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Vertical pattern and strategies in egg laying by fresh water fish louse *Argulus siamensis* (Crustacea: Branchiura) in carp culture ponds of SPSR Nellore District, Andhra Pradesh, India

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Abstract
The present study was conducted to study the vertical pattern and strategies in egg laying by fresh water fish louse *Argulus siamensis* as it is the most common *Argulus spp* and devasting fish parasite affecting fresh water fish culture in India. This study revealed that a greater number of egg clutches were found in the middle zone (107.15 ± 57.27) which is the habitat zone of common host fish rohu, next greater number of egg clutches were found in upper zone (78.43 ± 43.77) followed by bottom zone(47.18 ± 25.13). At the same time *Argulus siamensis*. used different substrates for egg laying non-living objects such as inlet pipes, feeding poles, chemical bottles, slippers. etc., similarly egg clutches were found on living organisms like aquatic weeds and snails indicating opportunistic egg laying behaviour, and it will help to control the population of *Argulus siamensis* by regularly removing eggs by using substrates.

Keywords: *Argulus siamensis,* fish culture, rohu

1. Introduction
Aquaculture is the farming of aquatic organisms, such as fish and aquatic plants in various production systems like ponds, lakes, swamps, floodplain wetlands, Mangroves, canals, rivers, lagoons, reservoirs 2000. India is a premier fish country and the world's second largest producer of fish through aquaculture. India contributes 7.7% to the global fish production and the country ranks 4th in global exports of fish products (Ministry of Fisheries, Animal Husbandry & Dairying, 2020) [21]. Generally, the cyprinids group of fish dominates global.
In India, as in many other countries around the world, the disease is a big constraint to aquaculture and a limiting factor for economic and socio-economic growth (Bagum *et al.*, 2013, Mohan *et al.*, 2002, and Sahoo *et al.*, 2013) [1, 24, 29]. Many diseases that affect modern aquaculture are the consequence of increased culture activities without a basic understanding of the delicate equilibrium between host, pathogen, and environment (Subasinghe *et al.*, 2001, Bondad-Reantaso *et al.*, 2005) [34, 4]. The diseases are mainly caused by bacteria and parasites with only a few cases of fungal pathogens and viruses being reported in Indian aquaculture (Mishra *et al.*, 2017) [23]. The infestation of numerous fish parasites has hindered the production of culture systems. In comparison to other pathogens, the parasitic disease has become a major concern and has resulted in substantial losses in Indian freshwater aquaculture industry (Sahoo *et al.*, 2013) [29]. and found on every continent except Antarctica (Rushton-Mellor, 1992) [27].
Among all fish parasitic infestations, argulosis is most common disease (Mishra *et al.*, 2017) [23]. Argulosis is caused by the parasite Argulus, also known as the fish louse, which belongs to the genus *Argulus*, order Argolida, and phylum Arthropoda. (Rafinesque, 1815). In terms of economic losses in the industry, it is one of the most devastating parasitic infections (Sahoo *et al.*, 2013) [29]. The genus *Argulus* has profound effect on the wellbeing of the host fish, as it is cosmopolitan in terms of host choice and found on every continent except Antarctica (Rushton-Mellore, 1992) [27]. Argulosis outbreaks in various culture systems have resulted in mass mortality events in Indian aquaculture (Walker *et al.*, 2008) [37]. Crustacean ectoparasites are combated by fish farmers using a range of approaches that are either time-consuming, expensive, or both and do not always avoid economic losses. To treat argulosis various chemicals like parathion, hydrogen peroxide, dichlorvos, cypermethrin, trichlorfon,

avermectin, doramectin, ivermectin, teflubenzuron, deltamethrin, pyrethrin, malathion, cypermethrin, chlorophenol and formalin were used (Pike and Wadsworth 1999; Toovey and Lynon, 2000; Goven *et al.*, 1980; Klinger and Floyd, 2002; Hemaprasanth *et al.*, 2012; Treves-Brown 1999) [25, 35, 7, 14, 10, 36]. Phytotherapeutic like azadirachtin (Kumar S *et al.*, 2012a, b; Banerjee and Saha, 2013) [16, 2], piperine (Kumar A *et al.*, 2013) [17], rotenone, and nicotine (Banerjee and Saha, 2013) [2] have been documented as a biodegradable and inexpensive herbal extract to treat *Argulus* infections. However, questions about the industry's long-term survival have emerged due to the possibility of bioaccumulation in the host and the advent of reduced sensitivity to chemical therapies with elevated doses used to combat parasites (Jones *et al.*, 1992) [12]. Due to the lack of pure extracts or synthetic substitutes, phototherapeutics is impracticable for application in large aquaculture systems. (Kumar A *et al.*, 2013) [17]. As a result, efforts have been undertaken to develop ecologically benign, sustainable, and financially feasible physical control measures for argulosis. So, *Argulus* egg-laying techniques were studied in-depth to improve biological control methods. In India, some study on egg-laying techniques has been done (Sahoo *et al.*, 2012) [28]. Besides Harrison *et al.*, (2007) [9], studied diel trends in *Argulus foliaceus* egg-laying behaviour in a natural population in Ireland. In Finland, preferences for various materials, colours, and positions of substrata for *A. coregoni* egg-laying were checked (Hakalahti *et al.*, 2004) [8]. The present study aimed to study Vertical pattern and strategies in egg laying by fresh water fish louse *Argulus siamensis* (Crustacea: Branchiura) in carp culture ponds of SPSR Nellore District, Andhra Pradesh, India.

