METHODOLOGY AND NEW TECHNOLOGY

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Influence of the light-dark cycle in the diel activity rhythms of sea lamprey's ammocoetes

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Abstract

Ten sea lamprey ammocoetes, tagged with passive integrated transponders (PIT), were released in a tank (2 m^3 capacity) and their position monitored twice a day, for a period of one month. Ammocoetes' locomotor activity appeared to be conditioned by circadian rhythms. Differences in the proportion of detected activity *versus* apparent inactivity resulted mainly from the observations registered during the night. Ammocoetes were more active throughout the night period, particularly in the first and second weeks of trials. After an initial period of enhanced activity, differences between daily activity patterns tended to disappear. During the entire experimental period, ammocoetes spent most of the time stationary. There were no significant differences found when comparing the distance moved by the ammocoetes during the dark and light periods, through which they display a similar diel locomotor behaviour. On the other hand, since movements were more frequent at night, the total distance moved by the lampreys is clearly higher during the dark period. This method was found to be a valuable and effective technique to locate and identify individual ammocoetes burrowed up to 10 cm deep in the substrate, with acceptable accuracy and minimum disturbance.

Introduction

Many behavioural, biochemical and physiological processes of organisms exhibit daily fluctuations, which sometimes persist under constant conditions showing that they are driven by an intrinsic daily or circadian clock. External stimuli from the environment are responsible for the entrainment or synchronization of such rhythms (Meissl and Brandstätter, 1992). Pineal organ, eyes and photoreceptors of the tail region are thought to be associated with the circadian organization within lampreys, mediating distinct photobehavioural responses (Hardisty, 1979).

In spite of the striking contrasts in the habits of the ammocoetes and adult sea lampreys, it is interesting to notice that both should show rather parallel innate rhythms of activity. Throughout the spawning migration sea lampreys exhibit a strong diel pattern, being active in the hours of darkness and avoiding light during the daytime (Hardisty and Potter, 1971a; Hardisty, 1979, Almeida *et al*., 2000; Almeida *et al*., 2002). Although ammocoetes are relatively sedentary burrowing animals, they may leave their burrows predominantly during the night period suggesting a similar circadian pattern of activity (Hardisty and Potter, 1971b; Hardisty, 1979; Potter, 1980).

Portable passive integrated transponders (PIT) tag reading units have proved to be efficient to characterize movements of small fishes (Roussel *et al*., 2000; Morhardt *et al*., 2000). To determine ammocoetes' activity rhythms during the light-dark cycle, a portable PIT tag reader prototype, similar to the one described by Bubb *et al*. (2002) to track crayfish in shallow rivers and streams, was used. This technique is an useful alternative to standard radiotelemetry in small-scale environments because PIT tags can be implanted in smaller-bodied fishes, therefore making possible the assessment of individuals' fine-scale movements (Roussel *et al*., 2000).

The objective of this study was to determine both the applicability and efficiency of PIT telemetry to characterize sea lamprey ammocoetes' movements and the influence of the light-dark cycle in ammocoets' diel activity rhythms.

Materials and methods

Telemetry equipment

Ammocoetes were marked with cylindrical PIT tags (122GL; Ukid Systems, Preston, U.K.). These transponders are housed in a biostable glass capsule, weigh 0.1 g in air and the external dimensions are 12 mm long by 2.1 mm diameter. The tags measured less than 3% of the experimental animals' body weight.

The portable PIT tags reader unit (UKID Systems, Preston, UK) is a full-duplex system operating at 125 kHz, powered by an integral 1500 mA/h NiMH battery pack, providing approximately seven hours of continuous use. It consists of a coil antenna (diameter: 180 mm), mounted on a pole (length: 1.5 m), connected to a decoding electronic module which displays the transponder's ID code when detected. The entire system weighs 2700 g, corresponding 800 g to the reader unit and 1900 g to the search antenna and pole.

