ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 3, pp: (44-52), Month: July - September 2019, Available at: www.researchpublish.com

Histopathological study of parasite infested *Channa punctata*.

Hena Parbin[1],Banasri Mech[2*]

Dept of Zoology, Gauhati University, Assam.

Email id: henaparbin12@gmail.com

Corresponding author email id: banasrimech530@gmail.com

Abstract: **The rich aquatic fauna of Assam especially the fishes often face the challenge of parasitic infestation. *Channapunctata* is often infected by different parasites which lead to different physiological changes. In present study, histological structure of gill, liver and kidney of parasite infested *Channapunctata* is studied. The liver of the infested fish showed vacuolar degeneration, necrosis and extensive tissue damage near portal vein. Gills showed degenerative and necrotic changes of gill lamellae, mucus cell and also epithelium detachment. Kidney also showed sign of infection, vacuole formation and tissue damage. The infestation also affects the behavior and growth of fish.**

Keywords: **Parasites, *Channapunctata*, necrosis, vacuole.**

I. INTRODUCTION

India is endowed with rich and varied aquatic resources amenable for fisheries and aquaculture. The state is fortunate to posses vast and varied aquatic resources from the part of global hot-spot of fresh water fish diversity and the water bodies harbor more than 200 species of fishes with a high degree of endemism. The snakeheads also known as murrels, belonging to the family *Channidae*, constitute the dominant group of air breathing fresh water fishes in terms of both culture and capture fisheries. Among the snakeheaded fishes, *Channapunctata* (Bloch 1979) is considered amongst the high demand food fishes as a source of protein. Fishes are the primary host of certain parasites so they are signified as zoonotic and biological hazards in prospective of human health (Chai, 2005)**.** The major groups of parasite in fresh water fishes are trematodes, cestodes, nematodes and acanthocephalons which cause infections. Beside these, there are many parasites which are transmitted to human being only through fish (Gupta, 1959). These parasites decrease the rate of growth as well as the reproduction rate of the fish, resulting in loss of potential food and economic loss to the culturist. In the context of India, parasitic diseases are most common (about 78%) and are encountered more frequently than microbial diseases; however the degree of severity and loss due to such diseases are highly variable (Abidi, 2002). Histopathology deals with the disease diagnosis of tissue. It provides a rapid and systematic way to detect any physiological changes which may be caused by irritants or pathogens in different organs of fish (Johnson *et al.* 1993). The histopathological analysis is a very sensitive aspect and is crucial in determining cellular changes that may occur in target organs, such as the gills, gut, liver and gonad(Dutta, 1996). A comparative study of histology is useful to distinguish abnormal cells from normal ones, which helps in diagnosis of many diseases (Majumdar 1980). Liver is one of the important glands concern with several important functions. It is the major organ of detoxification and plays important role in the metabolism of all important elements of the body (Pardeshi *et al.* 2012). Therefore the histological study of liver is a reveals the health issue of the fish and is helpful in knowing the possible effect of any parasites on the functions of the body systems (Kaur *et al.* 2012). Gills are the organ of breathing and it plays a major role in detecting the quality of the environment of the fish. Gills are generally considered as a good indicator of water quality. Kidneys are involved in excretion and regulation of the water balance within the fish. Polluted water and presence of parasites affect the kidney also (Tilak *et al.* 2004, Butchiram *et al.* 2007). Present study was attempted to compare the histological structure of gill, liver, and kidney of parasite infested and non infested*Channapunctata*along with recording their physical and behavioral changes.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 3, pp: (44-52), Month: July - September 2019, Available at: www.researchpublish.com

II. MATERIALS AND METHODS

Live specimen of *Channapunctata* with an average weight of 18 to 20 gm and standard length 13 to 15 cm were collected from Bor Bazar, Pandu, Guwahati during the month of February. The specimens were brought to the laboratory for observation. They were maintained at room temperature in plastic aquarium containing water. Before the commencement of the experiment the external as well as the internal body organs were thoroughly examined for the parasites. The fish was dissected to examine the internal organ. An incision was made along the mid ventral line of the fish and the liver, gill and kidney were dissected out for fixation. Parasites, collected from liver of *Channapunctatus*were preserved in 5% formalin. Liver, kidney, gill tissues were dissected out from the parasite infected fish and immediately kept in normal saline, cleared off fats and then fixed in carnoy's fixative for 3-4 hours. After fixation the tissues were processed for dehydration through a series of ethanol grade (30%, 50%, 70%, 90% and 100%) and cleared them with xylene before infiltration with several changes of hot paraffin wax. Subsequently, tissue were embedded in paraffin wax and cut into 5 micron thickness using a microtome. The sections were stained with double staining method, using heamatoxylin and eosin stain (Bancroft and Gamble, 2002) and were then examined under microscope.

