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Study of parasitic effect of *Argulus sp.* on Indian major carps in pond culture

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Abstract
The current investigation was undertaken to study the parasitic effects of Argulus sp. on Indian major carps. Only symptomatic and moribund samples of diseased fishes comprised of fingerlings and adults of Labeo rohita, Catla catla and Cirrhinus mrigala were collected for experiments. The affected fishes showed weight loss, retarded growth, restlessness, erratic swimming behaviour and loss of appetite. The skin showed abnormally pigmented while gills, kidney, liver and spleen of the affected fishes were pale in colour. The most significant histopathological lesions in the gills were the secondary lamellar hyperplasia, haemorrhages in the tips of the primary lamellae and fusion of both secondary and primary gill lamellae. Skin showed epidermis was lost but the epidermal cells in the vicinity of the sites of attachment were hyperplastic, muscular layer showed degenerative and necrotic. Kidney showed multifocal enlargement of glomeruli, glomerular tufts in many places shrunken, fragmented and necrosed, in renal tubules vacuolar degeneration. Fingerlings and adults of rohu were found highly susceptible to this disease than other IMC.

Keywords: Argulosis, histopathology, crustacean parasite, necrosis, Indian major carps, Labeo rohita, Catla catla, Cirrhinus mrigala

Introduction
The parasitic infestations are reportedly playing a major role in disease occurrences (78%) in Indian freshwater aquaculture. There are around 21% production loss due to diseases, poor farm management practices and impaired growth. In most of the fish farms, it was observed that among all fish pathogens, parasitic infestation has been the major cause of concern because of high morbidity and slow growth rate. These parasites were difficult to be removed from the culture system, causing significant setback to freshwater aquaculture. Under poor water quality conditions or stressful environment, these parasites multiply rapidly there by affecting fishes with high morbidity (Mishra, *et al.* 2017) [8].
Argulosis is a disease caused by crustacean parasite (*Argulus sp.*) and commonly known as "Fish louse" The larvae and adults of argulus are parasitic to fish. This parasite penetrates the upper layers of the host's kin and feeds on blood and body fluids (Van Der *et al*, 2000) [17]. From Indian subcontinent eight species of Argulus have been reported. Das and Das (1997) [2] reported *Argulus foliaceus*, *Argulus bengalensis* and *Argulus siamensis* infesting Indian major carps *L. rohita*, *C. mrigla* and *C.catla*. The skin and fins had brownish grey points and hemorrhagic areas. *Argulus* infestations lead to secondary parasitic infestation of the skin (Soulsby, 1982) [15]. Carps were more suitable host for *Argulus*. Mainly rohu infestation rate was l00%. This result was almost similar to Bakshi *et al.* (2006) [1]. So the objectives of the present study were to isolate, identify and *Argulus sp.* infestation in Indian major carps.

Materials and Method
Frequent incidences of various naturally occurring diseases were recorded in the cultivated ponds/tanks/hatcheries in Darbhanga's various State Govt. ponds and also private fish culturists during the period of observation. Collected samples were comprised of fingerlings and adults of Indian major carps in most of the occasions. Only symptomatic and moribund samples of diseased fishes were collected and were brought to the laboratory for patho-morphological and Patho-anatomical examinations. Small bits of tissues (3-4 mm thick) from the vital organs like skin, gills, kidney, liver and spleen dissected and fixed in ten percent

Neutral Buffered Formalin for 18-24 hours. Fixed tissue samples were then processed using the standard histological methods (Luna, 1968) [7]. Tissue blocks were cut into serial sections (5-7 thick) by a rotary microtome. For routine staining of the histological sections, Ehrlich's Haematoxylin (H.) and alcoholic Eosin (E.) stains were prepared. And Photomicrographs of the most characteristic regions of histopathological lesions in the stained tissues of diseased fish samples were taken.

Result

This disease was observed to be a serious problem for fish culturists in the brood and large fish culture ponds especially during the summer months. The disease is caused by the crustacean parasite; *Argulus* sp. which causes large scale mortalities of fish in case of acute infection. *Argulus* sp. is obtained from warm freshwater ponds of Darbhanga city (Figure-1). *Argulus* sp. measures a total length of 4-7 mm and a width of 2.5-5 mm. Argulid females are generally larger than males, and the growth of the parasite may be influenced by the size of the host. In warm summer temperatures, the whole life cycle may be completed in less than 40 days.

