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Prevalence of crustacean parasite (*Argulus japonicus*) in cultivable carp fishes in tarai region of Uttarakhand, India: Treatments and management

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Abstract
The present study delineates *Argulus japonicus* infestation in Indian and Exotic major carps from Udham Singh Nagar district of Uttarakhand. A total of 2695 specimens were examined for the presence of the parasite out of which 378 specimens found to be infected. The maximum prevalence of the parasite was recorded from *L. rohita* and least from *C. idella* as 15.2% and 12.0%, respectively. Furthermore, the results showed highest infestation in rohu as 18.5% from Kalinagar and lowest in grass carp as 6.0% from Pantnagar. Besides, the analyses indicated that the mean intensity of the parasitic infestation found to be established highest as 0.24±0.02 in Kalinagar while lowest as 0.12±0.02 in Pantnagar, however, as for as fish species is concerned, the mean intensity of Argulus infestation was recorded maximum from *L. rohita* and *C. carpio* as 0.84±0.22 and 0.84±0.24, respectively. The monthly variation in prevalence, abundance and mean intensity of the Argulus infestation have insinuated that the prevalence, abundance and mean intensity of the parasitic infestation recorded to be highest as 22.78±1.79%, 0.18±0.02 and 1.38±0.07, respectively, during the month of October among all the six fish species. The bigger size specimens (>25 cm) exhibited more parasitic infestation than the smaller ones. The treatment analysis has shown complete detachment of the parasite from the hosts when common salt's 3.0% solution for 5 minutes bath treatment applied. Also, the bath treatment with 0.1% solution of $KMnO_4$ for 5 minutes caused complete removal of the parasite from the host fishes.

Keywords: Indian and exotic major carps, *Argulus japonicus*, prevalence, salt, $KMnO_4$, Uttarakhand

Introduction
India is the second largest fish producer in the world with the share of 5.43% of global fish production and also the second largest country in total aquaculture production [11]. Fish is generally considered to be the most important source of animal protein, vitamins, minerals and other elements for the human health [1]. The importance of the fisheries sector in India can be realized by the fact that it employs more than 14 million people and contributing significantly to achieve millennium development goals [2]. Alike other animals, fish can also suffer from different diseases. Generally, in the freshwater habitats, the various fish-disease causing organisms like bacteria, virus, fungus, molds and parasites co-exist and live concurrently with the fishes [18]. Hence, the severity of a given disease is dependent on the interaction of numerous variables of the host, the pathogens and the environment [9]. In the process of intensification of aquaculture practices, the complex balance between the host (fish), the pathogens and the environment is often disturbed and the organisms under culture become stressed and prone to diseases. In recent years intensive fish farming is being practiced in the freshwater ponds of India and therefore sometimes, poor water quality invites different sort of fish diseases. The advanced techniques of intensive fish culture involve higher stocking and supplementary feeding which has in turn substantially enhanced the incidence of outbreak of various diseases in fishes [14]. In the freshwater cultured fishes, the major fish diseases reported are bacterial (fin and tail rot, columnaris, dropsy, bacterial kidney disease, eye disease), viral (Spring viraemia of carps or swim bladder inflammation, koi herpes virus), fungal (Saprolegniasis or cotton wool or water mould disease, branchiomycosis or gill rot, icthyophonus or swinging disease or reeling disease), protozoan (Ichthyophthiriasis or ich, castiasis, trichodiniasis, epistylis or red sore disease), crustacean (Argulosis, lernaeosis) and epizootic ulcerative syndrome or red spot disease or mycotic granulomatosis etc., furthermore,

nutritional, environmental and hereditary diseases are also known from the Indian freshwater fishes [6, 9, 14, 15, 18].

The *Argulus* species (Family: Argulidae) commonly known as fish lice, are members of a large group of branchiuran ectoparasite that infest and cause Argulosis disease in the carp fishes, if unchecked, virulence of this disease may lead to the mass mortality in fishes [10, 12, 14]. However, fish mortality is not the only criterion to evaluate the impact of disease. The morbidity due to loss of blood leads to weight loss and poor growth in fish, contributes substantial losses to the farmers. This parasite inhabits on the skin, fins and gills of the host and cause extensive pathological lesions in the skin showing circular haemorrhagic patches which become ulcerated subsequently, mucous cells get proliferated and copious mucous is produced [7]. The integument of the affected fish shows epithelial degeneration, hyperplasia of dense connective tissue whereas in kidneys change in tubular degeneration and necrosis [7]. These lesions promote secondary infections like fungi, pathogenic bacteria, viruses etc.