III. RESULTS

Present investigation was carried out to compare the histology of liver, gill and kidney tissues of normal and parasite affected *Channapunctatus* respectively. Behavioral and physical changes have also been recorded.

Behavioural and PhysicalChanges:

*Channapunctata*which were infested with parasite showed whitish spot on their body (Fig.2), also an increase in mucous secretion. The detrimental growth and sluggish movement was also observed for the infected. When compared with normal one the average weight of diseased fish was found to be declined, also they showed reduced swimming activity. The lengths of all the experimental fishes were found in between the range 13-15cm.

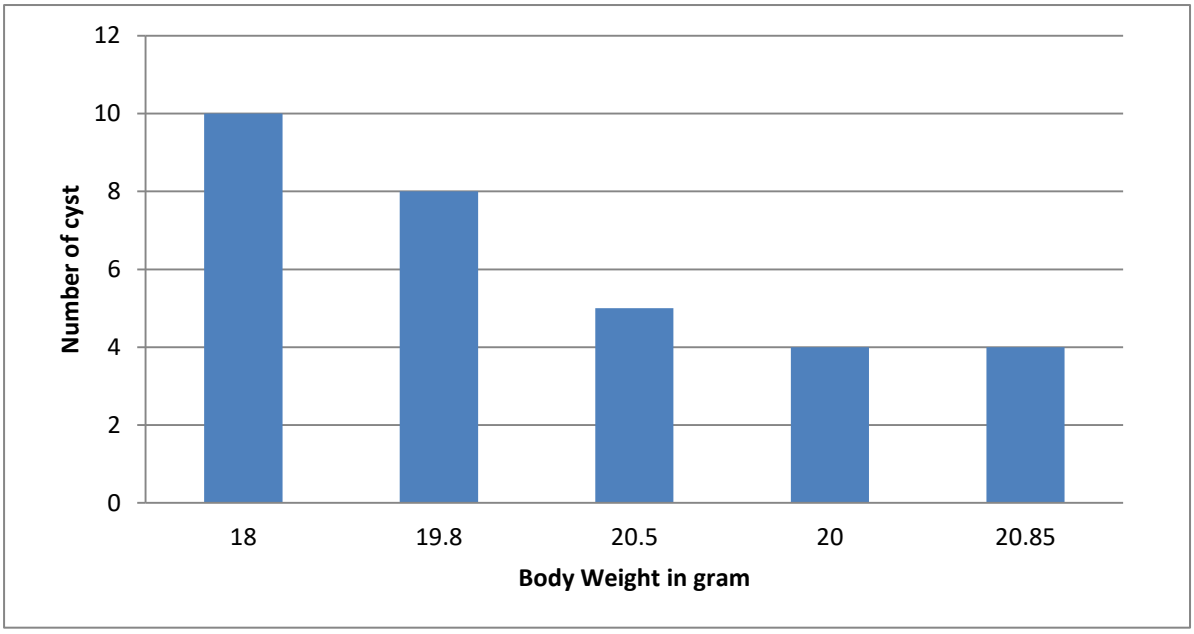


Fig. 1: Correlation between body weight (in gm) and number of cyst present in *Channapunctata*.

Table No. 1: The average weight of non infested and infested *Channapunctata.*

S. No.	Infested or uninfested	Number examined	Mean weight (g)	Loss of weight (g)
1	uninfested	5	23.04	-
2	Infested	5	19.83	3.21

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 3, pp: (44-52), Month: July - September 2019, Available at: www.researchpublish.com

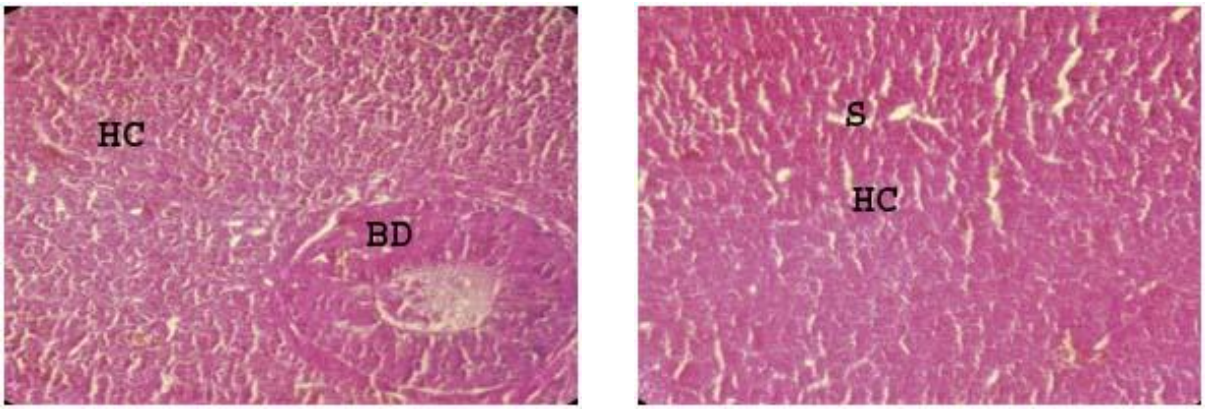
Fig. 2: White patches observed on the body of parasite infested *Channapunctata.*

Fig. 3: Larval trematode parasiteon the liver of *Channapunctata*.

