



## ARTICLE

# Prevalence of *Argulus* sp. in Indian Major Carps from Bhangore Block of South 24 Parganas District, West Bengal, India

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### ABSTRACT

The study was carried out to find the prevalence and severity of *Argulus* sp. in Indian Major Carps (IMCs) collected from Bhangore block of South 24 Parganas district of West Bengal during November 2018 to August 2019. January (2019) recorded the highest parasitic prevalence (PFI, 83.33%) and the month of August (2019) recorded the lowest parasitic prevalence (PFI, 9%). The identities of selected parasites were further confirmed by molecular identification through 18S rDNA analysis. The study revealed that *Argulus* sp. infestations had great economic implications especially in the winter months and is one of the most prevalent problems in fresh water aquaculture systems.

## 1. Introduction

The aquaculture sector of India is a potent food producing sector providing nutritional security, supporting livelihoods, providing employment and contributing to a major portion of agricultural exports. West Bengal's complete water area supports the state's potential fish farming and provides a variety of nutritional and dietary resources. West Bengal is popularly known as "rice – fish society" for the love of the people towards fish. Farming of the IMCs mainly takes over the state's freshwater aquaculture system in promoting fish culture

and development in West Bengal. The availability of safe and unique pathogen-free fingerlings and carp fry is of utmost importance. Literature cautioned that diseases, especially the parasitic diseases caused great damage to carps affecting their fry and fingerlings<sup>[1]</sup>. The intensity and severity of parasitic contamination in fishes show substantial variation within the distinctive environmental situations wherein they live<sup>[2]</sup>. Certain environmental conditions encourage diseases, among which water temperature is a crucial criteria<sup>[3]</sup>. Disease being the key problem for the fish culture causes a catastrophic effect both in economic and social growth. The upliftment and pro-

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motion of healthy and sustainable aquaculture practices face a number of problems, of which, emergent parasitic diseases pose a major threat to the production of fishes. Such emergent parasitic diseases endanger the economic, financial and nutritional security of the fish farmers and the consumers of the aquaculture products.

Carps are the mostly cultured fish species in West Bengal. One of the key problems for productive aquaculture is parasitic infestations of cultured freshwater carps causing severe economic losses in the industry. To study fish diseases, it is important to attain knowledge on different parasites, their biology and life cycle. Cultured carps are susceptible to myriad parasitic diseases of which *Argulus* sp. predominates.

The current study investigated the prevalence of *Argulus* sp. in IMCs from Bhangore block of South 24 Parganas District, West Bengal and the molecular identification of the same.

## 2. Methodology

### 2.1 Species and Area of Study

The study was carried out on Indian major carps namely Mrigal (*Cirrhinus mrigala*), Catla (*Catla catla*) and Rohu (*Labeo rohita*) for a period of 10 months from November' 2018 to August' 2019. The diseased fish samples were collected from Bhangore block [22°30'45.36"(N); 88°36'34.92"(E)] of South 24 Parganas District of West Bengal.

### 2.2 Sampling

100 numbers of each species were collected for a period of 10 months. A total of 300 species of Catla (*Catla catla*), Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*) were screened for the experiment of juvenile stage (Average 250 – 500 gm weight). The strategies used for collection and preservation of the samples for parasitic examination were followed as described by Soota, 1980<sup>[4]</sup>. The fishes were examined immediately after collection. Prior to collect the affected fish samples, its behaviour and clinical signs were recorded.

### 2.3 Parasitic Prevalence Study

The length and body weight of the fishes along with date and site of collections were recorded. The gills and body surface were checked thoroughly. Microscopic examinations were done from the smears taken from gills and body surface & photomicrographs of ectoparasites were taken using Olympus microscope (model no. BX51, made of Japan) with in-built digital camera (top view version

3.5). Phenotypic characterizations of parasites have been studied as defined and described by Soulsby (1982)<sup>[5]</sup>.

The Parasitic prevalence has been calculated with the aid of Parasitic Frequency Index (PFI, %), the formulae suggested and proposed by Margolis et. al. (1982)<sup>[6]</sup>.

$$\text{Prevalence (\%)} = \frac{\text{No. of hosts infected}}{\text{No. of hosts examined}} \times 100$$

Srivastava (1980)<sup>[7]</sup> suggested that the frequency index could be further classified into rare (0.1-9.9%), occasional (10-29.9%), common (30-69.9%) and abundant (70-100%). Determination of the Severity of infection was characterized for assigning numerical qualitative value to severity grade of infections, surface infestations and disease syndrome severity, through the following scale suggested by Lightner (1993)<sup>[8]</sup>:

**Table 1.** The Scale by Lightner (1993)

Disease Syndrome Severity	Remarks
0.5	Non infective
1	Mild
2	Moderate
3	Infective
4	Excessive

### 2.4 Parasitic DNA Extraction and PCR Amplification of 18S rDNA Gene

Genotypic identification of selected parasitic isolates was done by 18S rDNA sequencing. The genomic DNA of parasitic isolates were extracted by using genomic DNA isolation kit (Macherey-Nagel, Germany) as per the manufacturer's protocol. The 18S rDNA gene was amplified through PCR reaction that was performed in a Master cycler Pro S system (Eppendorf, Germany). The universal primers (forward primer UEP-F and reverse primer UEP-R) of amplification size 1900 - 2100bp were used.

### 2.5 Agarose Gel Electrophoresis & DNA Analysis

The PCR products were analysed on 1.0% agarose (HiMedia, India) gels containing 0.5 µg/ml ethidium bromide in 1X Tris-acetate- EDTA (TAE) buffer. The parasitic isolates were randomly selected for further identification through 18S rDNA analysis. This assay involved DNA isolation, amplification and sequencing of the gene coding for 18S rDNA. The result of sequencing is yet to derive (awaiting results).

