Prevalence and effects of *Lernaea cyprinacea* (anchor worm) on the growth, skin histopathology and hematology of *Catla catla*

Tufail H.[1]; Qureshi N.A.[2]; Khan N. [1]; Iqbal K.J.[5*]; Khan M.R.[4]; Maqbool A.[3]

Received: June 2015 Accepted: January 2016

Abstract

The experiment was conducted to evaluate the prevalence of *Lernaea cyprinacea* and its effects on growth, hematology and skin histopathology of *Catla catla* in four experimental earthen ponds (P1, P2, P3 and P4) for 90 days. The growth performance, prevalence of *Lernaea cyprinacea* and ulceration was recorded on a fortnightly basis. The highest final body weight was observed in P4 (413.7 g) followed by P2 (378.7 g) and P1 (359.8 g) compared to P3 (357.8 g). Increase of *Lernaea cyprinacea* infection in the first half of the study was from 20.51% - 36.25% in P1, 17.75% -25.25% in P2, 6.5% - 10% in P3 and 14.75% - 13.1% in P4. Ulceration percentage in fish was significantly higher during August and September than May to July. Histopathological studies of skin showed a huge difference between healthy and infected fish skin. Hematological parameters such as hemoglobin (Hb), total erythrocytes count (TEC), total leukocyte counts (TLC) and packed cell volume (PCV%) of non-infected fish exhibited significantly higher values than that of infected fish. Other parameters like erythrocytes sedimentation rate (ESR), neutrophils, eosinophils, lymphocytes, basophils, monocytes and thrombocytes of infected fish showed significantly higher values than those of non-infected fish. In conclusion the exposure to parasites illustrated significant decrease in the Hb, TEC, TLC and PCV% and increase in ESR and other blood cells concentration showed significant severity of parasitic infection.

Keywords: *Catla catla*, Growth, Lernaea, Histopathology, Hematology.

1-Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore, P.O Box. 54000, Pakistan
2-Department of Wildlife and Fisheries, Government College University, Faisalabad, Pakistan
3-Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, P.O. Box. 54000, Pakistan
4-Department of Pathology, University of Veterinary and Animal Sciences, Lahore, P.O. Box. 54000, Pakistan
5-Department of Life Sciences, The Islamia University of Bahawalpur, Pakistan
*Corresponding author's Email**:** khalid.javed@iub.edu.pk

Introduction

Fishes are at the apex of the predator – prey pyramid within freshwater as well as in seawater and therefore tend to be infested by a considerable range of parasites which occur in large numbers. The ectoparasites of fish constitute one of the most important problems associated with pond fish culture. Among them some are the most harmful parasites of cultured fishes consequently; and have become species of interest (Yin *et al.,* 1963; Kabata, 1985; Tasawar *et al.*, 2007a). Copepods are the most numerous among parasitic crustaceans and may be the most common group of fish parasites. They have been found parasitizing skin, gills, eyes, fins and even inside the mouth of fishes, near the palate and nostrils (Eiras, 1994). Damage caused by fish parasites includes hemorrhagic and ulcerated lesions, with potential for secondary infection. Some additional effects may be: anemia, retarded growth, loss of weight and loss of equilibrium.

Lernaeidae is a major family of cyclopoid copepods associated with freshwater fishes. Many species of the *Lernaea* parasitize freshwater fishes and have worldwide distribution. Economic losses due to ectoparasite infestations not only result from direct harm to the fish but also from disfigurement and secondary bacterial infection. Countless pounds of fish have been affected because of this parasite **(**Bauer *et al.*, 1973).