Histopathological changes in liver, gill and kidney structure:

Normal histology of liver showed polygonal hepatic cells containing clear spherical nucleus (Fig.4a). They are located among sinusoids (Fig.4b) forming cord like structure known as hepatic cell cords. Bile duct is also present (Fig.4b). Teleosts have 5 pairs of gill arches. In the front four pairs, the slender gill filaments form two lines facing towards the back and these two lines are joined to each other at the base by a gill septum. Numerous semicircular secondary gill lamellae are lined up along both sides of the gill filament that are seen in normal gill histology along both sides of the primary gill lamellae (Fig.5a). The primary gill lamellae consist of centrally placed rod like central axis with chloride cells and with blood vessels on either side. The secondary lamellae are also termed respiratory lamellae as were highly vascularized and covered with a thin layer of epithelial cells. Blood vessels are extended into each of the secondary gill filament. The blood cells of secondary gill lamellae have a single nucleus which is flattened in appearance. The region between the two adjacent secondary gill lamellae is known as inter lamellar region (Fig.5b). The pillar cells and mucus cells were also observed (Fig.5b).Teleosts kidney consists of head and body kidney. The head kidney is the anterior part of kidney, consists of lymphoid tissue. Body kidney composed of many nephrons and interstitial lymphoid tissue. The interstitial tissue is the major hematopoietic tissue in the body. Each nephron consists of two parts, renal corpuscle and renal tubule (Fig.6a). The renal corpuscle is composed of a glomerulus and its capsule. The renal tubules are thin and short and consist of single layer of epithelial cells. The proximal convoluted segment, one part is composed of cuboidal epithelial cells. The distal convoluted segment are stained with eosin more faintly then those of the proximal convoluted segment.

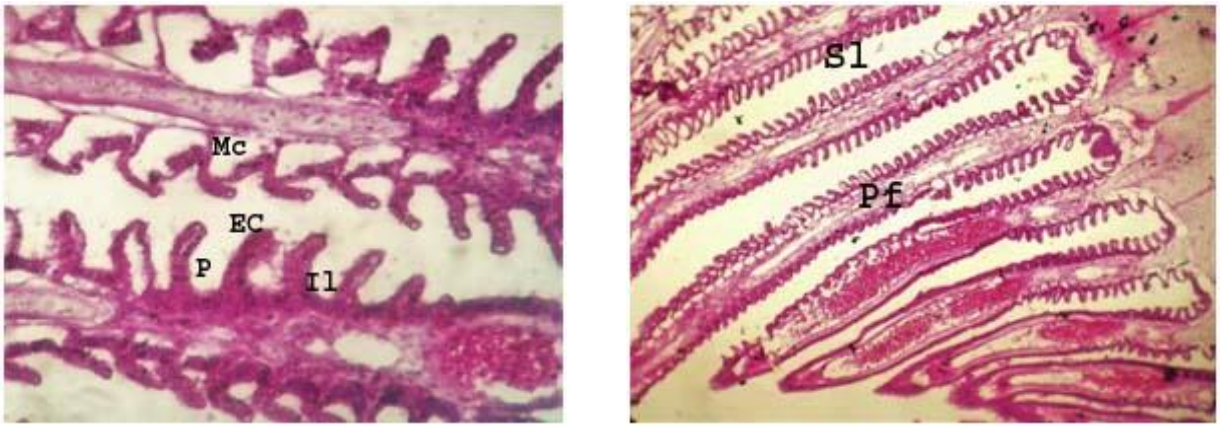
ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 3, pp: (44-52), Month: July - September 2019, Available at: www.researchpublish.com



4(a) **4(b)**

Fig.4. Photomicrograph showing the histological section of control liver of *Channa punctata.*

4(a) shows hepatic cells (HC) and bile duct (BD) in 40×10x resolution. 4(b) shows the sinusoids (s) and Hepatic cords (H) in 40×10x resolution.



5(a) **5(b)**

Fig.5. Photomicrograph showing, the histological sections of control gill of *Channapunctata*.5(a) shows primary filament (Pf), secondary lamellae (Sl) in 10×10x resolution, 5(b) shows Epithelial cell (E), Pillar cell (P), Mucous cell (M), Blood cell (B), Chloride cell (Cl), Inter lamellar region (Ir) in 40×10x resolution.

