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Infestation of sporocysts of parasite *Leucochloridium* in the snails *Succinea daucina* and *Indosuccinea semiserica*

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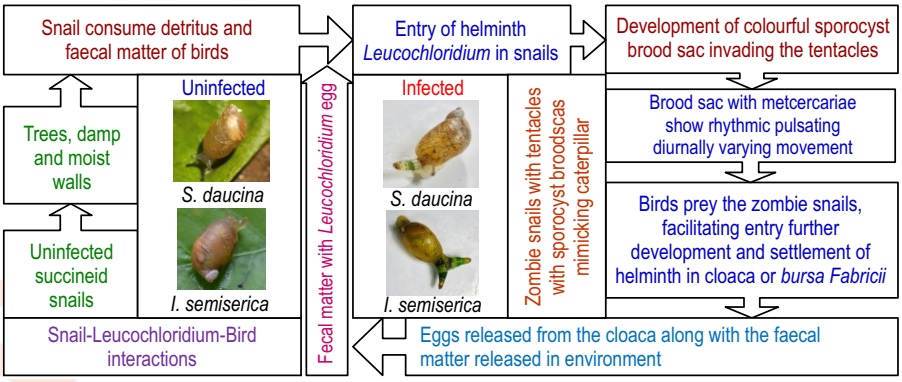
Abstract

Aim: The aim of the present study was to evaluate the differential beating rate of the parasite *Leucochloridium* within the tentacle of the infected snail *Succinea daucina* and *Indosuccinea semiserica* under different photoperiodic conditions.

Methodology: In this study, the rhythmic beating pulse of sporocyst of the parasite invading the tentacle of the snail was recorded from multiple snails, periodically in light and dark hours. The numbers of beat per minute were subjected to univariate ANOVA using day and photoperiod as explanatory variables. A two-sample t-test was performed with the mean of pulse rate in light and dark phase to estimate the significant differences between the same.

Results: The observations revealed that sporocyst brood sac of *I. semiserica* beat mean 99.23 ± 1.91 SE per minute (90 – 101) in day hours and mean 76.57 ± 4.64 SE per minute (48 – 88) in the night hours (n = 7 observations). In *S. daucina*, the sporocyst brood sac beat 65.85 ± 2.17 SE per minute (25 – 94) during day and 30.5 ± 3.55 SE per minute (0 – 80) in night (n = 9 separate observations). The beats of the sporocyst varied significantly diurnally and with the consecutive days revealed through t-test and ANOVA.



Interpretation: Apparently, the colourful sporocyst broodsac of *Leucochloridium* faster beating rate under light may attract definite host, the insectivorous birds which prey on the *Leucochloridium* sporocycst disrupting the snail tentacle. Thus, the pulsating sporocyst inside the tentacle makes the infected host snail more conspicuous for the birds, linking the snail- *Leucochloridium*- bird cycle.

Key words: *Indosuccinea semiserica*, Intermediate host, Land snail, *Leucochloridium* sp., *Succinea daucina*, Trematoda

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Introduction

The Gastropod snail species are renowned for living intermediate hosts of a range of disease-causing helminth parasites (Opisa *et al.,* 2011; Caron *et al.,* 2014; Igbinosa *et al.,* 2016). Owing to wide distribution and adaptability to a diverse range of microhabitats and ecosystems, gastropod snail species can host several parasites including nematodes and trematodes (Giannelli *et al.,* 2015). Freshwater snails are a major transmitter of parasitic species of *Schistosoma* causing schistosomiasis in certain tropical and subtropical countries (Knight *et al.,* 2014; King and Bertsch, 2015). Apart from the freshwater snail, land snails are also ideal host for several helminth species including rat lungworms *Angiostrongylus cantonensis* and *A. costaricensis*, *Strongylus stercoralis, Fasciola gigantica, Drocoelium dendtriticum* (Igbinosa *et al.,* 2016; Giannelli *et al.,* 2015). In the present investigation, the snails *Succinea daucina* (Pfeiffer, 1855) (Gastropoda: Succineidae) and *Indosuccinea semiserica* (Gould) (Gastropoda: Succineidae) are being represented as the intermediate host of trematode parasite *Leucochloridium*, in India. The description is expected to fill the required information on the intermediate hosts of the species in the Indian context.