Parasitic infection tends to decrease the growth rate resulting in the stunting of fish. Kinds and number of parasites may vary seasonally, geographically and with age of host. *L. cyprinacea* is a common parasite of freshwater cyprinid fishes. Measurement of blood parameters has been used for many years as a tool for monitoring the health of fish. The influence of parasitic crustacean inhabiting fish upon the hematological parameters of the host was mentioned by Nair and Nair (1983), Guillaume *et al.* (1985) and Natarajan and Felix (1987). Few studies are available about the histopathology of gills and skin of fish in Indian major carps (Sanaullah and Ahmed, 1980; Ahmed and Shahid, 2000). The alarming economical losses due to *Myxobolus* spp. and *Lernaea* spp. infestation of major carp in the nursery ponds of Bangladesh especially on *C. catla*, has been reported (Sanaullah and Ahmed, 1980). Keeping in view the importance of these parasites, the present study was therefore planned to investigate the lernaeid parasites on farm raised *C. catla*. The parasite burden, its effects on growth, skin histopathology and blood chemistry were studied.

Materials and methods

Location

The experiment was conducted at Fish Farms Complex, Research and Training Facilities for Fisheries and Aquaculture at Ravi Campus, University of Veterinary and Animal Sciences, Pattoki, Pakistan. Histopathological and

hematological studies were carried out at the Department of Pathology, City Campus, UVAS, Lahore, Pakistan.

Experimental design

In this study, 720 specimens of *C. catla* were observed for the prevalence of *Lernaea* infestation in the fisheries research and training experimental ponds designated as (P1, P2, P3 and P4) each having an area of 0.03 ha. Artificial feed containing 42% crude protein (CP) was applied in P1, P2 and P4, designated as treated ponds while in P3 (Control) no feed was given. This study was conducted for 90 days in summer months (April-June).

Collection of Lernaea samples

Fish was collected with the help of a drag net and kept in a plastic bucket to avoid stress on fish and keep them alive. The ectoparasites were collected with the help of forceps and kept in the bottles containing fixative (10% formalin). The parasites were collected with extreme care to prevent any damage to fish or parasites. The collected parasites were brought to the laboratory for further examination. The permanent mounts of the parasites were made (Cable, 1985) and identified with the help of keys (Kabata, 1985). After collection of parasites, the morphometric measurements (total body weight and length) of fish were recorded and they were released back to their respective ponds. In addition, ponds were treated with $KMnO_4$ @ 1ppm and common salt @ 1% of total water volume when *Lernaea* cases were observed.

Hematological parameters

Blood chemistry of the infected and non-infected fish was determined to investigate the effects of a natural infestation of *Lernaea* spp. on *C. catla*. Blood was taken from the caudal vein and heart in vacutainer containing EDTA. Differential leukocytes count, erythrocyte sedimentation rate, haemoglobinometry, haematocrit/packed cell volume, numerical counting of erythrocytes and leukocytes tests were performed according to Silva-Souza *et al.* (2000).

Histology of skin

Skin ulceration caused by the *Lernaea cyprinacea* and its histopathological studies were also performed to investigate the effect of *Lernaea cyprinacea* following the method reported by Ahmed and Shahid (2000).

Statistical analysis

The data obtained were analyzed using Minitab statistical package. Results were expressed in percentages and the values between various body weights and body length groups were compared and parasite prevalence was estimated (Ghiraldelli *et al.*, 2006). Analysis of Variance (ANOVA) and Duncan's Multiple Range Tests (DMR) was performed to find out statistical differences among various variables of infected and non-infected fish under study. Correlation and regression

analyses were performed to find out relationship among various parameters.

Results

Growth studies

Initial average body weight of *C. catla* was 65.7 g, 85.5 g, 95.15 g and 98.2 g in P 1, 2, 3 and 4, respectively. At the end of experimental study final average body weight was recorded as 425.5 g, 464.2 g, 453 g and 511.85 g in P 1, 2, 3, and 4, respectively (Fig. 1).

Lernaea prevalence

L. cyprinacea infestation was observed (5 *Lernaea* specimens per fish) after fifteen days of stocking with gradual increase in the months of May and June irrespective of the environmental changes in all ponds. The percentage *of Lernaea* infestation increased from 20.51% to 36.25% in P1, 17.75% to 25.25% in P2, 6.5% to 10% in P3 and from 14.75% to 13.1% in P4 (Fig. 2). Application of $KMnO_4$ @ 1ppm and common salt @ 1% of total water volume resulted in a significant gradual decrease in the *Lernaea* infection (F=43.5***). Ulceration increased as the rate of infestation increased however, when ponds were treated with $KMnO_4$ and common salt, the ulceration percentage also decreased (Fig. 3).