Fig 1: Light micrographs of live specimen of *Argulus* sp. (Magnification 40x)

Clinical Symptoms and gross pathology

The adult parasites which are leaf-like in appearance were found attached to the body of the host fish in the skin, fins or gills. Fin bases, ventral surface of the body and skin over the head and operculum were the favourite sites of attachment of the parasites to the host. The affected fishes carrying a heavy load of parasites were highly mucus laden with very slimy surface and pale appearance. These sites were haemorrhagic, ulcerated and mucus-studded. The skin of *Argulus* affected fishes was pale and sometimes abnormally pigmented. Gills were generally pale in colour. The affected fishes showed weight loss, retarded growth, restlessness, erratic swimming behaviour and loss of appetite. Kidney, liver and spleen of the affected fishes were pale in colour and somewhat abnormal in consistency.

Histopathology

Skin: *Argulus* parasites caused extensive damage to skin epithelium by insertions of stylets into the epidermal cells. At the sites of attachment of the parasites, the epidermis was lost but the epidermal cells in the vicinity of the sites of attachment were hyperplastic. The dermis showed inflammatory reactions and haemorrhages. Scales became loose in the scale pockets and fell because of the damage of the dermal cells. The dense connective tissue of the dermis became oedematous and hypertrophic and showed large vescicles. Multifocal zones of inflammatory cell infiltrations particularly by lymphocytes and macrophage cells were found in the dermis, hypodermis and muscular layers (Figure-2.) Muscular layer showed degenerative and necrotic changes in the muscle fibres. The influx of lymphocytes and macrophage cells in the ulcerated areas indicated severe inflammatory response of the host due to the parasite infestation. Some of the blood vessels in the dermis were highly congested and dilated (Figure-3.).

Gills: The most significant lesions in the gills were the secondary lamellar hyperplasia, haemorrhages in the tips of the primary lamellae and fusion of both secondary and primary gill lamellae. The fusion of secondary lamellae in some places was along the lengths while in other places it was restricted only to the tips of the primary gill lamellae. In many places of the section, the epithelial lining of the secondary gill lamellae appeared swollen and oedematous. In some other areas the epithelium was found detached from the underlying core of pilla cells. In some areas of the gill section few telangiectatic gill lamellae were also observed. In telangiectasis or aneurism of the lamellae, the tips of many secondary lamellae became swollen and ballon-like in appearance containing mostly red blood cells inside (Figure-4.).

Kidney: Kidney showed multifocal enlargement of glomeruli but having the glomerular tufts in many places shrunken, fragmented and necrosed. In some highly affected renal tubules, the epithelial cells showed large vacuoles indicating vacuolar degeneration and basement membrances of these cells were desquamated. The kidney haematopoetic tissue exhibited extreme degree of proliferation and showed infiltration of mostly macrophage cells in the tissue indicating acute inflammatory reactions. In some areas of kidney section, large melanomacrophage centres were seen in the tissue (Figure-5.).

Liver: In the liver, most of the central veins were engorged with red blood cells and some fibrinous materials. The heapatocytes were swollen and hypertrophied in many regions of the section (Figure-6). The hepatocyte nuclei in these swollen cells were found pycnotic and dark stained. In other regions of the section some focal necrotic areas were seen in the parenchyma and inflammatory cell infiltration particularly by lymphocytes were noticed. Cord-like arrangement of the hepatocytes was disrupted in many places and there was dilatation of the sinusoidal spaces particularly around the central veins. The sinusoidal spaces were congested with blood cells particularly red blood cells. Some focal areas of haemorrhages were also seen in the liver. The pancreas also showed focal necrotic changes.

Spleen: The spleen showed extensive necrosis of both the red and white pulp and there were multifocal areas of haemorrhage in the tissue (Figure-7.). Also there were many large melano-macrophage centres in certain areas of the tissue sections and these centres contained haemosiden pigments (Figure-8.). In some areas there was infiltration of neutrophils in the spleen parenchyma indicating inflammatory reactions.

Diagnostic histopathological lesions identified

At the sites of parasite attachment the typical skin lesions were epidermal desquamation, dermal necrosis, inflammatory reactions through infiltration of lymphocytes and macrophages, congestion and dilation of blood vessels. Characteristic gill lesions in the disease were gill hyperplasia, hypertrophy, and fusion of lamellae and presence of telangiectatic lamellae. Glomerular shrinkage, tubular degeneration and necrosis and proliferation of haematopoetic tissues in the kidney, hypertrophied hepatocytes, disruption of cordal arrangement of hepatocyts, sinusoidal and central vein congestion in the liver and necrosis of red and white pulp, multifocal haemorrhagic areas and presence of many melano-macrophage centres in the spleen were the most important diagnostic lesions in the disease.

