Histology of gills of *Labeo rohita* infected by crustacean parasite *Argulus* sps.

*Palaq, Seema Langer and Irfan Ahmed Noorani.*
Department of Zoology, University Of Jammu, Jammu and Kashmir-180006, India.

**Abstract**

The present study was conducted on the gills of *L. rohita* of river Tawi parasitised by a crustacean parasite *Argulus* to reveal the histopathological characteristics of the gills of these fresh water carps. Histopathology provides a rapid method to detect effects of pathogens on different tissues and it can be considered as an indicator of the extent of deterioration of fish environment. In Indian major carps argulosis disease is caused by the crustacean parasite. The parasites are leaf like in appearance and were found to attach with skin, fins and gills of the fishes. The affected sites were haemorrhagic, ulcerated and mucus studded. The histopathological changes in the skin of affected fish exhibited hypertrophy, telangiectasis, aneurism and fusion of lamellae along with the shortening and degeneration of gill lamellae accompanied by edematous or haemorrhagic spots. The histopathological observations due to the presence of pathogens in fish also play a vital role in disease control and health management in aquaculture.

**Introduction:**

Parasites are one of the major group of organisms that may or may not cause infection in fishes depending on a number of factors. The occurrence of both ecto and endoparasites in fish is very common (Moyle and Cech, 2004). Fish parasites result in huge economic losses as they increase mortality and also increase farm inputs via increased treatment expenses and cause reduction in growth rate due to disease outbreak (Kayis, 2009). Gill parasites are common on cultured and wild fish. Many of these have long been recognized to have the potential to affect the growth; fecundity and survival of hosts (Johnson *et al.*, 1996). Gills are specialized tissues for gaseous exchange, circulation, ionic balance, hormone production and nitrogenous waste excretion (Pelster, 2010). Gill parasites attach to the gills of fish and feed on the host blood and tissue (Ojha and Hughes, 2001). The organs of attachment such as suckers and hooks cause extensive tissue damage and inflammation and may render fish susceptible to secondary infection by bacteria, fungi and viruses (Dezfulli *et al.*, 2003). Thus the damage to gill tissue can reduce the ability of the fish to maintain normal oxygen uptake by hindering water flow (Ojha and Hughes, 2001). Parasites play an important role in determining the health status of the fishes (Ferguson, 1989). Present study was therefore, undertaken to reveal the effect of different parasites on histology of gills of freshwater carps.

Parasitic infections often give an indication of the quality of water, since parasites generally increase in abundance and diversity in more polluted waters (Poulin 1992, Noga 2010). Parasites are capable of causing damage to the fish through injury to the tissues or organs. Fish parasites result in economic losses or mortality, treatment expenses, growth reduction during and after outbreak of disease. There are no specific pathognomonic clinical signs for parasitic diseases in fish, although a group of clinical signs may be specific for some parasitic infestation (Reavill and Roberts, 2007). Most external parasites however, can be readily identified on direct observation and wet mount preparations while some parasitic infections need specific paraclinical examinations for confirmations.

*Argulus* species (Family: *Argulidae*), more commonly known as fish lice, are members of a large group of branchiuran parasites infestation that cause disease in fish. The argulids are crustaceans and are related to crabs,
lobsters and shrimp (Mohamed and Kenewy, 2013). Approximately 100 different species of Argulus are distributed worldwide depending upon species, can infest freshwater and saltwater fishes. The three most studied species are Argulus foliaceus, A. japonicus, and A.coregoni that are found in freshwater systems. This parasite penetrates the upper layers of the host skin and feeds on blood and body fluids (VanDer et al., 2000). The major fishes affected with this disease are fry, fingerlings and adults of Indian major carps (Sheila et al., 2002). The affected fishes become restless with erratic swimming movements and attachment sites show signs of ulceration. Adult parasites are oval, flat and leaf like in appearance with transparent to whitish color along with two conspicuous black spots (Sheila et al., 2002). Generally, they are found to attach with the skin, fins or gills of Indian major carps but sometime, kidney, liver and spleen are also found to distress with Argulus parasites (Hassan, 2005).

Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs such as the gills, muscle, liver and kidney (Dutta, 1996). It however, proves to be a cost effective tool to determine the health of organisms which in turn reflects the health of entire ecosystem. It is a rapid method to detect the effects of irritants and pathogens in different organs (Johnson et al., 1993) and it can be considered as the indicator for abnormal condition for fish environment (Roberts, 2001). It helps to identify the extent of damage in the organs of diseased fish and also the etiological agents harbored in target organs of the fish (Sheila et al., 2002). It plays a significant role for understanding the mechanism of disease processor and the course of diseases ranging from acute and chronic stages through fish level reactions in host fish by pathogens.

Materials And Method:-
The present study was conducted in River Tawi, Jammu from April 2015-December 2015. The sampling was done using the cast nets of one inch mesh size and the fishes were brought live to the Fisheries Lab. at P.G. Deptt. Of Zoology, University of Jammu. The total length, standard length and the weights were recorded. The samples were also studied for patho-morphological and anatomical examinations. The patho-morphological examinations comprised of identifying and locating any visible external lesions, haemorrhages, and formations of vista and patches on body surfaces, gills and fins. The patho-anatomical examinations were thus carried out for finding any viable lesion or inflammation in internal organ.

The specimens were dissected and the infected gills were immediately fixed in alcoholic Bouin’s fluid for 24 hours. After complete removal of picric acid, the tissue was dehydrated in alcoholic series, cleared in xylene and processed for preparation of paraffin wax blocks. Sections of 4-5µm were taken using a microtome and stained using haematoxylin eosin. Stained histopathological sections were examined under Olympus research microscope O/C 91525. Microphotography was done both at low and high magnification.

Results And Discussion:-
The present observations have been taken on the histopathological changes taking place in response to the infection of Argulus in gills of Labeo rohita. Many variations were found during the microscopic examination of the specimens. The visual observations showed that parasites are found attached to the skin with head and operculum being the favourite sites of attachment. The affected fishes carrying a heavy load of parasites were highly mucous laden with very slimy surface and pale appearance (Fig. 5). The fins showed the corroded fin filaments which might prove a hinderance in fish movement (Palaq et al., 2016). Clinical signs and behaviors observed in infected fish were in accordance with the cases reported by Toksen (2006), Yildiz and Kumantas (2002) and Noaman et al. (2010).

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 (Fig 1), severe degenerative and necrotic changes in gill filaments and secondary lamellae, curling of secondary lamellae and mucous cells proliferation. Hyperplasia of the filaments was found which was expressed in the expansion of their end sections. Edematous changes, characterized by epithelial detachment, were observed in gill filaments and secondary lamellae. Moreover, aggregations of inflammatory cells were noticed in gill filaments. Marked proliferation of mucous cells, shortening of secondary lamellae, and hemorrhage between gill filaments, dilatation and congestion in blood vessels of gill filaments were also observed. Also the tips of the secondary filament were enlarged to form balloon like swellings (telangiectasis) in severe infection (Fig. 3). Edematous changes, characterized by epithelial detachment, were observed in gill filaments and secondary lamellae (Fig 2). Atrophy of secondary lamellae was also observed. The hyperplasia of the epithelial cells caused cloudiness of the skin with excessive mucous secretion. This may lead to...
hypoxia of the gills (Darwesh et al., 2014). Similar results have been reported by Taylor et al. (2005) which is in agreement with the one obtained from the present study.