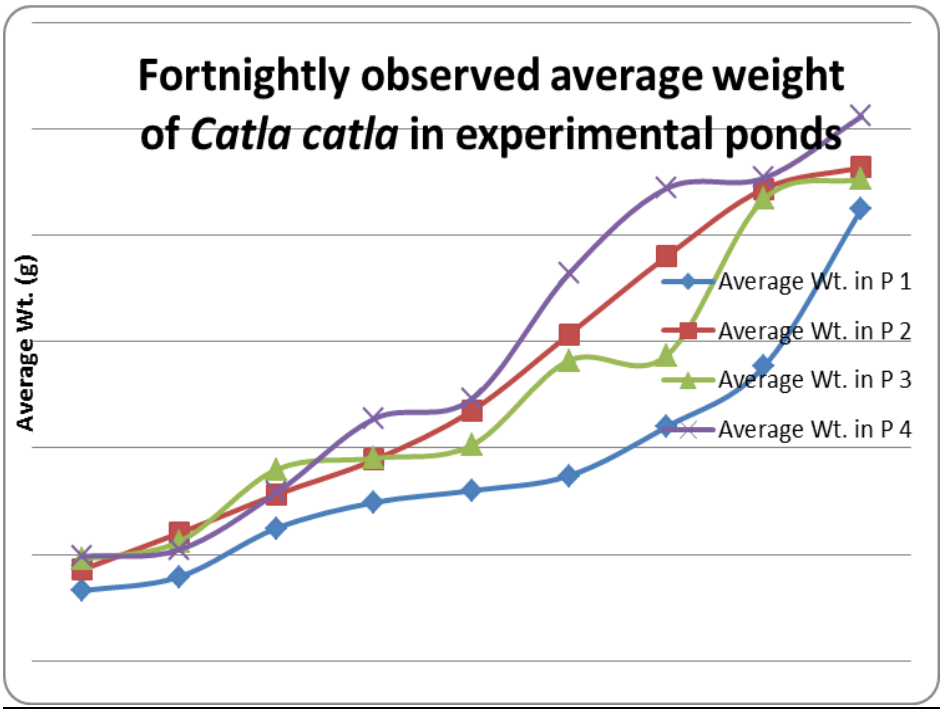

Figure 1: Fortnightly average weight of *Catla catla* in experimental ponds.

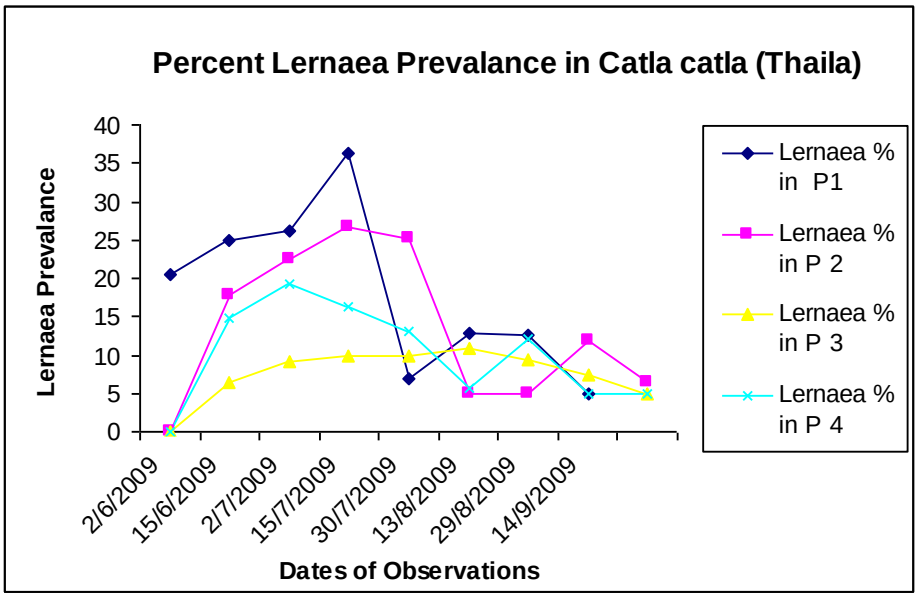

Figure 2: *Lernaea* prevalence in *Catla catla*.