2. Materials and Methods

The study was carried out for a period of about 25 days ina fresh water fish farm at mollur village of Sri potisriramulu Nellore District (SPSR Nellore). For examining the egg laying strategies of freshwater fish parasite *Argulus siamensis* the carp culture ponds that are infested by *Argulus* were observed in Mollur village of Muthukur mandal and Komarika, Indukuripeta, Nidimusali, Somarajupalli villages of Indukuripeta mandal in Nellore district.

2.1 Samples analysis

To analyse the species of *Argulus*, samples were collected and sent to Central Institute of Freshwater Aquaculture (CIFA) Bhubaneswar for analysis. The report reveals that *Argulus siamensis*is the species.

2.2 Statistical analysis

Statistical analysis of the experimental data was carried out using statistical software SPSS version 20 *(*IBM 2012*)* and graphs obtained using Microsoft Excel - 2007**.** One way analysis of variance *(*ANOVA*)* was used to test the significance of differences for vertical pattern in egg laying. Statistical significance level was set at $P < 0.05$ and the difference between mean number of egg clutches in each zone were tested using Duncan**'**s multiple range test. All the parameters and the analysed data are presented in the form of tables and graphs wherever necessary.

2.3 Strategies in egg laying

To evaluate the depth and pattern of egg laying activity of *Argulus siamensis.* carp culture farm of 2.5-acre pond and depth of 4.5 feet approximately was selected. The stocking density of pond was 3500 nos. per acre; stocked with 2500 rohu, 600 catla, 350 mrighal and 50 grass carp. The pond was stocked with *Catla catla* (catla), *Labeo rohita* (rohu), *Cirrhinus mrighala* (mrighal) and *Ctenopharangdon idella* (grass carp) which was infested with *Argulus siamensis*in the month of December, 2020. The average body weight of fish in the pond was 600 grams and above.

In order to evaluate the depth and pattern of egg laying activity Eucalyptus poles were placed 1-2m away from the dyke in vertical position to provide substratum for the egg laying at 4 different locations in the pond. The entire length of the Eucalyptus pole was divided in to three 3 vertical zones namely upper zone (Zone-1), middle zone (Zone-2) and bottom zone (Zone-3); each zone was of about 1.5 feet height approximately. The poles were cross examined daily for a period of about 25 days and egg clutches laid in each of the zone were enumerated and noted. The abundance of egg clutches in each zone were estimated by calculating average number of eggs clutches enumerated in each zone.

In order to study the different substrates used by *Argulus siamensis* for egg laying carp culture farms infested with argulosis was cross examined and different substrates were observed.

3. Results and Discussion

Strategies in egg laying

Vertical pattern in egg laying by *Argulus siamensis*

The current study was conducted in argulosis infested carp pond by fixing wooden poles in four different locations in the pond. The wooden poles were divided in to three vertical zones (each zone 1.5 feet approximately) i.e., zone 1 (Upper zone), zone 2 (Middle zone) and zone 3 (Bottom zone). The number of egg clutches were enumerated daily. One way ANOVA was carried out to check vertical pattern in egg laying for a particular day and to check significant difference between days within a particular zone. Results revealed that number of eggs laid significantly differed ($P<0.05$) that means laying of egg clutches had a preference of zone. Duncan posthoc test further revealed that during the entire study period number of egg clutches laid in each zone significantly differed. In case of particular zone preference there is a significant difference of egg clutches laid ($P<0.05$). The highest mean number of egg clutches i.e., 107.15 ± 57.27 was observed in zone-2 (middle zone) followed by zone-1 (upper zone) (78.43 ± 43.77), and the lowest mean number of egg clutches i.e., 47.18 ± 25.13 was observed in zone-3 (bottom zone).