**Figure 1:** Hyperplasia (H) causing the fusion of secondary lamellae (100x)

**Figure 2:** Fusion of the secondary lamellae with balloon like tips of the filaments (Telangiectatic T) (40x), prominent hyperplasia with edematic spots

**Figure 3:** Showing the disintegration of the cells of gill filaments (10x) and eating up cell stage
Pathological effects also revealed the proliferation of bronchial tips, thinning, deshaping, shortening and fusion of secondary gill lamellar epithelium. Extensive disintegration of the gill lamellae was also reported (Fig 4). The maximum histopathological damage were induced by scraping and sucking activities of the parasites on host tissues Derwa (1995), Endrawes (2001), Hanna (2001) and Osman (2001). The sections of gills revealed that the functional respiratory components of the gills namely the gill lamellae were also affected badly by the parasitic infection. Swelling of the host tissue was observed particularly at the attachment site of the parasites. There were no cellular changes in the adjacent uninfected tissues. Large sized argulids hamper the normal respiratory function of the gills by replacing the lamellar spaces with the argulids, thereby reducing the surface of air exchange and thus leading to stress and anoxic conditions. Also changes were found in the primary and secondary lamellae due to the attachment of parasites. The parasites were found attached to the tips of gill filaments. Jalali (1997) revealed that lesions caused due to attachment and feeding of parasites may be secondarily infected with fungi and bacteria whereas Fadaei et al. (2001), Barzegar and Jalali (2004), Shamsi et al. (2009), Raissy and Ansari (2011), Raissy et al. (2011), Campos et al. (2001) have recorded hyperplasia of the epithelial cells and subsequent lamellar fusion to be the characteristic feature of the infection.

The parasites pierce the host tissue with the pre-oral stylet, inject a cytolytic toxin and feed on the blood released by the resultant wound. The surface of the host at the point of stylet entry can become erythemic and hemorrhagic. A hemorrhagic factor is produced by some species. Several parasites feeding in close proximity may cause edema and
localized swelling of tissues. However, repeated piercing of the skin by stylet may inject a toxic enzyme causing irritation, in addition to the mechanical damage caused by hooks and spines. This may result in the development of inflammatory lesions characterized by increased mucus secretion, hemorrhages and necrosis of the injured areas (Toksen, 2006; Noaman et al., 2010; Noga, 2010; Purivirojkul, 2012; Sharma et al., 2012; Vasilean et al. 2012 and Mayer et al. 2013). Argulus parasites released some toxic substances from the proboscis glands and certainly have adverse effects on the fish and localized reddening or haemorrhages and also swelling of tissues at the site of infection (Dulin, 1979). Hyperplasia of epithelial cells and fusion of some secondary lamella are examples of defense mechanism of gills. The most significant lesions of Labeo rohita were secondary lamellar hyperplasia, haemorrhages in the tips of primary lamellae and fusion of both primary and secondary gill lamellae. Argulus parasites released some toxic substances from the proboscis glands and certainly have adverse effects on the fish and localized reddening or haemorrhages and also swelling of tissues at the site of infection (Dulin, 1979). Severe infection by Argulus can cause significant mortality in Indian Major Carps (Nandp and Das, 1991).

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. The findings accomplished that disease argulosis are serious threats in Indian major carps and can cause extensive damage to the yield of fishes. The infection may be visualized with naked eyes and need to manage promptly to secure the health of Indian major carps.

Acknowledgements:
The corresponding author is indebted to the University Grants Commission, New Delhi for providing financial support under CSIR-NET-JRF fellowship. The authors are equally thankful to the Head, Deptt. Of Zoology for providing necessary facilities.