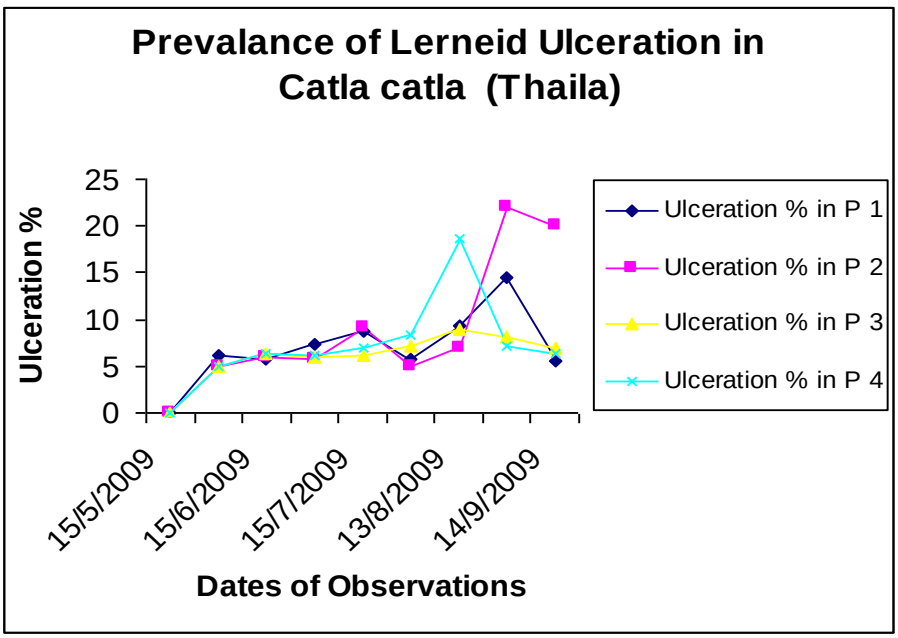

Figure 3: Ulceration prevalence in *Catla catla*.

Skin histopathology

Histopathological study of the skin of *C. catla* showed huge difference between healthy and lernaeid skin. Healthy fish skin had normal structure, while skin of infected fish showed deep variation and ulceration, skin burst from epidermis and cutis to the lower connective tissues with the infiltration of leukocytes including neutrophils, lymphocytes, and eosinophils (Figs. 4 and 5). Fish skin is composed of four different layers; epidermis, corium, cutis (the actual skin) and skeleton

muscles. Histological studies show, all the components of skin were intact in healthy fish (Fig. 4) but were found to be damaged in infected fish from epidermis to skeleton muscle (Fig. 5) that resulted in heavy ulcerations. This study reveals that there is a strong relationship between intensity of ulceration and tissue damage in fish.

Hematological parameters

Comparison of hematological parameters exhibited a significant increase in neutrophils, eosinophils, lymphocytes, basophiles, monocytes and thrombocytes count in infected fish compared to non infected fish (Table 1). There was a significant increase in total leukocyte counts (TLC) and erythrocytes sedimentation rate (ESR) of infected and non infected fish, but a decrease in total erythrocytes count (TEC) and packed cell volume (PCV%).