The Indian major carps (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*), contributing most to the total freshwater fish production in the country are found susceptible to the Argulosis disease, but *L. rohita* reported to be the most susceptible fish species [19]. Since last decade, there has been an increased realization of the necessity to avoid infectious agents to maintain healthy stocks to enhance fish production. In view of this, the present research work was undertaken to evaluate the prevalence of Argulus infestation in cultivable carps and to find out its effective treatments and management measures which can easily be adopted by the farmers.

Materials and Methods

In the present study, to know the extent of prevalence of fish lice (*Argulus* sp.) in cultivated carps fish species i.e. *C. catla, L. rohita, C. mrigala, H. molitrix, C. idella* and *C. carpio*, live specimens were collected from October 2012 to March 2013 from the selected fish farms at the Udham Singh Nagar District of Uttarakhand, India. The sampling was done once in a month from each of the selected sites. The geographical area of the district is 3055 km^2 and location 28° 53' N latitude and 78° 45' E longitudes. The district is bounded by Nainital and Champawat districts of Uttarakhand on the north, Moradabad, Rampur, Bareilly and Philibhit districts of Uttar Pradesh on the south, Bijnor district of Uttar Pradesh on the west and Nepal on the east. There are three climate seasons i.e. summer, monsoon and winter. The maximum temperature in the district goes up to 42 ºC during the summers and the minimum temperature is between 1 and 4 ºC, further north of the district, temperature comes down to 0.4 ºC in winter season. The average rainfall was recorded to be 1296.85 mm in the year of 2012. Soils are sandy to loamy texture, poorly sorted, comprising mainly of gravel, sand, silt, clay with pebbles.

For the current research work, nine fish farms were randomly selected from three different regions namely Dineshpur, Kalinagar and Pantnagar of Udham Singh Nagar district. The fish ponds having an area ranged from 0.2 ha to 0.5 ha and depth 1.15 m - 1.45 m wherein Indian and Exotic Major Carps were stocked together at the rate of 8000 – 12000 fingerlings/ha. The collected specimens were externally examined for the Argulus infestation. The parasites which could be seen easily with the naked eyes were picked up from the hosts with the help of a blunt forceps and camlin painting brush. After collection the parasites were preserved in 70% ethanol and examined under the microscope for their identification.

The treatments of Argulus infestation in fishes was carried out under laboratory condition for which 3 replicates were set up in which healthy five specimens of each fish species kept in 40 L capacity plastic tubs, separately. The fishes were made infected with eight to ten individuals of the Argulus parasite. The therapeutic measures used for the treatment of infected fishes were common salt (NaCl) with 1.0%, 2.0% and 3.0% concentration and potassium permanganate ($KMnO_4$) with 0.025%, 0.05% and 0.1% concentration, respectively. The prevalence, abundance and mean intensity of occurrence of the Argulus infestation were estimated using the following formulae proposed by Margolis *et al.* [8, 13].

$$1.\ \text{Prevalence} = \frac{\text{Total no. of infected fishes}}{\text{Total no. of fishes examined}} \times 100$$

$$2.\ \text{Abundance} = \frac{\text{Total no. of parasites recovered}}{\text{Total no. of fishes examined}}$$

$$3.\ \text{Mean intensity} = \frac{\text{Total no. of parasites recovered}}{\text{Total no of infected fishes}}$$