Tagging procedures

The lampreys were anaesthetised in 1 ml 2-phenoxyethanol l^{-1} water, and surgery was initiated when lamprey stop reacting to stimulus. The abdominal region was disinfected with an iodine solution (Betadine®) and a 3 mm incision was made in the midventral line, 15 mm from a point corresponding to the projection of the anterior insertion of the dorsal fin. The PIT tag was then implanted into the peritoneal cavity and the incision was closed with one independent monofilament synthetic absorbable suture (Byosin® Glycomer 631, USP 6/0) and finally disinfected with Betadine®. Since ammocoetes are burrow dweller animals, a parafilm bandage was applied and fixed with cyanocrilate glue to ensure the protection of the incision's wound. The surgery procedure was conducted with the help of a dissecting microscope (Leica MZ6) and took 3 to 5 minutes. Ammocoetes were revived in 500 litres holding aquariums with a bottom sediment composed of medium sand (0.25 mm ≤ MS < 0.5 mm), and were left to recover for a period of one month to ensure complete cicatrisation of the wound. There was no registered mortality during the recovering period.

Experiment trials

Experiment trials were conducted with ten sea lamprey's ammocoetes with an average total length (TL) of 154 mm (range: 135 – 168 mm), and an average total weight of 4.9 g (range: 2.9 – 6.5 g). Animals' adaptation to the experimental conditions was tested in a 2 m^3 indoor fiberglass holding tank, kept under a constant light-dark cycle (LD 10:14) for a period of one month. The bottom sediment of this tank was composed of a 10 cm layer of sand (59%, 0.5 mm ≤ coarse sand < 2 mm; 38%, 0.25 mm ≤ medium sand < 0.5 mm; 3%, 0.063 mm ≤ fine sand < 0.25 mm) to ensure the location of all tagged individuals. The water temperature was approximately 15 ºC during the entire laboratory experiment. Captive ammocoetes were fed weekly with powdered yeast *Saccharomyces cerevisae*.

Ammocoetes' positions were determined twice a day, at the end of the light and dark periods, using a simple structure located on the top of the tank. This location system consisted of a pole, placed on the top of a two axis structure, rotating as a clock hand, on which a plumb line was attached (Fig. 1). Ammocoetes' co-ordinates were determined according to the Pythagoras Theorem. When a tagged individual was detected, its position was identified with the plumb line and then both the distance to the centre of the axis (i.e. hypotenuse) and the projection of the ammocoetes' position to one of the axis (i.e. cathetus) were measured (Fig. 1).

During the tracking surveys, it was considered a valid move or activity whenever, on two consecutive detections, a minimum apparent distance moved (ADM) of 20 cm was covered by the tracked lamprey. Conversely, every time that the ADM was inferior to 20 cm it was considered inactivity or halt. The determination of the ammocoetes' position

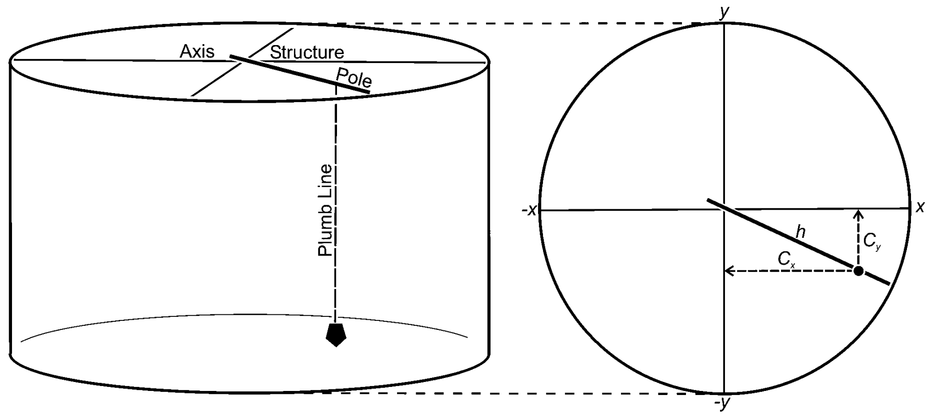

Fig. 1 – Diagram of the experimental tank with detail on the location system. Schematic representation of the method used for ammocoetes co-ordinates location. *h* (hypotenuse) – distance between the ammocoete position and the centre of the axis; c_x (cathetus x) – distance between the ammocoete position and the abscissa axis; c_y (cathetus y) – distance between the ammocoete position and the ordinate axis.

with the portable PIT tag reader unit had an associated average (± SD) detection error of 3.4 ± 1.9 cm. This detection error was calculated using a 2x2 m long opaque board on which 30 PIT tags were randomly attached and detected one by one. The positions of the tags were blind-searched by an operator unfamiliar with their locations. This equipment was also tested with success by Bubb *et al*. (2002) to identify and track crayfish in their natural environment. According to this author the operator could locate tagged individuals burrowed up to 15 cm deep on the stream bed with high efficiency (> 80% detection).