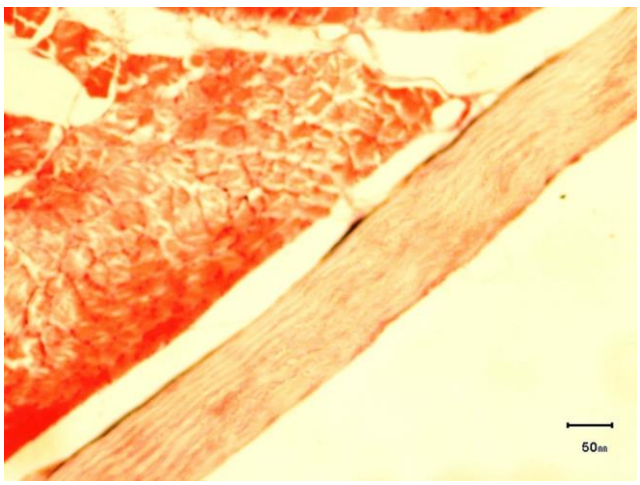

Figure 4: Skin of healthy C*atla catla*.

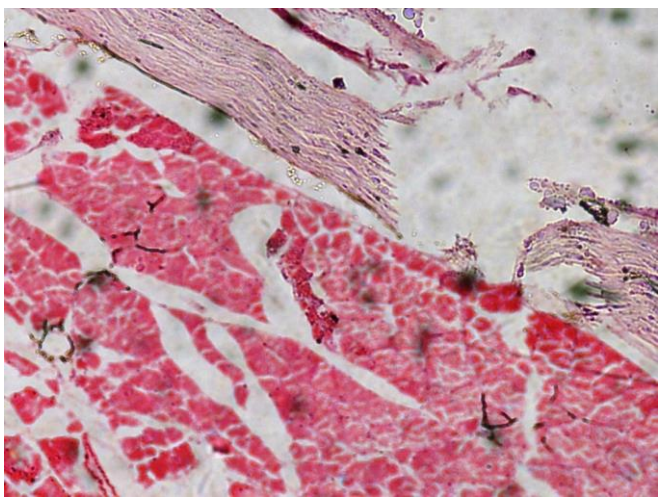

Figure 5: Skin of infected C*atla catla.*

Table 1: Hematological parameters of infected and non infected fish.

Sr.#	Parameters	Non infected (n=21) Mean±SD	Infected (n=21) Mean± SD
1	Hb (g dL^{-1})	4.47 ± 0.8^a	3.6 ± 0.7^b
2	TEC (No of cells/cubic mm of blood)	$512x\ 10^5 \pm 735.10^a$	$3.0x10^6 \pm 6798026^b$
3	TLC (No of cells/cubic mm of blood)	67950 ± 250.2^a	$48X10^5 \pm 10057.55^b$
4	PCV (%)	19 ± 1.22^a	5 ± 1.19^b
5	ESR (mm hr^{-1})	90 ± 3.6^a	110 ± 13.4^b
6	Neutrophils (%)	13 ± 4.96^a	31 ± 4.9^b
7	Eosinophils (%)	5 ± 0.86^a	11 ± 0.96^b
8	Basophiles (%)	7 ± 0.29^a	22 ± 0.79^b
9	Lymphocytes (%)	9 ± 0.44^a	15 ± 0.622^b
10	Monocytes (%)	11 ± 0.8^a	20 ± 0.710^b
11	Thrombocytes (%)	13 ± 1.4^a	15 ± 1.02^b

*Figures with different superscripts are significantly different (p<0.05)

*Hb = hemoglobin; TEC = total erythrocytes count; TLC = total leukocytes count; PCV = packed cell volume; ESR = erythrocytes sedimentation rate.

These results indicated that due to parasitic infestation, host response to infection increased with an increase in immunity level (white blood cells) against lernaea infestation in *C. catla*. Destruction of red blood cells caused hemolytic anemia in infected fish and enhanced the value of ESR that released more hemoglobin. The present study exhibited significant changes in various hematological parameters of infected fish blood compared to non infected fish blood. Erythrocytes and particularly Hb have been postulated to function as oxygen sensors and controllers of local blood flow and oxygen delivery. Destruction of red blood cells in infected fish results in hemolytic anemia. However, increase in white blood cells showed an increased immunity level of fish; the cell swells and breaks to increase ESR value.