Results and Discussion

The current study revealed that the prevalence of Argulus in cultivable carp fishes was severe in the studied region. During the course of investigation, it was found that this parasite generally gets attached to the base of the fins (mostly pectorals and dorsal), operculum, body surface and the caudal peduncle region of the host [5, 10]. The species of the parasite was identified as *Argulus japonicas* [16, 20, 22]. There were a total of 2695 specimens examined which incorporated *C. catla* (469 nos.), *L. rohita* (482 nos.), *C. mrigala* (436 nos.), *H. molitrix* (434 nos.), *C. idella* (422 nos.), and *C. carpio* (451 nos.). The numbers of infected specimen were recorded as 67, 73, 63, 58, 51 and 65 of catla, rohu, mrigal, silver carp, grass carp and common carp, respectively. Moreover, the average numbers of the parasite were recovered as 20, 21, 20, 17, 17 and 20 from the hosts *viz*., *C. catla, L. rohita, C. mrigala, H. molitrix, C. idella* and *C. carpio*, respectively. Further, the analysis revealed that 14.0±1.1 percent population (including all the six species) was infected with the parasite with the maximum average level of infestation in *L. rohita* and minimum in *C. idella* as 15.2 and 12.0 percent, respectively [5]. Furthermore, results showed highest percentage of infestation in *L. rohita* (18.5%) from Kalinagar and lowest in *C. idella* (6.0%) from Pantnagar. The mean value of prevalence of infestation was calculated as 14.4±3.2, 15.2±3.8, 14.5±4.4, 13.4±4.2, 12.0±5.2 and 14.0±2.9 in *C. catla, L. rohita, C. mrigala, H. molitrix, C. idella and C. carpio* respectively. In addition, it was observed that fish farms in Pantnagar had lower prevalence of Argulus infestation i.e. 9.5% than that of Kalinagar i.e. 17.0% when populations of the six species together taken as a whole, Fig. 1.

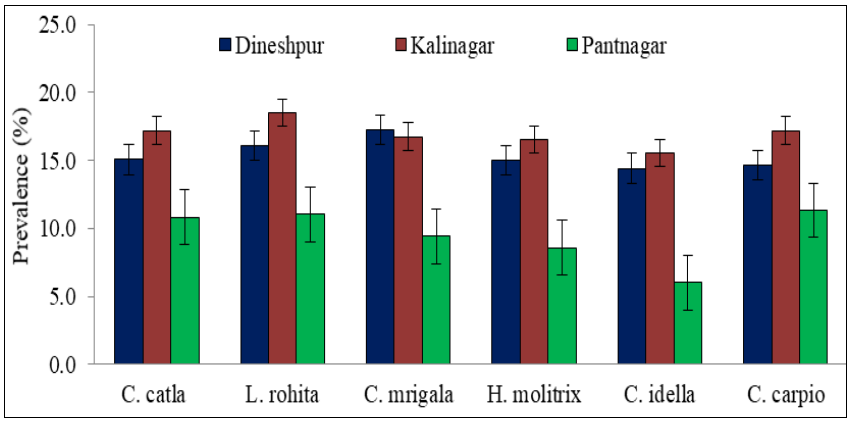

Fig 1: Prevalence of Argulus infetation on different fishes

The abundance of Argulus parasite on different fish species at different locations was also examined and recorded highest average abundance of the parasite i.e. 0.24±0.02 from Kalinagar whereas lowest i.e. 0.12±0.02 from Pantnagar. Though, the abundance of parasite on different fish species was analyzed and found to be highest in *C. carpio* followed by *L. rohita*, *C. catla*, *H. molitrix*, *C. mrigal* and *C. idella*, respectively Fig. 2.

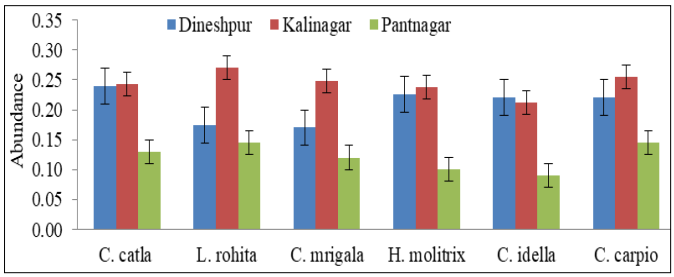

Fig 2: Abundance of Argulus infestation on different fishes

Besides, the analyses indicated that the mean intensity of the infestation was established to be highest (0.24±0.02) in Kalinagar while the lowest (0.12±0.02) in Pantnagar, however, as for as fish species is concerned, the mean intensity of Argulus infestation was recorded maximum in *L. rohita* and *C. carpio* as 0.84±0.22 and 0.84±0.24, respectively, Fig. 3.