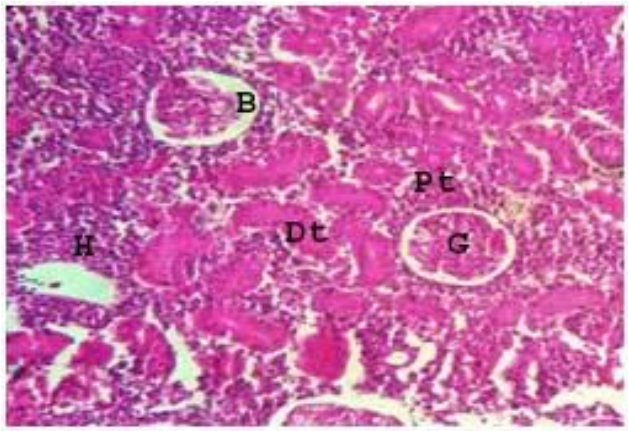


Fig.6. Photomicrograph showing the histological section of kidney of *Channapunctata*in 40×10x resolution. bowman space (B), glomerulus (G), proximal tubule (Pt), distal tubule (Dt), hematopoietictissue (H).

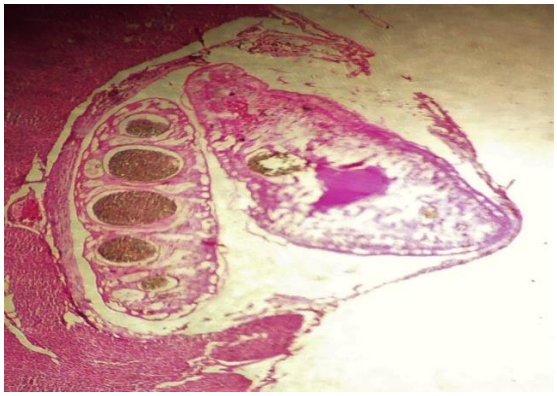
The liver of *Channapunctata* was infested with cyst of larval trematode, revealed different histopathological and cellular lesions. The section of infested liver showed the presence of trematode larvae (Fig.7a), covered under a capsule due to which a large area of liver tissue was damaged. The section of liver showed vacuolar degeneration in the hepatocytes (Fig.7b), focal areas of necrosis (Fig.7d) and melanomacrophage (Fig.7e) were identified as round aggregate of cells containing dark yellowish granules of various sizes normally close to the vessel. The presence of intense leucocytes

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 3, pp: (44-52), Month: July - September 2019, Available at: www.researchpublish.com

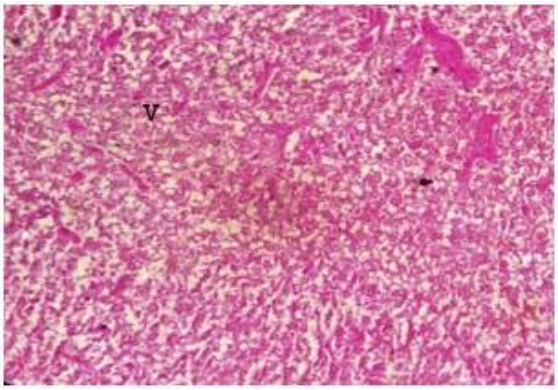
infiltrations (Fig.7c) throughout the cyst wall and in surrounding tissue is clear evidence of host immune response to the parasite infection. Extensive tissue damage near portal vein was also observed (Fig.7e). Liver sinusoids were distended (Fig.7f).

The gills of *Channapunctata* were also affected, degenerative and necrotic changes in gill lamellae were observed. Destruction of mucous cells (Fig.8a), epithelium detachment (Fig.8c) were recorded. Blood vessel dilation in primary gill filament (Fig.8b)), atrophy of secondary lamellae, fusion of secondary lamellae, curling of secondary lamallae andhyperplasia (Fig.8a) in secondary lamellae were also observed in the section of gill when viewed under light microscope.

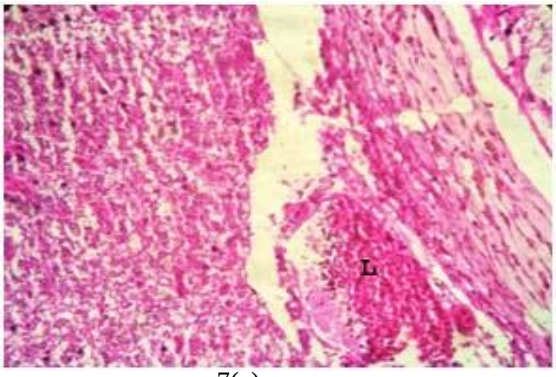
The section of kidney tissue of *Channapunctata* when observed under microscope showed vacuole formation (Fig.9a), necrosis and tissue damage in some part (Fig.9b).



