity of the underlying electrical events (Fig. 2; Table 1). The regularity of the observed pattern in both free swimming and tethered cercariae suggests the existence of a pattern generating mechanism that is centrally, rather than reflexly, controlled. This suggestion is supported by the observation that removal of the tail furcae does not alter the pattern of swimming. If sensory feedback were responsible for control of the rhythm, removal of the furcae would be expected to alter the pattern due to the absence of position detectors and a reduction of viscous drag on the cercariae (Wilson, 1961; Mellon, 1969). Other common tests that aid in distinguishing between central and peripheral control of rhythmic behaviour include sensory denervation and complete restriction of movement. It is impossible to denervate cercariae but we could restrict movement by pinning them securely to the bottom of the recording chamber. In this situation, in which no movement was observable, we recorded patterned activity identical to that recorded from freely moving cercariae. This observation also eliminated the possibility that we were recording movement artifacts.

In order to localize the pattern generating mechanism, we sequentially cut away various portions of the cercariae (including removal of the distome) while recording swimming bursts. Cessation of rhythmic activity occurred only when the transverse band of the tail was removed. Moreover, the potentials were conducted from the region of the transverse band anteriorly along the tail. These observations independently support the suggestion that this region is the site of generation of the swimming activity.

One of our initial observations was that swimming behaviour continued unaltered following detachment of the distome. This is particularly interesting in that it illustrates the complete autonomy of the tail with regard to the generation of the patterned behaviour. In contrast, the control of swimming in cercariae of at least two other digeneans, *Cryptocotyle lingua* and *Transversotrema patialense*, seems to involve input from the brain which is located in the distome (Chapman, 1974; Whitfield, Anderson & Bundy, 1977).

There have been numerous reports of the responses of cercariae to various stimuli, including temperature, mechanical (tactile), photic and chemical (e.g. Miller & Mahaffy, 1930; Wheeler, 1939; Chapman, 1974). There has been, however, no specific account of the relative responsiveness of cercariae during the various phases of swimming (possibly due to the irregular swimming behaviour of the species studied). The regularity of swimming in *P. macrostoma* cercariae allowed us to analyse generation of the rhythm as well as the responsiveness of cercariae to stimuli during both swimming and sinking periods.

Initiation of swimming is not a rigid phenomenon. We found that a burst of activity could be initiated at any time in the swim-sink sequence (Figs. 5, 6). Contact with other cercariae, vegetation or debris in a natural environment could, therefore, trigger a burst of swimming. This might be particularly adaptive if, as suggested by Chapman (1974), swimming activity enhances the probability of contact between cercariae and potential host fish.

While the frequency of swimming bursts declined in cercariae aged 12 h or more,

the duration of swim bursts was essentially unaltered. Similar results have been reported by Whitfield *et al.* (1977) who made a detailed analysis of free-swimming *Transversotrema patialense* cercariae. They reported that although the mean frequency of swimming events declined from 26 to less than 5 per 2·5 min during the first 12 h of life, the mean duration of the swimming events did not change significantly. In discussing the energetic significance of the activity patterns of cercariae, Whitfield and his colleagues proposed the existence of a quantum of activity, that is, 'a stereo-typed, relatively unchanging packet of locomotory behaviour', somewhat analogous to the activity quantum of the larval nematode parasites described by Croll (1970) and Croll & Al-Hadithi (1972). Our observations of *P. macrostoma* cercariae provide a physiological correlate of such behaviour. The fact that the swimming patterns of 'embryonic' and naturally shed *P. macrostoma* cercariae were the same suggests that the mechanism that initiates and maintains such activity is established before emergence and varies little throughout its free-living existence.

The tail of *P. macrostoma* cercariae is an autonomous locomotor organ specialized for the brief free-living period between emergence from the snail and infestation of the primary host. The rhythmic swimming pattern of this cercaria is a splendid example of the highly adaptive behaviour so often observed in parasitic forms that have solved the problem of transfer from one host to another.

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