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Egg laying strategies and effect of temperature on egg development of *Argulus siamensis*

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Abstract *Argulus siamensis* is the most damaging fish parasite prevalent in the freshwater aquaculture systems of India. In an attempt to further understand the behavior of this economically important parasite, the means of biological transmission, egg laying strategies and effect of temperature on development of eggs was studied. *A. siamensis* showed opportunistic egg laying behavior where in it used both living and non-living substrata for egg laying. It was marked that the parasites used the shells of freshwater snails of the family Viviparidae, the runners of the water weeds of genus *Nymphoides* and dead fish in the culture ponds for laying of eggs. This study confirmed that the maximum eggs were laid by the parasite in the habitat usage zone of the host fish. The optimum temperature for development of the eggs of *A. siamensis* into the infective naupliar stage and hatching was found to be 28 °C. These new insights into the behavior of *A. siamensis* would be helpful to devise biological control methods against the parasite.

Keywords *Argulus siamensis* · Egg laying · Biological transmission · Temperature

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Introduction

The diversification and intensification of aquaculture has led to an increased interest in the study and control of the parasitic diseases of fish and shellfish. Crustacean ectoparasites from the genus *Argulus* are regarded as the most widespread and problematic parasites in freshwater fish culture (Kearn 2005; Walker et al. 2004) which can be responsible for significant economic losses in aquaculture (Menezes et al. 1990; Saurabh et al. 2011). *Argulus* spp. can have deleterious impact on the host by their attachment and feeding leading to dermal ulcerations. However, the situation intensifies when these feeding sites become a gateway for secondary infections from fungi and bacteria (Stammer 1959; Shimura 1981; Singhal et al. 1990) and when argulids act as vectors for other pathogens like nematodes (Moravec 1994; Molnar and Szekely 1998) and viruses (Dombrowski 1952; Ahne 1985; Cusack and Cone 1986). There exist several instances when argulosis has also been associated with fish mortality (Gopalakrishnan 1964; Singhal et al. 1990; Sheila et al. 2002).

Among the species of *Argulus*, *Argulus siamensis* infection has specifically emerged as a significant problem of composite carp culture ponds in India (Mishra 1991; Saurabh et al. 2011). *A. siamensis* is the most dominant species of the Indian subcontinent having been found in the major aquaculture zones of India (Sahoo et al. 2012). In spite of this there is a great paucity of data regarding the ecology and behavior of this parasite species. In the absence of effective chemical control methods and vaccines against this parasite, biological control could be a viable option. A novel biological control method using plastic egg laying boards was developed for the control of *A. foliaceus* in a rainbow trout fishery (Gault et al. 2002), however; its effectiveness relies on knowledge of the egg

laying patterns of the parasite. The egg laying patterns of *A. coregoni* (Hakalahti et al. 2004) and *A. foliaceus* (Harrison et al. 2006; Taylor et al. 2009) are well documented. However, the egg laying strategies of *A. siamensis* are yet to be studied. In this report we look into the egg laying habits of *A. siamensis* and its biological transmission in culture ponds. This report also aims at identifying suitable temperature that serves as a major factor influencing the developmental stages of the parasite. Such knowledge is important in order to devise biological control methods against *A. siamensis.*

Materials and methods

The carp culture ponds infested by *A. siamensis* in the farm of the Central Institute of Freshwater Aquaculture, Bhubaneswar, India were closely monitored in order to study the egg laying habits of *A. siamensis*. Visual inspections on the egg laying habits of *A. siamensis* were conducted in these ponds during netting and after draining. All possible substrata options available in the ponds were inspected for presence of eggs of *A. siamensis*.

To assess the depth and pattern of egg laying activity by *A. siamensis*, a carp culture pond of 4 hectare area and average depth of 146.25 cm was selected. The pond was stocked with all the major carp varieties, i.e. *Labeo rohita* (rohu), *Catla catla* (catla), *Cirrhina mrigala* (mrigal), *Hypophthalmicthys molitrix* (silver carp) and *Ctenopharyngodon idella* (grass carp) and was reportedly infected with the parasite during the month of January 2011. The stocking density of the pond was 7,500 fish/ha in the ratio of rohu:catla:mrigal:silver carp:grass carp::30:25:25:10:5. Fish were captured by drag netting and at least 20 individuals (most of them weighing above 500 g) from each species were inspected for the presence of *A. siamensis* and the degree of infestation in different species of the carps recorded. In order to assess the depth of egg laying activity, bamboo poles (approximately 180 cm long) were placed in three different locations of the pond vertically to provide substratum to the parasites for egg laying. The entire length of the poles in water was divided and marked into three zones namely the bottom zone, middle zone and the upper zones each of approximately 60 cm. The poles were investigated everyday for a period of 5 days, and clutches of egg laid in each zone were counted and recorded. The abundance of eggs in each zone was estimated by calculating the average number of egg clutches in each zone.