Average number of egg clutches laid in each zone by *Argulus siamensis* at the end of the study

Zone	Mean number of egg clutches
Upper (Zone-1)	78.43 ± 43.77
Middle (Zone-2)	107.15 ± 57.27
Bottom (Zone-3)	47.18 ± 25.13

Fig 1: Egg clutches laid by *Argulus siamensis* found on A) Feeding pole B) Weed plants

Fig 2: Egg clutches laid by *Argulus siamensis* found on the Inlet pipe

Fig 3: Egg clutches laid by *Argulus siamensis* found on the experimental pole

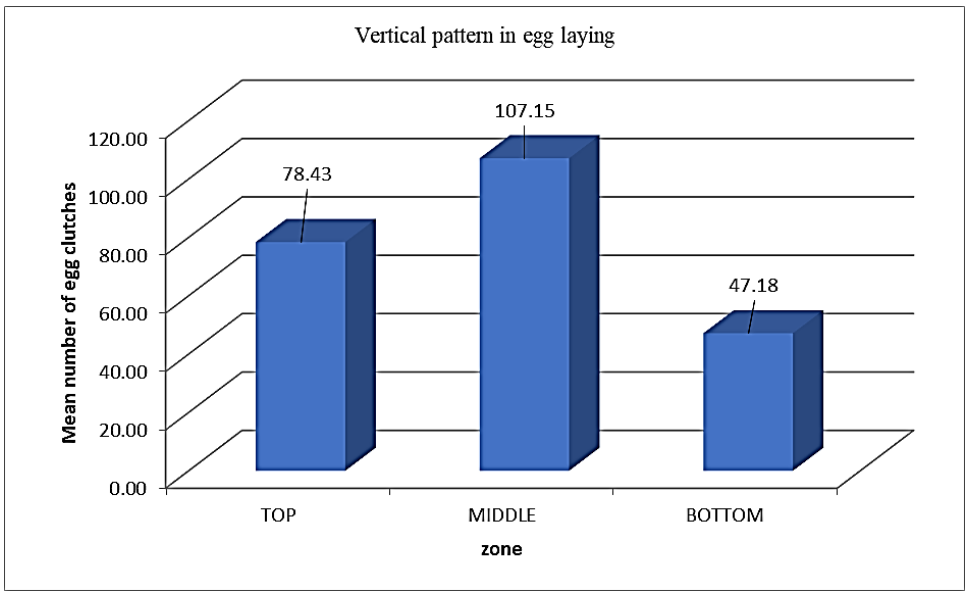

Fig 1: Average number of egg clutches laid in three zones by *Argulus siamensis* at the end of the study

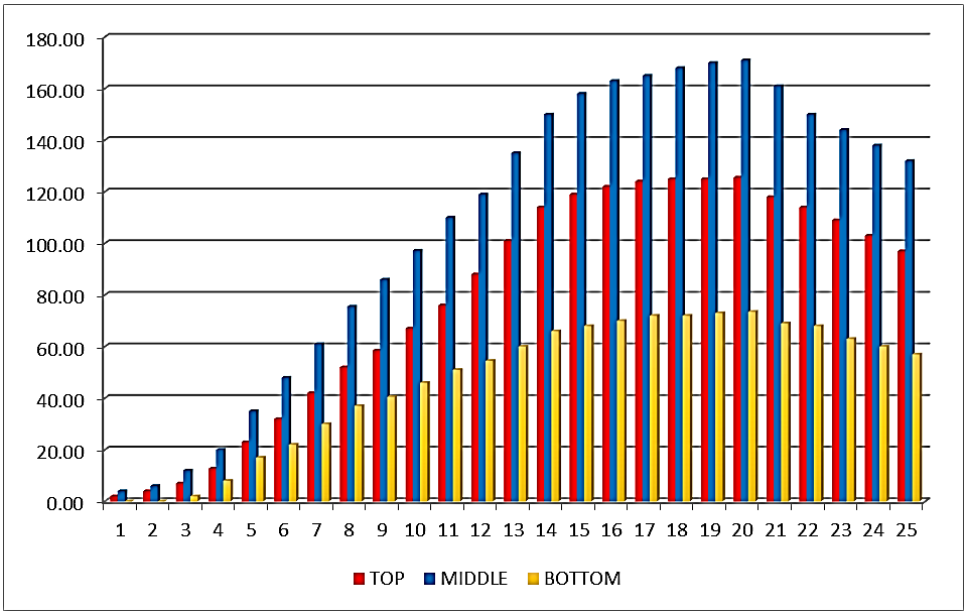

Fig 2: Average number of egg clutches laid in each zone by *Argulus siamens is* on daily basis

Different substrates used by *Argulus siamensis* for egg laying present in the pond

In the present study, when *Argulus* infested ponds were examined for the substrates used by *Argulus* for egg laying, it was noticed that the following substrates present in the pond were utilized: Aquatic weeds, grass found on the inner periphery of pond, snail shells, disposed plastic bottle found in the pond, inlet and outlet pipes, plant litters like coconut leaves, coconut, disposed chemical bottles, slippers and plastic covers which were accidentally fallen in the pond, feeding poles.