The adult *Leucochloridium* parasite inhabits the cloaca or intestine of birds, while the sporocyst of this parasite occurs within the tentacle of several species of Succineid snails. The presence of *Leucochloridium* in *S. ovalis* (Robinson, 1947), *S. retusa* (Woodhead, 1935) and *Oxyloma retusa* (Kagan, 1951; Gower, 1936) from North America; *S. putris* (Ataev *et al.,* 2013, 2016) from Russia; *S. latua* (Nakao *et al.,* 2019; Ohari *et al.,* 2019; Sasaki *et al.,* 2022) from Japan has already been reported in previous studies. Recently, the parasite was recovered from the cloaca of the bird *Jacana jacana* from South America (Núñez *et al.,* 2020). The sporocysts of the parasite beat continuously (Wesenberg-Lund, 1934) during the day, thereby, making the infected host snail more conspicuous for to predator birds. The colorful and bulging *Leucochloridium* sporocysts change the appearance of the tentacles of the infected snails which are commonly termed as 'zombie snail'. Pulsating sporocyst within the brood sac resembles like crawling caterpillar which attracts its definite host, insectivorous birds (Wesołowska and Wesołowski, 2013) which preyed on the *Leucochloridium* sporocycst disrupting the snail tentacle (Nakao *et al.,* 2019). In this study, the pulsating rate of the sporocyst within the tentacles of *S. daucina* and *I. semiserica* was recorded separately in dark and light to estimate the significant difference in pulse rate in two photoperiodic conditions.

Materials and Methods

A thorough survey work was conducted during the monsoon period of the year 2015 to 2017, to enlist the land snail species of Kolkata and adjoining areas. During this survey period, we collected *S. daucina* from the amphibious habitat of sewage drain and *I. semiserica* from the leaves and trunks of several tree species. Post field collection, both snails were brought to the laboratory and kept in different glass terrarium measuring 38 cm x 29.5 cm x 13 cm. The base of the terrarium was filled with a moist soil layer and the terrarium was covered with a perforated plastic sheet. The infected snails were monitored regularly and color pattern of the sporocyst was observed visually and from photographs also. The number of beats of sporocyst per minute within the tentacle of both snail species was counted separately under dark and light phases. The observation was made on both species for five days at an interval of one minute between each record. The light intensity was kept same at each recording time for two species. The numbers of beating per minute were subjected to univariate ANOVA using day and photoperiod as explanatory variable. The two-sample t-test was performed with the mean pulse rate in light and dark phases to estimate significant differences between the same. The data of the experiments were subjected to statistical analysis (Zar, 1999) using XLSTAT software following the methodology described by Krebs (1999).

Results and Discussion

In the course of the survey, approximately 15 to 20 snails out of 200 *S. daucina* were observed to be infested with the sporocyst of *Leucochloridium* parasite. The sporocyst number in the infested snails varied between 01 and 03 with varying levels of development and colouration. In case of *I. semiserica,* about 20 out of 300 snails collected from different habitats of North 24 Parganas were observed to be infested with the sporocyst of *Leucochloridum* sp. The sporocyst brood sac invaded the antenna of the snails and the pulsating beats provided the appearance mimicking that of a caterpillar (Núñez *et al.,* 2020). Although colourful, the tentacles increased multiple times in size rendering the snails appear as 'zombie', an odd shape compared to that of other uninfested snails.