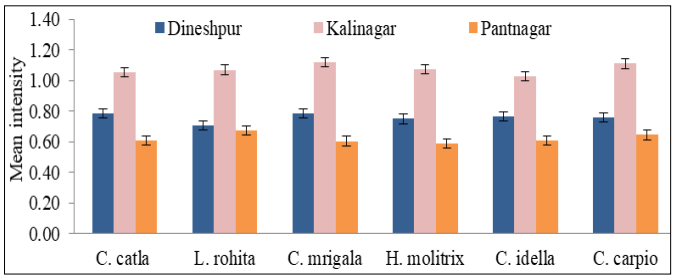

Fig 3: Mean intensity of the parasitic infestation on affected fishes

The monthly variation in prevalence, abundance and mean intensity of the Argulus infestation was also studied. The results have insinuated that the prevalence, abundance and mean intensity of the Argulus infestation recorded to be highest as 22.78±1.79%, 0.18±0.02 and 1.38±0.07, respectively, during the month of October among all the six studied fish species and lowest in the month of January. It was also found that the fishes of bigger size (>25 cm) had greater severity of the parasitic infestation than smaller ones (<25 cm). Also, there was found positive correlation between prevalence and mean intensity of the parasite with host's body size (r=0.89, r=0.78) [4, 5, 8, 17, 21].

It is a well known fact that Argulosis disease can be fatal for the fishes, if not, then also causes morbidity and stress in fishes and hence in turn gets in loss in weight and growth of the fishes. Therefore, fish growers in the region are experiencing substantial amount of monetary losses by having low fish productivity. Therefore, the research work on treatments and managements of the Argulus infestation was also carried out simultaneously. The treatment of the infected fishes was done with NaCl (Sodium Chloride) and $KMnO_4$ (Potassium Permanganate) by applying their various doses for the different durations. The treatment analysis revealed that all the individuals of the parasite got detached from the infected fishes when bath treatment with 3.0% common salt's solution was given for 5 minutes whereas the same solution used for 10 minutes revealed the complete removal of the parasites, though fishes were observed to become lethargic. The 1.0% salt's solution for 2, 5 and 10 minutes bath treatments did not show any impact on the parasitic detachment from the infected fishes. Furthermore, 2.0% salt's solution for 2 minutes bath treatment produced no effects, but, when same solution was used for 5 and 10 minutes showed partial detachment of the parasites. The bath treatment with 0.025% $KMnO_4$ solution for 2, 5 and 10 minutes produce no effects on the parasite. Though, treatment of the infected fishes with 0.05% $KMnO_4$ for 2 and 5 minutes caused partial detachment of the parasite from the host fishes, moreover, 10 minutes bath treatment has resulted in complete removal, but, fishes became lethargic and got stained. On the other hand, 0.1% $KMnO_4$ bath treatment for 2 minutes resulted in complete removal of the parasite from the host, furthermore, it was observed that the fishes were in a critical condition and showed staining when exposed for 10 minutes in the same solution.

Conclusion

The Argulosis is one of the major fish diseases caused by the *Argulus spp* commonly known as fish louse. The severity of this disease in India is well known as it can cause mass mortality in fishes, if not, then also, morbidity due to loss of blood leads to weight loss and poor growth. Moreover, the leftover hemorrhaged red or black patches on the body surface of the affected fishes resulting in their blemished appearance which let their market price get reduced due to the devourers' reluctance and hesitation to purchase such fishes that ultimately brings in substantial monetary losses to the fish farmers. The current investigations have demonstrated that 3.0% common salt's solution for 5 minutes or 0.1% $KMnO_4$ solution for 2 minutes as bathing medium can be used

successfully to treat the fishes affected with the Argulus parasite. Moreover, other researchers have also reported that bath treatment with 2.5% NaCl for 5 hours or 3.0% for 5 minutes or piperine at the rate of 9.0 ppm can cause complete elimination of the Argulus parasites from the carp fishes [3, 10]. During the course of investigation, it was found that the key factors responsible for the Argulus infestation in the studied regions were lack of awareness about the preventive measures need to be taken and the better management practices to be adopted to avoid parasitic infestation in fishes. The best ways to control Argulus infestation in the fish ponds are adherence to better management practices like proper water quality maintenance, stocking of healthy fingerlings which are free from such parasitic infestation, optimum feeding and manuring, liming, draining the ponds and subsequently allowing them to dry off at least once in a three years, and prescribed doses of lime and $KMnO_4$ may be applied intermittently in the ponds as precautionary measures to avoid parasitic infestation.

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