## 3. Results

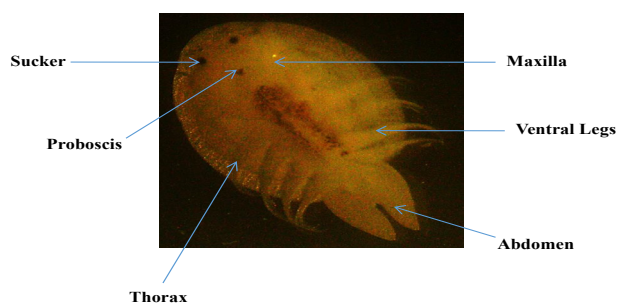
The Prevalence, Frequency Index and severity of infection

of the *Argulus* sp. (Figure 1) were presented in Table 2. The infestation reached its peak in the winter (from December to February) which was described as “abundant”. January recorded the highest infestation showing a PFI % of 83.33% (abundant) and lowest in the month of August (PFI, 9%) which was “rare” in terms of frequency index. During the ten (10) months study period Argulosis was almost always present in IMCs, which was “common” in most of the cases (Table 2).

**Table 2.** Prevalence, Frequency Index and Severity of Infection of *Argulus* sp. in Indian Major Carps (IMCs)

Months	PFI (%)	Frequency Index	Severity of infection
November 2018	66.67 ± 11.30 <sup>c</sup>	Common	2
December 2018	75.00 ± 10.10 <sup>d</sup>	Abundant	2
January 2019	83.33 ± 11.21 <sup>d</sup>	Abundant	3
February 2019	72.30 ± 09.30 <sup>d</sup>	Abundant	2
March 2019	44.00 ± 10.30 <sup>c</sup>	Common	2
April 2019	41.68 ± 11.04 <sup>c</sup>	Common	2
May 2019	36.67 ± 10.62 <sup>c</sup>	Common	2
June 2019	33.33 ± 09.70 <sup>c</sup>	Common	2
July 2019	28.67 ± 10.43 <sup>b</sup>	Occasional	1
August 2019	09.00 ± 10.23 <sup>a</sup>	Rare	0.5

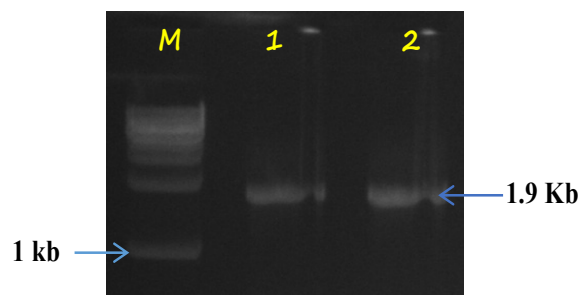
**Note:** Parasitic Frequency Index: a – rare (0.1-9.9%); b – occasional (10-29.9%); c – common (30-69.9%); d – abundant (70-100%).



**Figure 1.** Identified Parasite – *Argulus* sp.

Following the research works of Lightner (1993) [8] severity grade of infection was calculated in *Argulus* sp. It was observed that severity of infection was “moderate” almost throughout the year (November, December, February-June). The month of January showed “infective” severity grade due to extreme seasonal variation. July and August recorded “mild” & “non-infective” severity grades respectively (Table 2).

Randomly selected *Argulus* sp. were further identified through 18S rDNA analysis. In 1% agarose gel electrophoresis, approximately 1.9 kbp bands were obtained with 18S universal primers for parasitic isolates through PCR (Figure 2).



**Figure 2.** 18S rDNA PCR product from *Argulus* sp. [M-1kb ladder, 1 & 2-Samples]

#### 4. Discussion

All fishes are potential hosts to different species of parasites. With the increase in the interests of the fisheries potential, the awareness of the fish farmers and their experience with parasites have also increased. Though meagre amounts of parasites are ubiquitous in the culture system, they do not pose serious threats. However, all piscine parasites have a tremendous reproductive potential and can, under ideal conditions, quickly overwhelm the host.

The parasitic infestation influenced by the seasons had been portrayed by many researchers. Due to changes in water temperatures and poor fish immunity during the months of Winter and Spring, parasitic infestations tends to increase. The results of the current study were in agreement with the works of Bhuiyan *et al.* and Hoole *et al.* [9,10], who worked on disease incidence in relation with seasonal variations in aquaculture. In this context it can be said that in winter due to fall in temperature, fish species were more susceptible to diseases. The period of November-February recorded the highest parasitic prevalence.

The identities of selected parasitic strains were further confirmed by molecular characterization through 18S rDNA analysis (Figure 2). Modern scientists have been using a variety of kits which provide better progress at molecular level and are highly sought in the field of academic research. This evolving field accomplish the needs of the both laboratory and clinical study.

A lot of works had been done by researchers based on parasitic infestations and their seasonal influences. Crustacean parasites dominated most of the seasons. The current study clearly indicated that the month of winter is the most vulnerable time for the fishes [9,10]. Fish parasites when in higher concentrations can cause heavy mortalities and huge economic loss. Argulosis is one of the problems in fish culture program in Bhangore, South 24 Parganas, West Bengal.

## 5. Conclusion

*Argulus* sp. were more severe in winter. The current study highlighted the infestations of *Argulus* including their molecular identity. In winter lower temperature along with other factors made the fishes more vulnerable towards parasitic infestations.

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