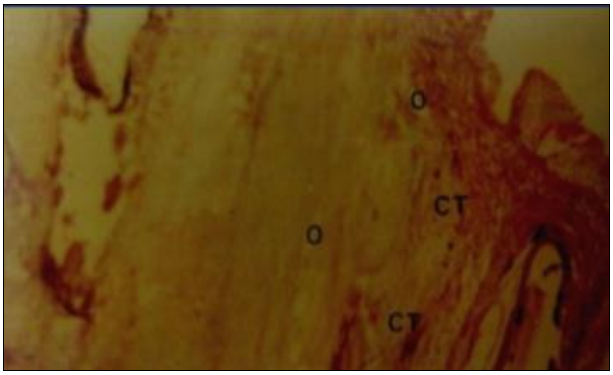

Fig 2: *Argulus sp.* affected skin of *Labeo rohita* (Ham) showing loss of epidermal layer, odema (O), in the dermis formation of hypertrophied dense connective tissue (CT) H. & E., X400.

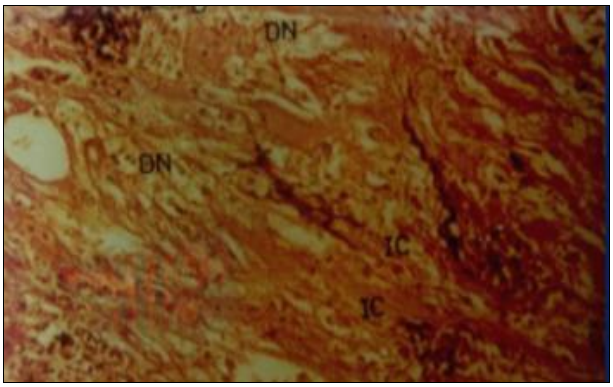

Fig 3: *Argulus sp*. affected skin of *Labeo rohita* (Ham) showing dermal necrosis (DN), congestion (C) and dilation (D) of dermal blood vessels and infiltrating cells (IC). H. & E., X 100.

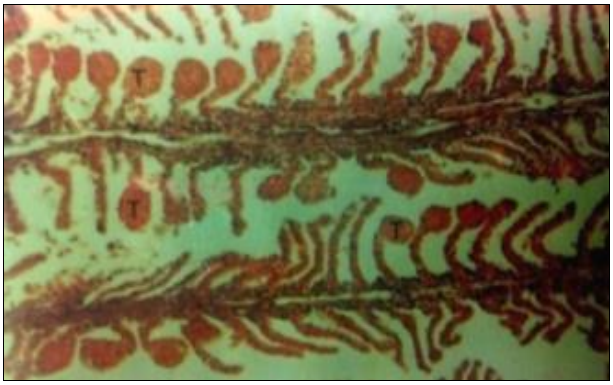

Fig 4: Gill of *Argulus* affected *Labeo rohita* (Ham) showing telangiectatic (T) secondary gill lamellae. H. & E., X 200.

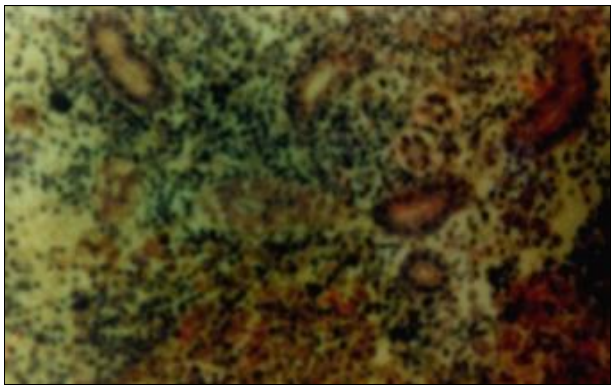

Fig 5: Kidney of *Argulus* affected *Labeo rohita* (Ham) showing large Melanomecrophage centre (MC) in the tissue (HT). H. & E., X200.

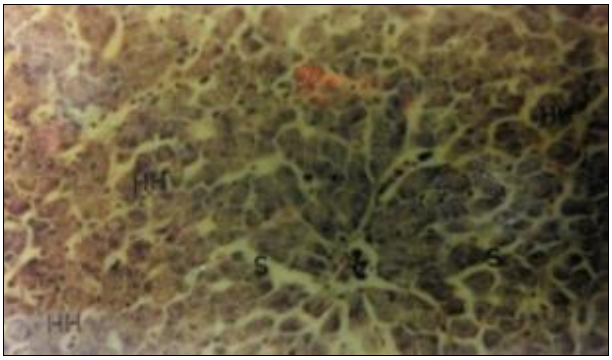

Fig 6: Liver of *Argulus* affected *Labeo rohita* (Ham) showing, hypertrophied hepatocytes (HH), congestion (C) of blood vessels and dilation of sinusoids (S). H. & E. X 200.