The *Lecochloridium* parasite was inserted within the tentacle of both snail species and 'pulsating brood-sac' (Woodhead, 1935) was conspicuous from the outside. In *S. daucina*, *Lecochloridium* sporocyst was intruded within one antenna while *I. semiserica* was infected with two sporocysts which were imbedded within the both tentacle (Fig. 1 a, b). The observations revealed that sporocyst brood sac of *I. semiserica* beat mean 99.23 ± 1.91 SE per minute (90 – 101) during day time and mean 76.57 ± 4.64 SE per minute (48 – 88) during night (n = 7 observations). In *S. daucina*, the sporocyst brood sac beat 65.85 ± 2.17 SE per minute, (25 – 94) during day and 30.5 ± 3.55 SE per minute (0 – 80) during night (n = 9 separate observations).

The coloring and banding pattern of observed sporocyst was similar with "The Green Sporocyst" color pattern described by Woodhead (1935). The beating rate of sporocyst within the tentacle varied with light intensity (Wesenberg-Lund, 1934; Wesołowska and Wesołowski, 2013). The pulse rate of sporocyst within *S. daucina* and *I. semiserica* varied significantly for day and photoperiod. The two sample t-test of mean of pulse rate for *S. daucina* ($t_{2,64}$ = 7.28; p<0.0001) and *I. semiserica* ($t_{2,73}$ = 28.71;