Discussion

It was generally observed during sampling in the current study which was conducted in summer season that *Lernaea* infestation was higher in small and medium sized fish compared to large sized fishes. Studies of Tasawar *et al.* (1999); Kir (2007) and Tasawar *et al.* (2007a) also reported that as the weight and length of the fish increased, infestation of copepod ectoparasite decreased. According to Marcogliese (1991), Medeiros and Maltchik (1999) who also supported our results reported that prevalence of *Lernaea* on fish typically increases during the summer when water temperatures exceed 25°C, although parasitized fish can be found during the fall and winter season (Bulow *et al.*, 1979). However, treatment with $KMnO_4$ and common salt can reduce the infestation. Parasitic females of *L. cyprinacea* appeared to be an important agent of the lesions, adult specimens attached to the skin, primarily on the ventral part of the body (mainly at the base of the pectoral and pelvic fins). Acute inflammation, necrosis and capsules (with collagen fibers) formation were the typical characteristics of infection (Ho and

Kim, 1997). The present study and the studies of Joy and Jones (1973) showed positive and significant correlation of ulceration percentage with *L. cyprinacea* infestation and symptomatic treatment with $KMnO_4$. It seems that application of $KMnO_4$ reduces *Lernaea* developmental stages and infestation in Indian major carps and can be used as a prophylactic measure against *L. cyprinacea*.

The histopathological results of the current study reveal a strong relationship between intensity of ulceration and tissue damage in fish. There was an inflammatory response in white bass (*Morone chrysops*) infected with *L. cruciata*, and the dermis and musculature were the only sites of attack by the copepod. No penetration to internal organs was observed. Contradictory to the previous studies most of the internal organs of small fish were invaded by *L. cyprinacea* indicating that *L. cyprinacea* is a more dangerous parasite, especially in juvenile *C. catla* (Joy and Jones, 1973).

Studies of Khalifa and George (1976) supported our results i.e., the pathological effect on fish resulting from an infection of *L. cyprinacea* was traumatic. Tissues in close contact with the head and neck of the adult female parasite were hardened and toughened, which helped to hold the parasite in place; the site of parasite penetration was always necrotic and red. The results indicated that severe attack of *Lernaea* on *C. catla* increased deep ulceration on skin due to which the fish feels itchy, and irritated, and rubs its body against hard objects. The fish looks weak and unhealthy. According to Tidd and Shields (1963), the alimentary tract of the parasites contains tissue debris and erythrocytes, indicating that the parasite feeds on both tissue and blood, especially in the early stages of penetration. This feeding may cause weakness of the host and finally its death. Extreme proliferative changes; cellular degeneration, necroses and disintegration of tissue observed in skin infection by ectoparasites are also reported by Ribelin (1975), Paperna (1980) and Robertson *et al*. (1981). Paperna and Van (1983) also proved that the epithelial hyperplasia was encountered by the extreme process of degeneration of epithelial layer.

The hematological study of the fish blood can provide an insight into potentially hazardous exposure experienced in the ponds with *L. cyprinacea* infestation or any other parasitic infection. Blood is an excellent indicator of infection and metabolic disorders in the body and functional state of many tissues thus can be assessed by analyzing changes in the blood chemistry. A significant change in various hematological parameters of infected fish blood compared to non infected fish was observed during the study. Erythrocytes and particularly Hb have been postulated to function as oxygen sensors and controllers of local blood flow and oxygen delivery. Destruction of red blood cells in infected fish results in hemolytic

anemia. However, increase of white blood cells showed an increased immunity level of fish. The cell swells and breaks to increase ESR value. Silva-Souza *et al.* (2000) also reported an increased number of leukocytes in lernaied fish blood. It can also be inferred that *lernaeaosis* affect directly the leukocyte population in circulating blood by inducing more production enhancing the host responses. Das *et al.* (2006) reported that change in water pH or disease condition exerted stress in fish characterized by swelling of erythrocytes, production of immature erythrocytes, and reductions in the total erythrocyte counts, hemoglobin and serum protein content. Ghiraldelli *et al.* (2006) observed similar hematological parameters in parasitized tilapia and stated that parasitized tilapia showed reduced number of erythrocytes and increased number of lymphocytes, neutrophils, monocytes and circulating thrombocytes. Significant alteration in hematological parameters such as decrease in Hb level, RBC and WBC counts were observed in grass carp with endosulfan toxicity that causes stress condition (Hassan *et al.,* 2015) and in *Cyprinus carpio* (Jenkins *et al.,* 2003).