Strategies in egg laying

Vertical pattern in egg laying by *Argulus spp.*

According to Kimura (1970) [13], Shimura and Egusa (1980) [32], *Argulus spp*. lay their eggs on a suitable surface distant from the host. Rows of Argulus eggs are held together by a gelatinous substance that hardens when exposed to water (Martin 1932, Bower-Shore 1940, Shafir & Van As 1986, Rahman 1995) [18, 5, 30, 38]. An important finding was noticed while studying egg laying strategies in *Argulus siamensis* infested carp culture ponds. The freshwater snails attached to the poles that were used for study have egg clutches of *Argulus*on their shells. This finding is similar to Sahoo *et al.*, (2012) [28] where snails with egg clutches of *Argulus siamensis* were observed during netting. In the present study the egg laying strategies in relation to depth were observed where maximum number of egg clutches were found in middle zone (Zone 2) followed by upper zone (Zone 1) and bottom zone (Zone 3). This depth pattern is in accordance to the earlier report by Sahoo *et al.*, (2012) [28] where middle zone was found to have the highest level of egg laying activity followed by upper zone and bottom zone. According to Taylor (2009) the location of egg laying is assumed to be determined by the host fish's habitat usage. Similarly, because of their relationship with their preferred host species, Mikheev *et al.*, (2003) [19] suggest that *Argulus foliaceus* lay their eggs in shallow water environments. The results of this analysis backed the aforementioned theory, as the most egg clutches were gathered from the middle region, which also happens to be the habitat of *L. rohita* which is a *A. siamensis* preferred host because rohu most common cultured species. According to Sahoo *et al.*, (2013) [29] *A. siamensis* was the most commonly found species *Argulus species* in India. Next second-highest number of egg clutches were recorded in upper zone which is the habitat of *C. catla* and it is the second most affected fish in the pond. Finally, bottom zone had the lowest number of egg clutches, since the bottom feeder *C. mrigala* had the lowest degree of infection as well as lowest number stocked in the pond. However, Das *et al.*, (2016) [6] reported that *L. rohita* was the first preferred host for *Argulus siamensis* followed by *C. mrigala* and *C. catla.* As per findings in the present study, *Argulus siamensis.* exhibits opportunistic egg laying behaviour and the depth of egg laying is might dependent on the host fish's habitat usage. Unlike *A. coregoni* which lays eggs in deeper parts of the canal and ponds (Mikheev *et al.*, 2001) [20], *Argulus foliaceus* was found to prefer shallow water (Kollatsch 1959, Bauer 1970) [15]. Similarly, researchers (Bauer 1959, Mikheev *et al.*, 2001, 2003) [3, 20, 19] reported that *Argulus foliaceus* choose top 1 or 2 m of the water column for egg laying. According to Mikheev *et al.*, (2003) [19], *Argulus foliaceus* lay their eggs in superficial water environment because of their dependence on their preferred host species, perch and roach, which are usually found near to the shore in boreal waters in the summer, whereas salmonids, the preferred hosts of *A. coregoni*, choose deeper water. However, Harrison *et al.*, (2007) [9] reported that *Argulus foliaceus* lays its eggs at deeper regions of the lake during the second half of the egg laying season these shift in egg laying is due to habitat shift of salmonid population at the experimental site.

Different substrates used by *Argulus siamensis* for egg laying present in the pond

When some of the *Argulus* infested ponds inspected, the gravid females of the *Argulus spp.* laid eggs on solid substratum available, such as feed poles, plastic bottle, used chemical bottles, plastic pipes, aquatic weeds, snail shell and coconuts as a substratum for laying eggs. These findings were similar to Sahoo *et al.*, (2012) [28] and Bauer (1959) [3] earlier researcher observed *Argulus siamensis* eggs on wood, plastic,concrete, water plants and weeds and latter researcher reports that mature A. *foliaceus* lay their eggs as clutches on hard substrata. Similarly, Hoffman (1977) [11] suggested that

Argulus spp. lay egg clutches on readily available substrate. Mikheev *et al.*, (2001) [20] also reported that stony bottoms are most chosen by *A. coregoni* for egg laying.

4. Conclusion

In India, *Argulus siamensis* infection has emerged as a significant problem in composite carp culture ponds. According to the findings of the current study, A. siamensis exhibits opportunistic egg laying behaviour patterns, and the depth of egg laying is dependent on the host fish's habitat usage. Infection of ectoparasites to carps can be overcome by certain extent by keeping the artificial substrate in the pond ecosystem, so that secondary infection as well as crop failure can be controlled.

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