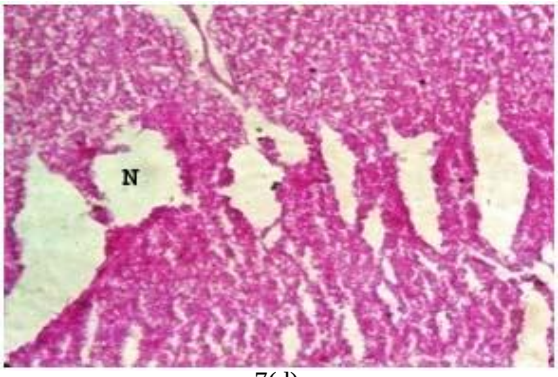
7(a)



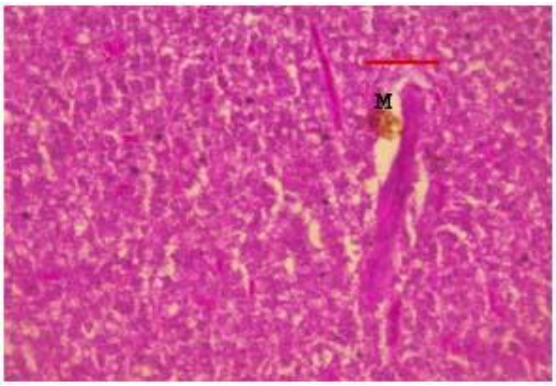
7(b)



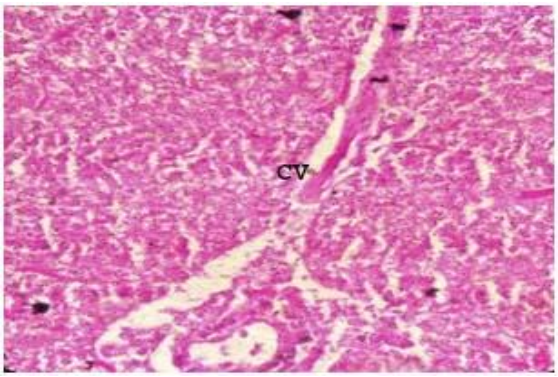
7(c)



7(d)



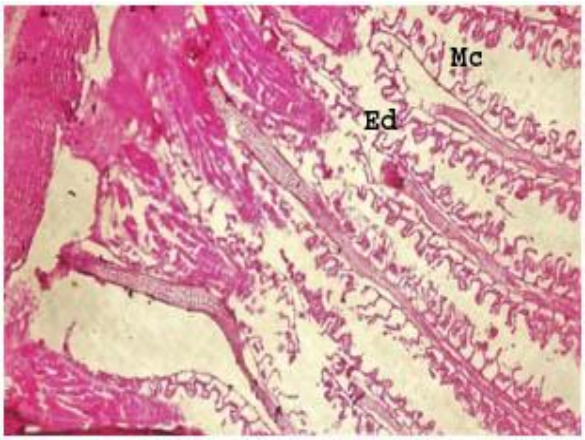
7(e)



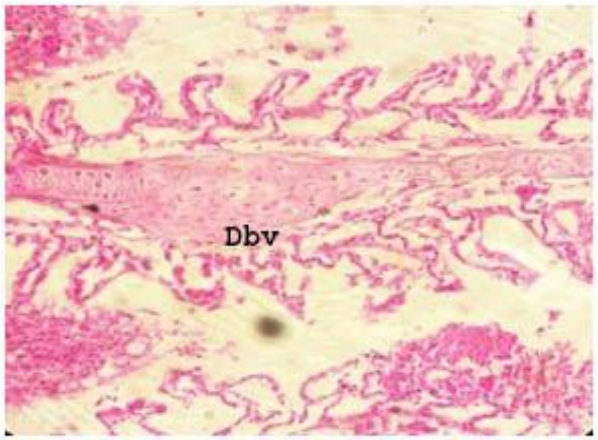
7(f)

Fig.7. Photomicrograph showing the histological section of liver of parasite infested *Channapunctata*. (a) Section shows larval trematode parasite, enclosed within a capsule in 10×10x resolution. (b) Vacuole (V) formation in 40X10x resolution. (c) Infiltration of leucocytes (L) in 40X10x resolution. (d) necrosis (N) of hepatic tissue in 40X10x resolution. (e) Aggregation of melanomacrophage (N) in 40X10x resolution. (f) Dilation of central vein (CV)in 40X10x resolution.