In order to study, if a biological gradient exists between *Argulus* infection and temperature; the effect of temperature on development and hatching of *A. siamensis* eggs was studied by varying temperature while keeping all other parameters (viz., source of eggs and water) constant. Populations of *A. siamensis* were maintained on *L. rohita* (approximately 1 kg in weight) in two glass aquaria of volume 360 L. Non-chlorinated tap water was used in all the systems with daily exchange. The pattern of egg laying was observed. Eggs deposited on the glass sides and bottom of the aquaria were scraped off gently using a scalpel and transferred to beakers containing the same non-chlorinated tap water. The eggs (approximately 100 in each beaker) were incubated at different temperatures ranging from 0 to 32 °C (0, 4, 15, 28 and 32 °C) for 3–20 days, in replicates with a daily refreshment of water in cooling incubators. The time taken for development of eggs into naupliar larvae and hatching was recorded.

Results and discussion

Argulus siamensis exhibited an opportunistic egg laying behavior. Inspection of some of the culture ponds revealed that the gravid females of the species laid eggs on any solid substratum available, i.e. rocks, wood, plastic and concrete. Water plants and weeds were also often found as substratum by the parasites for laying eggs. A preference for wood over plastic and dark colored over light colored substratum was also observed. However, as the wood surfaces gathered silt, the deposition of eggs on them also reduced showing a preference of the parasite for surfaces free from silt. Similarly, earlier studies have also shown deposition of less or no eggs of *Argulus* spp. on silt or fine gravel (Hakalahti et al. 2004; Taylor et al. 2009).

It has been described that *Argulus* spp. use hard substrata, rough irregular stones (Hakalahti et al. 2004) and plant stems for laying of eggs (Walker et al. 2004). An interesting observation was made while netting in some *Argulus* infested carp culture ponds. The freshwater snails

Fig. 1 Egg clutches of *Argulus siamensis* (*arrow*) on the shell of the freshwater snail of the family Viviparidae

dragged out by netting from the ponds had clutches of eggs of *A. siamensis* on their shell (Fig. 1). The hard shell of these snails belonging to the family Viviparidae provided an excellent substratum to the parasite for laying of eggs. The added advantage of laying eggs on the shell of snails might be the active biological transmission of the parasite in the pond systems. A check on the population of these snails in the culture ponds possibly would reduce the transmission of the parasite from an infested pond to other and thereby reduce the incidence of *Argulus* infestation. This could be achieved by use of various available chemical control methods like treatment with copper sulphate. However the risks associated with such chemical treatments should be well considered.

The culture ponds in the farm were found to harbor some plant species of the genus *Nymphoides*. These plants produce runners which snake around in the pond water. In some of the ponds, egg clutches of *A. siamensis* were collected from the runners of these water weeds (Fig. 2). This indicated that the presence of submerged water weeds in ponds also aids the transmission of these parasites.

Further, clutches of eggs were found laid on the scales of dead fishes which were collected from ponds heavily infested with *A. siamensis* (Fig. 3). Most probably before leaving the dead host the gravid females would have used it as a substratum and laid eggs on it. This behavior also probably aids the successful transmission of the parasite, in case the dead fish being lifted up and dropped in nearby culture systems by birds.

The fish from the site selected for the assessment of pattern and depth of egg laying when checked for the presence of *A. siamensis* revealed that *L. rohita* was the most affected species. It harbored the maximum quantum of parasites and was followed by *C. catla* and *C. mrigala*. Other species of fish stocked in the pond did not show significant infestation by the parasite. This infestation pattern is in accordance with earlier reports which found rohu to be most susceptible and silver carp relatively resistant to *Argulus* infestation (Sahoo et al. 2011). The degree of egg laying activity was found to be maximum in the middle zone of the pond followed by the upper zone and lower zone (Fig. 4). It is hypothesized that the location of egg laying is determined by the habitat usage of the host fish (Taylor et al. 2009). The findings of this study were in accordance with the above hypothesis as the highest numbers of egg clutches were collected from the middle zone which happens to be the habitat of *L. rohita*, the most preferred host of *A. siamensis*. *C. catla* the second most affected species of the pond is a surface feeder and therefore the upper zone had the second highest number of egg clutches. Lastly, the bottom zone had the least number of egg clutches as the bottom feeder *C. mrigala* showed the lowest degree of infection.