Succinea daucina infected with *Leucochloridium* parasite

Indosuccinea semiserica infected with *Leucochloridium* parasite

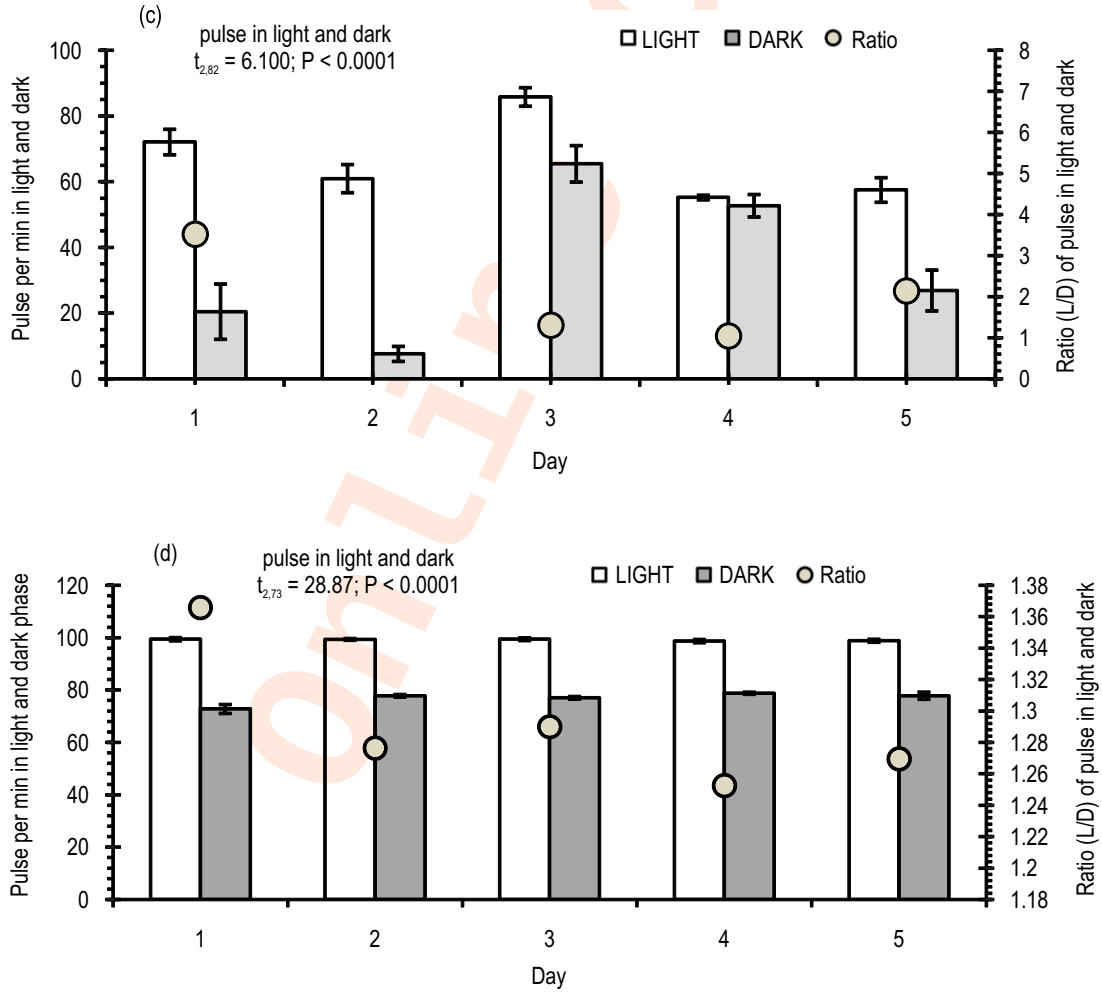

Fig. 1: (a) *Succinea daucina* (Pfeiffer, 1855) with *Leucochloridium* (Trematoda) infection; (b) *Indosuccinea semiserica* (Gould) with *Leucochloridium* infection; (c) Pulse rate of *Leucochloridium* within host *S. daucina* and (d) Pulse rate of *Leucochloridium* within host *I. semiserica*.

Table 1: Result of the ANOVA showing significant difference of pulse rate of the parasite in light and dark conditions. The values in bold are significant at p<0.0001

Succinea daucina				
Source	DF	SS	MS	F
DAY	4	25780	6445	**25.75**
Photoperiod	1	38678	38678	**154.56**
DAY*Photoperiod	4	11443	2861	**11.43**
Error	114	28529	250	
Total	123	1044293		
Indosuccinea semiserica				
Source	DF	SS	MS	F
DAY	4	147	37	**3.506**
Photoperiod	1	16426	16426	**1566.421**
DAY*Photoperiod	4	203	51	**4.832**
Error	118	1237	10	
Total	127	18012.88		

p<0.0001) depicted that the number of beating per minute differed significantly under light and dark conditions (Fig. 1c, d). The analysis of variance (ANOVA) test also revealed significant variation in beating rate of parasite under dark and light conditions (Table 1). Thus, the result of the present study was compatible with the previous statements that beating of sporocyst within the snail tentacle varied with light intensity.

The bizarre appearance of the parasitized snails is approximately named 'zombie snails' in common nomenclature. *S. daucina* is amphibious (Raut *et al.,* 1997) in nature preferring moist wall of sewage drain and *I. semiserica* (Raut and Ghose, 1984) has arboreal adaptation mostly inhabiting in tree leaves and tree trunk. Though both species inhabit different habitats, but are equally prone to get infected with the parasite. During the grazing of snail species over the soil and detritus, the sporocyst of this parasite enters the snail body and then intrudes within the tentacle of a snail. The strategy of the parasites *Leucochloridium* is to utilize the amber snails as a vehicle to increase in number and manipulate them for reaching to the ultimate host – a bird species. With the aid of environmental factors like light and soil conditions, the helminth parasite modulates the behavioural activity of the snail (Ataev and Tomakova, 2015) and thus increased the possibility of reaching the final host and completion of life cycle.

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Add-on Information

Authors' contribution: G. Nandy: Snail collection and counting of beating, analysis and primary drafting; **H. Barman:** Snail collection and counting of beating and primary drafting; **S. Pramanik:** Snail collection and counting of beating; **K. Paul:** Snail collection; **D. Kundu:** Snail collection; **A. Ash:** Identification of the parasite and counting of beating; **G. Aditya:** Statistical analysis, compilation and primary drafting.

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Ethical approval: Not applicable.

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Data from other sources: Not applicable.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology*.

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