Non parametric statistics (G-test of independence, Mann-Whitney and Kruskal-Wallis tests) were applied according to Sokal and Rolf (1981).

Results

There were no deceased specimens during the experiment and all transponders remained operational. The experiment indicated that PIT tags can be surgically implanted in sea lamprey ammocoetes with success. The telemetry equipment was successful in locating and identifying individual ammocoetes burrowed up to 10 cm deep in the substrate.

Ammocoetes' locomotion activity appeared to be conditioned by circadian rhythms, being more active during the night period, particularly in the first ($G = 16.6$, $df = 1$, $p < 0.001$) and second ($G = 5.0$, $df=1$, $p<0.05$) weeks of trials. Nevertheless, after an initial period of enhanced activity, differences between daily activity patterns tended to disappear. Thus, throughout the third ($G = 0.1$, $df = 1$, $p = 0.74$) and fourth ($G = 1.8$, $df = 1$, $p = 0.18$) weeks no significant differences in the activity patterns during dark and light periods were identified (Fig. 2).

The ammocoetes' detected movements were caused either by swimming or burrowing activity. No significant differences were found in the apparent distance moved (ADM) by the ammocoetes between dark and light periods in each of the four weeks of study ($U_{1^o week} = 193.0$, $df = 33,13$, $p = 0.60$; $U_{2^o week} = 72.0$, $df = 19,9$, $p = 0.50$; $U_{3^o week} = 78.0$, $df = 14,12$, $p = 0.76$; $U_{4^o week} = 50.0$, $df = 15,9$, $p = 0.29$) (Fig. 3). Similar results were obtained for both the total ADM in the dark and light periods ($U=2274.0$, $df = 95,48$, $p = 0.98$), and the total ADM during the four weeks of study in the dark ($KW = 3.0$, $df = 3$, $p = 0.39$) and light periods ($KW = 1.9$, $df = 3$, $p = 0.58$), suggesting that even though the frequency of movements may differ within the light-dark cycle, the movements' covered distance remains identical.

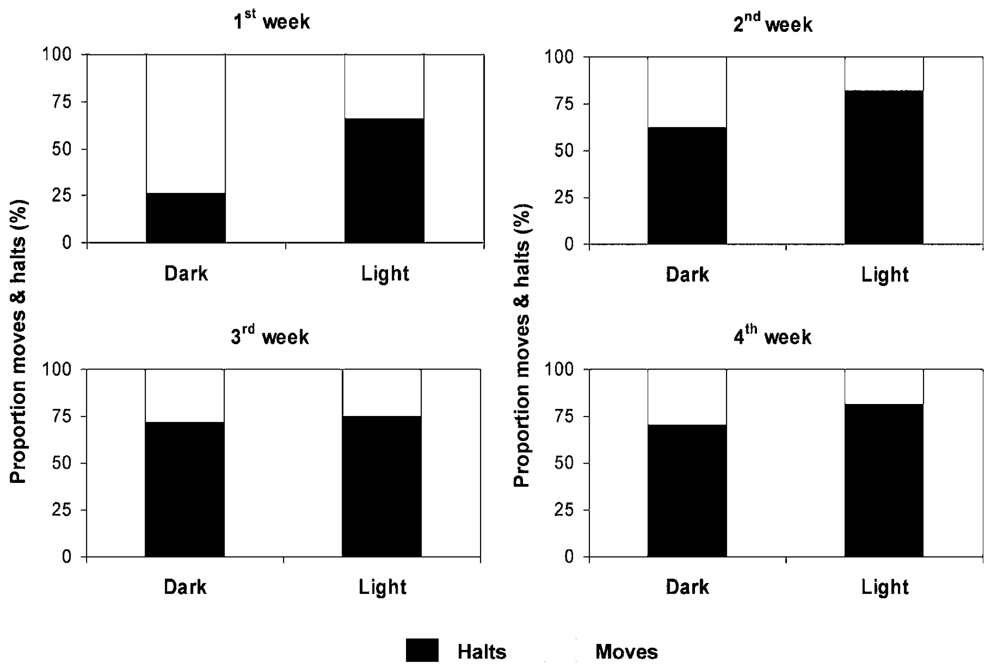

Fig. 2 – Proportion of moves and halts during the dark-light cycle throughout the four weeks of experiment.