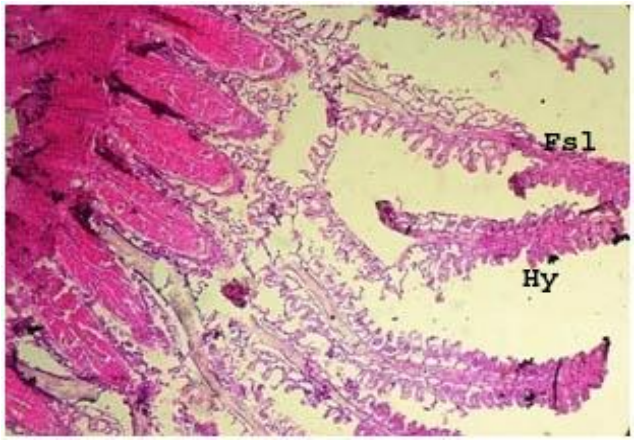
ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 3, pp: (44-52), Month: July - September 2019, Available at: www.researchpublish.com



8(a)

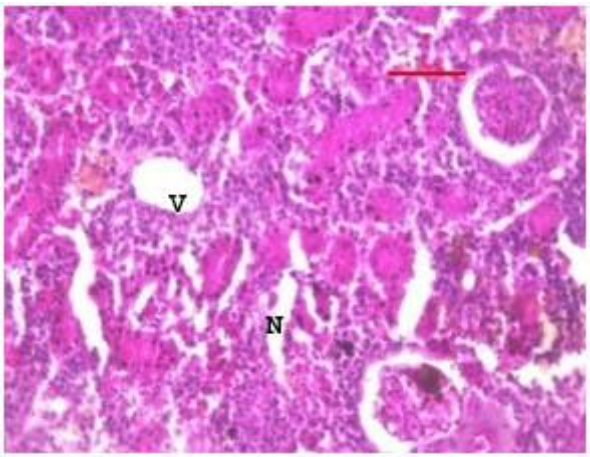


8(b)

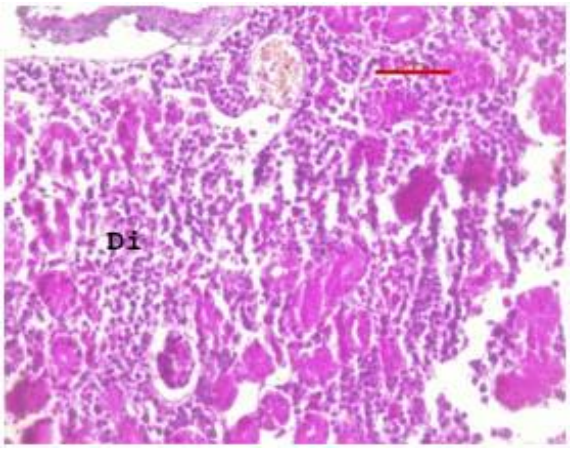


8(c)

Fig.8. Photomicrograph showing the histological section of gill of infected *Channapunctata.* (a) Shows epithelium detachment (Ed), destruction of mucus cell (Mc), and fusion of secondary lamellae (Fsl) in 10×10x resolution. (b) shows dilation of blood vessel (Dbv) in 40×10x resolution. (c) Shows curling (cl) and hyperplasia (HY) in secondary lamellae in 10×10x resolution.



9(a)



9(b)

Fig.9. Photomicrograph showing the histological section of kidney of infected *Channapunctata.* (a) shows the necrosis (N) in kidney tissue section and vacuole (V) formation in 40×10x resolution. (b) Shows the degenerate interstitium (Di) in 40×10x resolution.

IV. DISCUSSION

Parasites of fish can either be internal or external. Every parasite of fish extents some degree of harmful influence on its host that leads to mechanical damage (e.g. fusion of gill lamellae, tissue replacement etc.) physiological damage (e.g. cell proliferation, altered growth, detrimental behavioral response etc.) and reproductive damage (Buchman and Lindstan, 2002) (Knudsen *et al.* 2009) (Al Jahd ali & Hassanine, 2010).In present investigation, adverse affect of parasite on its host species was recorded. The parasite was a trematode larvae inhabiting on the liver of *Channapunctata*. The histopathological alteration of liver, gill and kidney showed that the host was affected adversely by the parasite. Behavioral changes were also observed with sluggish movement and altered growth. The body weight of infected fish was found to be decreased with the presence of cyst number which may be due to the absorption of nutrient by the parasite from the host species. Earlier investigation with parasite also showed detrimental growth and decreased body weight (Mamun *et al*. 2016). Parasites cause different tissue damage with detrimental behavioral response and altered growth (Iwanowicz, 2000). In this study the infected fish showed whitish spot on its body. Histopathological changes have been used as an important biomarker in environmental monitoring that allows examining specific target organs. In present case the liver histology showed the presence of trematode larvae in the fish *Channa punctata.*Similar finding was recorded in the same fish infected with trematode *Euclinostomumheterostomus*(Shareefet *et al.* 2015).Some studies also showed the effect of helminth parasites on the liver of fishes (Maghrabi2017). Results of this study shows infection in liver with vacuolar degeneration in the hepatocytes, necrosis and dilation in central vein. The vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the circulation system. These findings show resemblance with that of previous studies (Mohamed, 2001) (Buthiram*et al*. 2009). This investigation also showed melanomacrophage proliferation that may be due to the presence of parasite. Melanomacrophages are phagocytic in nature. Proliferation of melanomacrophage was also recorded in some earlier investigations (Maghrabi, 2017).In present study infiltration of leucocytes throughout the cyst wall and surrounding tissue is seen in liver histology. This is may be due to the host immune response against the parasite infection. Similar finding was also observed in some other studies (Shareef *et al*.2015).