References:
1. Barzegar, M and Jalali, B. (2004): Helminthes Acanthocephala and crustacean parasites of fishes in Vahdat reservoir. Iran J Vet Sci., 2:229-234.
2. Campos, C.M., Moraes, J.R.E and Moraes, F.R. (2001): Histopathology of gills of Piaractus mesopotamicus (Holmberg, 1887) and Prochilodus lineatus (Valenciennes, 1836) infested by monogenean and myxosporrea, caught in Aquidauana River State of Mato Grosso do Sul, Brazil. Rev Bras. Parasitol Vet Jaboticabal., 20(1): 67-70.
3. Darwesh, A., Maytham, A., Shabbani, A and Faris, B.H, (2014): Diagnostic and pathological study of Argulus japonicas in Goldfish Carassius auratus. G.J.B.B., 3(4): 384-387.
4. Derwa, H.I.M. (1995): Some studies on gill affections of some freshwater fishes M. sc thesis Faculty of Veterinary Medicine Suez Canal University.
5. Dezfuli, B., Sayyaf, L.G., Robert, K., Paul, J and Maurizio, M. (2003): Immunohistochemistry ultrastructure and pathology of gills of Abramis brama from Lake Mondsee Austria infected with Ergasilus sieboldi (Copepoda). Diseases of Aquatic Organisms., 53:257–262.
6. Dulin, M. (1979): Fish diseases TFH publications, Neptune City, N.J.
7. Dutta, B. (1996): Coalition governments and Fisecal Policies in India, IRIS India, Working paper No. 29.
8. Endrawes, M.N.(2001): Observations on some external and internal parasitic diseases in Nile catfishes. A Master thesis submitted to Dept. of fish diseases and management. Fac of Vet Medicine Zagazig Univ.
9. Fadaei, F., Mokhayer, B and Ghorbani, H. (2001): Identification of fishes and their parasites in Choghakhor Lagoon. J Facul Vet Med Uni Tehran., 56:109-113.
10. Ferguson, W.H. (1989): Gills and pseudobranchs In Systemic Pathology of Fish A text and Atlas of Comparative Tissue Responses in Diseases of Teleosts. MCK, SH, F 42.
11. Hanna, M.I. (2001): Epizootiological studies on parasitic infections in fishes cultured under different fish cultural systems in Egypt. A Master thesis submitted to Dept. of fish diseases, Fac of Vet Med Cairo Univ.
12. Hassan, N. (2005): Study on some aspects of parasitic diseases of some fresh water fishes. PhD thesis, L. N. M Univ., Darbhanga, 5(2):71-76.
13. Jalali, B. (1997): Parasites and parasitic diseases of Iran’s fresh water fishes. Iranian Fishery Institute Publications.Tehran Iran., 312-407.
14. Johnson, S.C., Blaylock, R.B., Elphick, J and Hyatt, K. (1996): Disease caused by the salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) in wild sockeye salmon (*Oncorhynchus nerka*) stocks of Alberni Inlet British Columbia. Can J Fish Aquat Sci., 53:2888-2897.

15. Johnson, L.L., Stehr, C.M., Olson, O.P., Myers, M.S., Mccain, B.B., Chan, S.L. and Varanasi, U.(1993): Chemical contaminants and hepatic lesions in winter flounder (*Pleuronectes americanus*) from the northeast coast of the United States. Environmental Science and Technology., 27: 2759-2771.

16. Kayis, S., Ozcelep, T., Capkin, E and Altinok, I. (2009). Protozoan and metazoan parasites of cultured fish in Turkey and their applied treatments. The Israeli J Aquac Bamidgeh., 61: 93-102.

17. Mayer, J., Hensel, P., Fava, J., Brandao, J and Divers, S. (2013): The Use of Lufenuron to Treat Fish Lice (*Argulus sp*) in Koi (*Cyprinus carpio*). Journal of Exotic Pet Medicine ., 22(1): 65-69.

18. Mohamed, A.H. and Kenawy, A.M. (2013): Studies on argulosis in some freshwater ornamental fishes with special reference to treatment trials. New York Science Journal ., 6(10) :37-41.

19. Moyle, P.B. and Cech, J.J.(2004): An introduction to ichthyology. Prentice Hall, Upper Saddle River, New Jersey., 356.

20. Nandp, N.C. and Das, S.R.(1991): Argulosis causing juvenile mortality in some fishes at Kakdwip, West Bengal. Indian J Fish ., 38(2) :132-133.

21. Noaman, V., Chelongar, Y and Shahmoradi, A.H.(2010): The First Record of *Argulus foliaceus* (Crustacea: Branchiura) Infestation on Lionhead Goldfish (*Carassius auratus*) in Iran. Iranian J Parasitol., 5(2):71-76.