It is concluded that this study contributes to current knowledge on the fish external parasite *L. cyprinacea* which does not have so much effect on the growth performance of *C. catla* but affects the skin histology, causes ulceration and bursting of skin layers. It also affects the blood cells and increases host response to increase immunity level. In addition, due to proper treatment this parasitic infestation can be cured at a proper time and economical losses for the farmers can be reduced.

Acknowledgements

The authors are extremely thankful to Dr. Muhammad Ishtiaq, Lecturer and Chairman Department of Pathology, University of Veterinary and Animal Sciences, Lahore, Pakistan for providing Laboratory facilities and technical guidance during lab work. Special thanks to field and laboratory staff of the Department of Fisheries and Aquaculture, UVAS for helping in fish netting, handling and sampling of experimental fish species.

References

Ahmed, S.M. and Shahid, A., 2000. Histological studies of the skin, affected by *Lerneae cyprinacea* infection in a freshwater fish, *Cirrhinus mrigala*. *Pakistan Journal of Fishes*, 1, 59-64.

Bauer, O.N., Musselius, V.A. and Streelkov, Y.A., 1973. Diseases of pond fishes. Jerusalem: Israel programmed for scientific Translation.

Bulow, F., Winningha, J.R. and Hooper, R.R., 1979. Occurrence of the copepod parasite *Lernaea cyprinacea* in a stream fish population. *Transactions of American Fish Society*, 108, 100-102.

Cable, R.M., 1985. An illustrated

laboratory manual of parasitology. Surjeet Publications, Delhi, India.

Das, P.C., Ayyappan, B,S, and Jena, J.K., 2006. Hematological changes in the three Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) exposed to acidic and alkaline water pH. *Aquaculture*, 256(**1**), 80-87.

Eiras, J.C., 1994. Elementos de Ictioparasitologia. Porto, Fundação Engenheiro Antônio de Almeida, 339P.

Ghiraldell, L., Mauricio L.M., Marcela, M.Y. and Abriela, T.J., 2006. Ectoparasites influence on the hematological parameters of Nile tilapia and carp cultured in the state of Santa Catarina, south Brazil, *Journal of Fish Aquaculture Science*, 3, 270-276.

Guillaume, Douëllou, L., Romestand, B. and Trilles, J.P., 1985. Influence d'un parasite hématophage: *Lernaeocera branchialis* (L., 1767) (Copépode, Pennellidae), sur les constantes erythrocytaires de son hôte Merluccius (L., 1758). *Rev Trav Inst Pêches Marit*, 47(**1-2**), 55-61.

Hassan, Z., Ghayyur, S., Hassan, Z.U. and Rafique, S., 2015. Histomorphometric and hematological profile of grass carp (*Ctenopharyngodon idella*) during acute endosulfan toxicity. *Pakistan Veterinary Journal*, 35(**1**), 23-27.

Ho, J.S. and Kim, I.H., 1997. *Lernaeid copepod* (Cyclopoida) parasites on freshwater fishes of Thailand. *Journal of Natural History*, 31, 69 – 84.

Jenkins, F., Smith, J., Rajanna, B., Shameem, U., Umadevi, K., Sandhya, V. and Madhavi, R., 2003. Effect of sub-lethal concentrations of endosulfan on hematological and serum biochemical parameters in the carp (*Cyprinus carpio*). *Bulletin of Environmental Contamination and Toxicology*, 70, 993-997.