Another finding of present work reveals pathology in gill showing epithelium detachment, mucus cells destruction and edema in gill filament that is due to presence of the parasite. Several studies mentioned that gills are well known target organ in fishes, being the first to react to unfavorable environmental condition (Devi *et al*. 2007) (Benli, 2008). Histology of kidney of the infected fishesdoesn't show any severe damage in this study. Vacuole formation and the hematopoietic tissue damage are only observed that shows similarity with some earlier investigations (Butchiram *et al*. 2007) (Koteswarerao, 2003) (Tilak *et al.* 2004).

V. CONCLUSION

The present study marked a difference in health condition between infested and non infested fish. The larval trematode found on the liver cause histopathological alteration in liver, kidney, and gill of *Channa punctata*, the hematological results also signifies the adverse condition and unfavorable environment. Since the fish are most sensitive aquatic fauna, any little change that occurs in their living media might immediately influence their physiology. The infestation also affects the behavior and growth of fish, as the parasite acquires its nutrients directly from the host tissue. Though it requires further study to know more elaborately about the infestation, and there must be some control measure to prevent this type of severe infestation as it is also concern with the health of human being.

ACKNOWLEDGEMENTS

The authors thank department of Zoology, Gauhati University and all the professors for their constant helpand support to complete the research work.

REFERENCES

[1] Anita, Susan T., & Tilak K.S. (2003). Histopathological changes in the vital tissues of the fish *Cirrhinus mrigala* exposed to fenvalerate technical grade. *Pollut. Res.*, 22, 179-184.

[2] Anderson, DP. (2003). Disease of Fishes. Narendra Publishing House, Delhi pp. 22-73.

[3] Barber, I., Hoare, D., and Krause, J., (2000). Effects of parasites on fish behavior: a review and evolutionary perspective. *Reviews in Fish Biology and Fisheries* 10, 131-165.

[4] Bashirullah, A.K.M. (1972) A brief survey of the helminth fauna of certain marine and fresh water fishes of Bangladesh, *Bang. J. Zool.*, **1(1),** 63-81.

[5] Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P ., Wahli, T. (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases*, **22**: 25-34.

[6] Bhalerao, G. D. (1936) Studies on the helminths of India Trematoda IV, *Journal of Helminthology*, 14(4), 181–206.

[7] Bhure, D. B., & Nanware, S. S. S. (2014). Studies on prevalence of cestode parasites of freshwater fish, *Channa punctatus*. *Journal of Entomology and Zoology Studies*, 2 (4): 283-285.

[8] Blaxhall, P.C., (1972). The haematological assessment of the health of the freshwater fish. *A review of selected literature. J. Fish Biol*. 4, 593–604.

[9] Blaxhall, P.C., Daisley, K.W., (1973). Routine haematological methods for use with fish blood. *Fish Biol*. 5, 771–781.

[10] Bone, Q., Marshall, N.B. & Blaxter, J.H.S. (1995) *Biology of Fishes*, (2nd ed.). Chapman and Hall, London, 332 pp.

[11] Brusle, J. & Anadon, G. (1996). The structure and function of fish liver. In: Fish morphology, Munshi and H.M. Dutta. Science Publishers Inc. (Eds) J.S.D. 77-93.

[12] Butchiram, M.S., Tilak, K.S., & Raju, P.W. (2009). Studies on histopathological changes in the gill, liver and kidney of *Channa punctatus* (Bloch) exposed to Alachlor. *J. Environ. Biol.* 30(2), 303-306.

[13] Camargo, M.M.P., Martinez, C.B.R. (2007). Histopathology of gills, kidney and liver of a neotropical fish caged in an urban stream. *Neotrop. Ichthyol*. 5: 327-336.

[14] Chai, J. Y., Murrell, K. D., & Lymbery, A. J. (2005). Fish-borne parasitic zoonoses: status and issues. *Int J Parasitol*, 35:1233–54.

[15] Chandra, K.J. (2006). Fish Parasitological Studies in Bangladesh: A Review. *J Agric Rural Dev* 4(1&2), 9-18.

[16] Chinabut, S., Roberts, R.J., Willough, L.G. & Pearson, M. D. (1995). Histopathology of Snakehead, *Channa striatus* (Bloch), experimentally infected with the specific *Aphanomyces* fungus associated with Epizootic Ulcerative Syndrome (EUS) at different temperatures. *J. Fish Dis*., 18: 41-47.

[17] Chowdhury, A.K. (1992). Helminth parasite infestation and histopathological changes in snakehead fishes, *M. Sc.Thesis*, Department of Zoology, University of Dhaka, Dhaka.