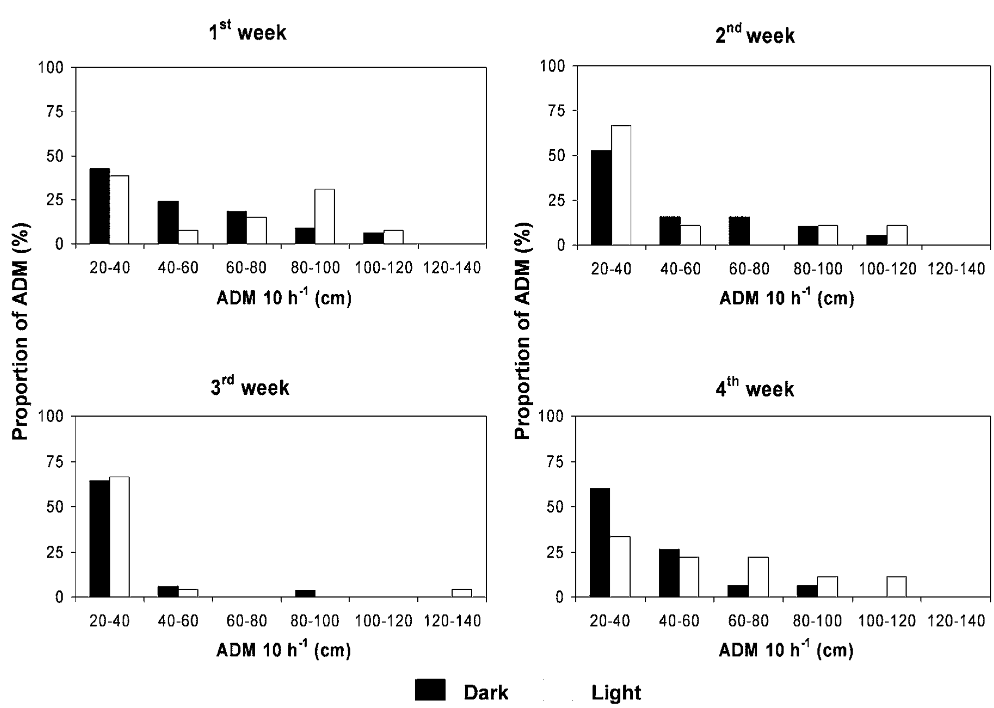

Fig. 3 – Proportion of apparent distance moved (ADM) during the dark and light period throughout the four weeks of experiment.

Discussion

The cryptic behaviour widely recognized for adult sea lampreys (Almeida *et al*., 2000; Almeida *et al*., 2002) also occurs among ammocoetes according to our results, supporting Hardisty and Potter (1971a) findings. This might indicates that after an adaptation period (i. e. first two weeks of experiment) during which the ammocoetes were more active, they tend to maintain their location, supporting earlier findings stating that lamprey larvae, living in stable and favourable environments, may remain in the same area for an undetermined period of time (Hardisty and Potter, 1971b).

There were multiple advantages and few disadvantages when using this new portable PIT tag detection method. As described by Morhardt *et al*. (2000), the prime advantage when using this technique on small fishes is that it avoids handling, which could influence their behaviour and health, every time individual fish positions need to be access. Unlike radio transmitters, PIT tags do not require batteries to work and continue to be functional throughout the life of the fish, can be implanted in smaller-bodied fishes, and are inexpensive making it possible to mark a large number of individuals (Morhardt *et al*., 2000; Roussel *et al*., 2000). Disadvantages of this system when applied to sea lamprey larvae include the need to use, due to tag size, ammocoetes larger than c. 120 mm; shallow water streams (<1 m deep) (Roussel *et al*., 2000); and the prolonged time needed to effectively probe a small area.

This method was found to be a valuable addition to the techniques available on the study of small fishes' movements. With this system it is possible to effectively locate and identify tagged ammocoetes in the sediment with acceptable accuracy and minimum disturbance. This experiment's results suggest that it might be viable to use this technique to monitor ammocoetes fine-scale spatial movements and assess microhabitat preferences in the natural environment.

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