Joy, J.E. and Jones, L.P., 1973. Observations on the inflammatory response within the dermis of a white bass, *Morone chrysops* (Rafinesque), infected with *Lernaea cruciata* (Copepoda: Caligidea). *Journal of Fish Biology*, 5(**1**), 21-23.

Kabata, Z., 1985. Parasites and diseases of fish cultured in the Tropics. Taylor and Francis, London, UK.

Khalifa, A.K., and George, P., 1976. Histopathological effect of *Lernaea cyprinacea* (a copepod parasite) on fish. *Journal of Wilderness Fish Biology*, 38, 110-113.

Kir, I., 2007. The effects of parasites on the growth of the crucian carp (*Carassius carassius* L., 1758) inhabiting the Kovada Lake. Turkiye Parazitol Derg, 31, 162-166.

Marcogliese, D.J., 1991. Seasonal occurrence of *Lernaea cyprinacea* on fishes in Belews Lake, North Carolina. *Journal of Parasitology*, 77, 326-327.

Mediros, E.S.F. and Maltchik, A.D.L., 1999. The effects of

hydrological disturbance on the intensity of infestation of *Lernaea cyprinacea* in an intermittent stream fish community. *Journal of Arid Environment*, 43, 351-356.

Nair, G.A. and Nair, N.B., 1983. Effect of infestation with isopod *Alitropus typus* M. Edwards (Crustacea: Flabellifera: Aegidae) on the haematological parameters of the host fish *Channa striatus* (Bloch.). *Aquaculture*, 30, 11-19.

Natarajan, P. and Felix, S., 1987. Studies on infestation and effects of certain parasites of *Mystus gulio* along the south-coast of India. *Aquaculture*, 67, 233-235.

Paperna, I., 1980. *Amyloodinium ocellatum* (Dinoflagellida) infestations in cultured marine fish at Eilat, Red sea: epizootology and pathology. *Journal of Fish Disease*, 3, 363-372.

Paperna, I. and Van, A.S., 1983. The pathology of *Chilodonella hexasticha* infection in cichlid fishes. *Journal of Fish Biology*, 23, 441-450.

Ribelin, W.E. and Migaki, G., 1975. The pathologies of fishes. The University of Wisconsin press LTD., Russell Street, London.

Robertson, D.A., Roberts, R.J. and Bullock, A.M., 1981. Pathogenesis and autoradiographic studies of epidermis of Salmonids infected with *Ichtyobodo necator*. *Journal of Fish Diseases*, 4, 113-125.

Sanaullah, M.A. and Ahmed, A.T., 1980. Gill Myxoboliasis of major carps in Bangladesh. *Journal of Fish Diseases*, 3, 349-354.

Silva- souza, A.T.S., Almeida, C. and Machado, P.M., 2000. Effect of the infestation by *Lerneae cyprinacea* (Linnaeus), (Copepoda, Lernaeidae) on the leukocytes of *Schizodon intermedius* Garavelo and Britiskia, (osteichthyes, Anostomidae). *Review in Brasilian Biology*, 60, 217-220.

Tasawar, Z., Razaq, A. and Ashraf, M., 1999. Investigations of lernaeid parasites of *Catla catla*. *Pakistan Journal of Zoology*, 14, 17-23.

Tasawar, Z., Zafar, S., Lashari, M.H. and Hayat, C.S., 2007a. The prevalence of lernaeid ectoparasites in mori (*Cirrhinus mrigala*). *Pakistan Veterinary Journal*, 27, 176-178.

Tidd, M.W. and Shields, R.J., 1963. Tissue damage inflicted by *Lernaea cyprinacea* Linnaeus, a copepod parasitic on tadpoles. *Journal of Parasitology*, 49, 693-696.

Yin, W.Y., Liang, M.E., Hsu, G.A., Chen, L.S., Kuang, P.R. and Chu, S.L., 1963. Studies on the lernaeosis (*Lernaea*, Copepoda parasitica) of freshwater fishes of China. *Acta Hydrobiologica Sinica*, 2, 48-117.