[18] Chubb, J.C. (1980). Seasonal occurrence of helminth parasite in fishes Part III. Monogenea. *Advances in parasitology.* Academic Press, York, USA. 18, 1-120.

[19] Cox, F. E. G. (1994) Immunology. *In*: Cox, F.E.G. (ed.), *Modern Parasitology*, (3rd ed.). Blackwell Scientific Publications,Oxford, 193–218.

[20] Das, D. and Goswami, M.M. (2014). The helminth infection in *Anabas testudineus* of three wetlands of Goalpara, Assam. *J. applied & Nat. Sci.* 6 (2), 677-679.

[21] Das, G., Sharma, B.B., Chakraborty, D., & Mukherjee, D. (2015). Parasitic study of Indian Major Carp *Catla catla* (Hamilton, 1822) in selected District of west Bengal, India. *Int. J. adv. Sci. & technical research*, 1(5). 75- 83.

[22] Devia, Y., & Mishrab, A. (2013). Histopathological Alterations in Gill and Liver Anotomy of Fresh Water, Air Breathing Fish *Channa Punctatus* after Pesticide Hilban® (Chlorpyrifos) Treatment. *Advances in Bioresearch*,4(2), 57- 62.

[23] Dutta, H. M. (1996). A composite approach for evaluation of the effects of pesticides on fish. In: *Fish morphology*, (eds) J.S.D. Munshi & H. M. Dutta. Science Publishers Inc.

[24] Ghani, M. O. & Bhuiyan, A. I. (2011). Community structures of endoparasitic helminths of *Channa punctatus* from a fresh water river and a polluted lagoon of Bangladesh. *Bangladesh J. Zool.* 39, 173-185.

[25] Giles, N. (1987). A comparison of the behavioural responses of parasitised and non-parasitised three-spined sticklebacks, *Gasterosteus aculeatus* L., to progressive hypoxia. *J. Fish Biol.*30, 631–638.

[26] Goel, K. A., Mishra, B. P., Gupta, K. & Wadhwa, S. (1984). A comparative haematological study on a few freshwater teleost. *Indian J.Fisheries*, 31(1),108-112.

[27] Gupta, S. P. (1961). Morphology life history and biophysical studies of helminth parasites.*D. Sc. Thesis*. Dept. Zool.,Lucknow Univ., Lucknow, India.

[28] Gupta, S. P. (1959). Nematode parasites of vertebrates of east Pakistan III: Camallanidae from fish, amphibian and reptiles. *Canadian Journal of Zoology* 37 (5), 771-779, 1959.

[29] Jayaprakash, C., & Shettu, N. (2013). Changes in the hematology of the freshwater fish, *Channa punctatus* (Bloch) exposed to the toxicity of deltamethrin. *Journal of Chemical and Pharmaceutical Research*, 5(6), 178-183.

[30] Khalil, L. F., & Polling, L. (1997). Checklist of the helminth parasites of African freshwater fishes. University of the North, Republic of South Africa. River Printers, Pieterburg, South Africa, 185.

[31] Khalil, S. M. I., Mamun, M. A. A., Bari, S. M., & Haque, M. N. (2014). Endoparasitic infestation in *Channa punctatus* (Bloch, 1793) collected from different water bodies in Sylhet. *J. Syl. Agril. Univ.* 1(1), 59-64.

[32] Khatun, F. A. (1995). Metazoan parasites of some freshwater fishes of Comilla, Bangladesh, *M.Sc. thesis*, Dept. of Zoo., Chittagong Univ.,162.

[33] Kumar, A., Singh, S., & Sharma, H. N. (2017). Changes in total protein in liver and kidney of freshwater fish, *Channa punctatus* (Bloch.) After intoxication of carbaryl. *Journal of Advanced Laboratory Research in Biology*, 8(2).

[34] Kumari, Anita & Kumar, R. (1997). Effect of polluted water on histochemical localization of carbohydrates in a freshwater teleost *Channa punctatus* (Bloch) from Hussain Sagar lake, Hyderabad, Andhra Pradesh. *Pollut. Res*., 16, 197-200.

[35] Shareef, P.A. A., & Abidi, S. M. A. (2015). Studies on the epidemiology and histopathology of *Euclinostomum heterostomum*(Trematoda; Digenea) infection in *Channa punctata*from North India. *Arch. Pol. Fish*., 23, 133-140 DOI 10.1515/aopf-2015-0015.

[36] Tilak, K. S., K. Veeraiah and K. Yacobu : Studies on histopathological changes in the gill, liver and kidney of Ctenopharyngodon idellus (Valenciennes) exposed to technical fenvalerate and EC 20%. Pollut. Res., 20, 387-393 (2001).

[37] World Health Organization, Control of food borne trematodes infections, Geneva: WHO, 1-107, (1995).