22. Noga, E.J.(2010): Fish disease diagnosis and treatment. 2nd ed., Mosby.

23. Ojha, J and Hughes, G.M. (2001): Effect of branchial parasites on the efficiency of the gills of a freshwater catfish *Wallago attu*. J Zool., 255:125-129.

24. Osman, H.A.M. (2001): Studies on parasitic gill affections in some cultured freshwater fishes. Master thesis submitted to the Faculty of Veterinary Medicine, Suez Canal University

25. Palaq, Langer, S and Noorani, I.A.(2016). Protozoan and metazoan parasites of cultured fish in Koi (*Cyprinus carpio*). Seria Zootehnie., 58: 203

26. Noaman, V., Chelongar, Y and Shahmoradi, A.H. (2010): The First Record of *Argulus foliaceus* (Crustacea: Branchiura) Infestation on Lionhead Goldfish (*Carassius auratus*) in Iran. Iranian J Parasitol., 5(2):71-76.

27. Poulin, R. (1992): Toxic pollution and parasitism in freshwater fish. Parasitology Today., 8(2) :58–61

28. Purivirojkul, W. (2012): Histological Change of Aquatic Animals by Parasitic Infection. "Histopathology – Reviews and Recent Advances" book edited by Enrique Poblet Martinez. 179-180.

29. Raisy, M and Ansari, M.(2011): Histopathological changes in the gills of naturally infected *Capoeta aculeata* (Cuvier and Valenciennes, 1844) with parasites. African Journal of Biotechnology., 10(68): 15422-15425.

30. Raissy, M., Ansari, M and Moumeni, M. (2011): Parasite fauna of *Aphanius vladykovi* Coad (Osteichthyes: Cyprinodontidae) in Gandoman Lagoon. Comp Parasitol., 78:104-106.

31. Reavill, D.R. and Roberts, H.E.(2007): Diagnostic cytology of fish. Veterinary Clinics of North America Exotic Animal Practice., 10:207–234.

32. Roberts, R.J. (2001): Fish Pathology 3rd ed, Roberts R. J., SaundersW.B(eds). 472.

33. Shamsi, S., Jalali ,B and Azhazadeh, M. (2009): Infection with *Dactylogyrus spp* among introduced cyprinid fishes and their geographical distribution in Iran. Iran J Vet., 10:70-74.

34. Sharma, M., Shrivastav, A.B., Sahni, Y.P. and Pandy, G. (2012): Overviews of the treatment and control of common fish diseases. International Research Journal of Pharmacy., 3(7) :123-127.

35. Sheilla, F.A.A., Sivakumar and Chandran, R. (2002): Infestation and prevalence of copepod parasite, *Argulus indicus* on some freshwater fishes. Nat Env Poll Technol., 1:201-206.

36. Taylor, N.G.H., Sommerville, C and Wootten, R. (2005): A review of *Argulus spp*. occurring in UK freshwaters. Journal of Fish Biology, 11:349.

37. Toksen, E. (2006): *Argulus foliaceus* (Crustacea: Branchiura) on Oscar, *Astronotus ocellatus* (Cuvier, 1829) and Its Treatment. Journal of Fisheries & Aquatic Sciences., 23(1-2): 177:179

38. VanDer Salm, A.L., Nolan, D.T., Spanings, F.T.A. and Wendelaar, S.E. (2000): Effects of infection with the ectoparasite *Argulus japonicus* (Thiele) and administration of cortisol on cellular proliferation and apoptosis in the epidermis of common carp, *Cyprinus carpio* L. skin. J Fish Disease., 23(3) :173–81.

39. Vasilean, I., Cristea, V and Dediu, L. (2012): Researches regarding the argulosis treatment to Huso Huso juveniles with NaCl Lucrări Ştiinţifice. Seria Zootehnie., 58: 203-207.

40. Yildiz, K and Kumantas, A. (2002): *Argulus foliaceus* infection in a goldfish (*Carassius auratus*). Isr. Journal Vet Med., 57(3) :118-120.