This opportunistic egg laying behavior of *A. siamensis* could be exploited for the biological control of the parasites. A two pronged method wherein any hard substrata like stones and plants which can be used by female lice for egg laying are removed from the ponds and instead egg

Fig. 2 The water weeds of the genus *Nymphoides* which acts as a substratum for egg laying by *Argulus siamensis*. The *inset* shows clutches of eggs of *Argulus siamensis* (*arrow*) laid on the runners of the water weed

Fig. 3 Deposition of clutches of eggs of *Argulus siamensis* (*arrow*) on a dead Catla

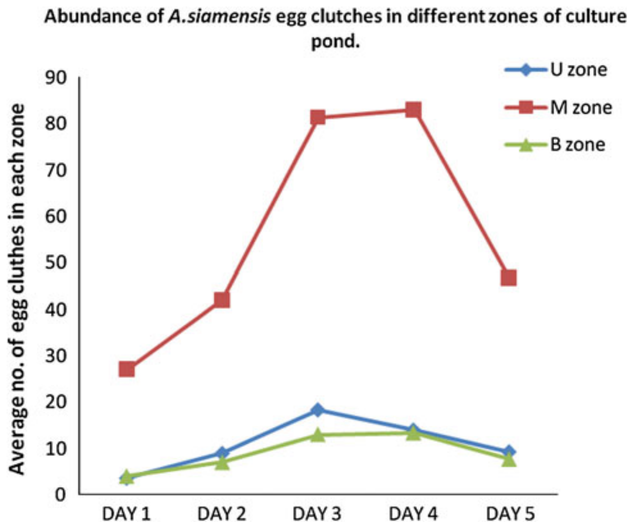

Fig. 4 Average number of egg clutches of *Argulus siamensis* in three zones of the culture pond under study

laying traps from which eggs can be harvested are introduced; would be effective in controlling *Argulus* infection. Egg laying boards have been found to be effective to some extent in controlling the population of *A. foliaceus* in commercial stillwater trout fishery (Gault et al. 2002). The placement of egg laying boards or other substratum strategically in the habitat zone of the host fish would be beneficial for the control of the population of the parasite to a greater extent.

The eggs collected and incubated at different temperatures showed that temperature greatly influences the rate of development of the eggs and also the quantum of eggs developing and hatching out as naupliar larvae. The eggs of *A. siamensis* incubated at 32 °C hatched out to naupliar larvae in a period of 10 days with a hatching percentage of 60 %. At an incubation temperature of 28 °C, 75 % of the incubated eggs developed into the naupliar larvae stage in a period of 15 days. Eggs incubated at 15 °C did not show any development. However when brought back to room temperature (25–28 °C), these eggs developed into the naupliar stage in 18 days. It was observed that eggs incubated at 15 °C for periods extending 20 days were not able to develop even after being brought back to the room temperature. The percentage of eggs developing into the larval stage in case of storage at 15 °C for 20 days was 30 %. The eggs stored at 4 °C for 3 days took 20 days to develop into the naupliar larvae when brought back to room temperature and the percentage of eggs hatching out was 20. The eggs stored at 4 °C for periods extending 3 days did not show any development. The eggs stored at 0 °C did not show any further development (Table 1).

The time required for hatching progressively decreases with an increase in incubation temperature showing the developmental dependency on temperature. The optimum temperature for development into the infective naupliar stage and hatching was found to be 28 °C. At temperature higher than 28 °C although the development was faster but the percentage of eggs hatching out as larvae was lower comparatively. At low temperature the development was slow and also the percentage of naupliar larvae hatching out was very low. It has been recorded that the parasite *A. foliaceus* is absent below 10 °C water temperatures in lakes (Taylor et al. 2009). In India, the incidence of argulosis is less in the months of extreme temperatures (P. K. Sahoo, unpublished observation). This finding provides a reasonable explanation for the seasonality of *Argulus* infection and its higher occurrence in the warmer months of the year. Since the development slows down at lower temperatures and hatching out occurs only when ambient temperature is provided, this suggest that eggs of *A. siamensis* also undergo overwintering in the colder months of the year as reported in many other species of this genus prevalent in northern latitudes (Bower-Shore 1940; Mikheev et al. 2001).

Table 1 Effect of temperature on egg development of *A. siamensis*

Temperature of incubation of *A. siamensis* eggs in °C	Hatching percentage of eggs	Time period required for hatching in days
0	Nil	Nil
4	20	20 (only when brought back to 28 °C within 3 days of incubation at 4 °C)
15	30	18 (only when brought back to 28 °C within 20 days of incubation at 15 °C)
28	75	15
32	60	10

The present study indicated that *A. siamensis* exhibited an opportunistic egg laying behavior and the depth of egg laying depends on the habitat usage of the host fish. This knowledge can be exploited for control of the parasite by providing artificial substratum and strategically placing them in the habitat zone of the host for removal of the laid eggs eventually. The study also explained the ways by which biological transmission of the parasite took place in the pond systems. Further, it also identified the optimum temperature for development of the parasite. This information is potentially important in developing a strategy for the control of *A. siamensis* and also would be of help to determine the time and frequency of anti-parasitic treatments in different water temperatures.

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