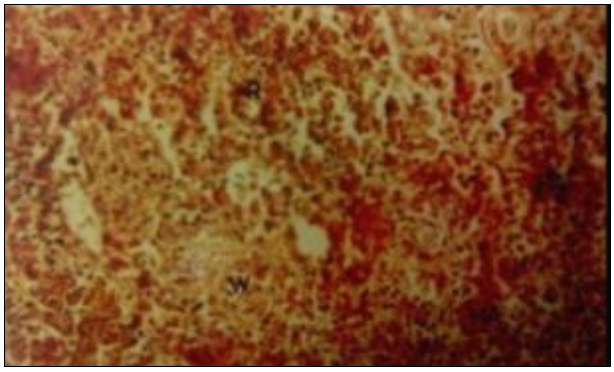

Fig 7: Spleen of *Argulus* affected *Labeo rohita* (Ham) showing necrosis of red (R), haemorrhagic areas (HA) and white pulps (W). H. & E., X 200.

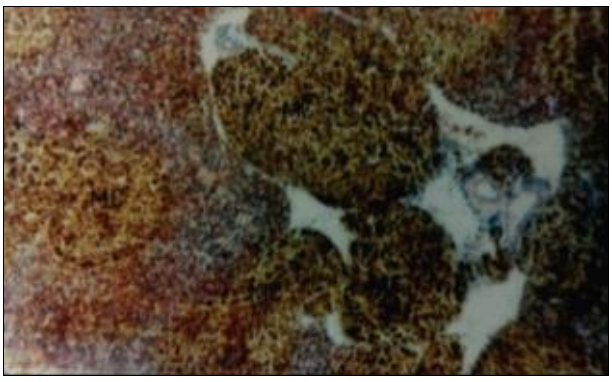

Fig 8: Spleen of *Argulus* affected *Labeo rohita* (Ham) showing large numbers of big Melanomecrophage centres (MC) in the tissue. H. & E., X 400.

Discussion

The present observations have been taken on the histopathological changes taking place in response to the infection of *Argulus* in skin, gills, liver, kidney and spleen of IMC. Many variations were found during the microscopic examination of the specimens. The fins showed the corroded fin filaments which might prove a hinderance in fish movement (Palaq *et al*., 2016) [11]. Clinical signs and behaviors observed in infected fish were in accordance with the cases reported by Toksen (2006) [16] and Noaman *et al.* (2010) [10].

The observed histopathological changes in the skin and gills of the infected fishes probably might have been due to the combined effected of mechanical damages and release of toxins by the parasites at the site of attachment reported by Feist & Longshaw, 2008. Dulin (1979) [4] studied that the toxic substances released from the proboscis glands of *Argulus* parasites certainly have adverse effects on the fish. He has reported localized reddening or haemorrhages and swelling of tissues at the sites of parasite infection and ultimate death of the fish due to the potent toxins liberated by the parasites.

Nandp and Das (1991) [9] reported in the liver of *Labeo rohita*, most of the central veins were encored with red blood gills and some fibrinous materials. Gills of *L. rohita* show the histopathological alterations which included proliferation in the epithelium of gill filaments and secondary lamellae, resulting in fusion of secondary lamellae, severe degenerative and necrotic changes in gill filaments (Palaq *et al*., 2016) [12]. The histopathological changes recorded in the liver, kidney and spleen of the diseased samples are in accordance to Kabata (1970) [6], Schaperclaus (1986) [13], Dey (1988) [3], Singh and Srivastava (1992) [14] and Hassan (2005) [5], and they reported hyperplasia of dense connective tissue Kidneys which exhibited marked glomerular changes and tubular degeneration and necrosis. Schaperclaus (1986) [13] reported that degenerations and lymphocyte aggregations can be observed histologically on the places where *Argulus* parasites infect. The present observations are found in conformity with the studies of earlier workers.

In culture system, more attention and necessary steps should be given in the rainy season when probability of parasitic infestations and disease outbreak increased in stocking pond. The infection may be visualized with naked eyes and need to manage promptly to secure the health of Indian major carps.

Conclusion

It may be concluded from the present investigation that *Argulus* sp. was found all over the body surface of the host fish. But the most preferable sites of *Argulus* infestation were at the base of pectoral, pelvic and anal fin. Histopathological observations as a whole, affect the health background of the host fish resulting in the depletion of growth, susceptibility for other diseases etc. The damage to the gills affects the respiration of the fish, resulting in hypoxia or anoxia and thereby affecting the health status of the host. Glomerular shrinkage, tubular degeneration and necrosis and proliferation of haematopoetic tissues in the kidney, hypertrophied hepatocytes, disruption of cordal arrangement of hepatocyts, sinusoidal and central vein congestion in the liver and necrosis of red and white pulp, multifocal haemorrhagic areas and presence of many melano-macrophage centres in the spleen were the most important diagnostic lesions in the disease. The findings accomplished that disease argulosis are serious threats in Indian major carps and can cause extensive damage to the yield of fishes.

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