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Effect of Ecto-parasitic Infestations on Histology and Growth of *Clarias gariepinus*

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ABSTRACT: Histological alterations in the skin and gills of *Clarias gariepinus* vis-à-vis effect on important growth parameters such as percentage gain in head length and percentage loss of body weight was studied in *Clarias gariepinus* infested with ecto-parasites. Out of 217 total specimens of *C. gariepinus* collected, 91 fishes were found infested with parasites marking overall percentage prevalence of 41.94%. Three parasitic species including *Gyrodactylus, Dactylogyrus* and *Lernea* were recovered in this study attached to gills and skin. Fishes procured were segregated and divided into small (<13cm), medium (14-18 cm) and large (>18cm) groups. It was found that percentage head length was increased in parasitized fishes when compared with the non-parasitized ones. The percentage gain in the head length was 99.2%, 118.27% and 109.47% in small, medium and large groups respectively. Similarly, percentage loss in weight recorded was 89.2%, 90.54% and 93.31% in small, medium and large groups respectively. Histological studies revealed profound skin erosion, gill hyperplasia, blood clots and distorted skins muscles.

Keywords: *C. gariepinus*, Ecto-parasites, Alterations, Gill, Skin, Growth indices.

INTRODUCTION

Waterbodies have undergone progressive pollution and deterioration of water quality (Akhter *et al*., 2015; Sharma *et al*., 2015; Akhter *et al*., 2018; Mehboob *et al*., 2018; Malik *et al*., 2022; Abubakr *et al*., 2022). Rise in water pollution has been linked with infestation of parasites in fishes (Qayoom *et al*., 2015a; Qayoom and Shah 2017). Parasitic diseases are secondary diseases, parasites get adhered to fish body when they are immune compromised, diseased or under stress due to pollution. Weather infested by ecto-parasites or endo-parasites, fishes undergo serious hematological (Qayoom *et al*., 2022), biochemical (Hudha *et al*., 2021) and histological (Feist and Longshaw 2008) changes. Moreover, various important morphometric and growth indices such as body weight and head length alterations has also been reported by various researchers in fishes infested with parasites.

Diseases and parasitic infestations act as limiting factors in aquaculture (Kabata *et al.*, 1985) as parasites, in addition of acting as disease causatives, are responsible for fish mortalities as well (Caira and Littlewood 2001). Lack of technical staff availability and the application of scientific technique both contribute to the spread of disease in fish (Qayoom *et al*., 2015b; Qayoom *et al*., 2014). Besides this, contamination results in alterations in the flora and fauna communities in aquatic system (Qayoom and Jaies 2022). It is generally known that parasites can alter the host fish immune system and cause a number of physiological dysfunctions (Qayoom and Jaies 2019). They reduce fish production yield, result in slower growth and weight gain, spread diseases to humans and other animals, delay fish sexual maturation, and raise fish mortality rates (Qayoom *et al*., 2022; Tabasum and Dixit 2021a).

Those parasites cause infections both in vertebrates as well as in invertebrates and the majority of these parasites have co-evolved with their hosts (Sitjà-Bobadilla, 2008). Ectoparasites frequently cause dermatitis, secondary infections, necrosis, anaemia, hypersensitive immunological reactions, irritability, and necrosis (Tabasum and Dixit 2021b). The successful co-evolution of the host and its parasite has led to the latter's adaptability through the development of evasive mechanisms to prevent extinction (Shah *et al*., 2015). Parasites provide an integrated measure of the health status of an organism, which with the passage of time manifests in changes in weight (Yaji and Auta 2007). Histology serves as one of the basic diagnostic tools for

gross examination and determination of diseased tissues and compares it with the normal tissues. Parasites affect wide range of fishes and are known to damage the tissues as well at the site of their attachment to the host. That site is worth studying and form the principle site of diagnosis for the histopathologists. Histopathological diagnosis forms one of the certain methods which validate the occurrence of disease and suggests the treatment for its rehabilitation to the normal. Therefore, the current study aimed to measure the effect of parasite on the health of host catfish (*C. gariepinus*). In addition to this, these parasites while infesting the host can result in appreciable change in the host growth indices, i.e., length and weight. Therefore, the percentage effects in growth indices were also studied in different sized fishes.

MATERIAL AND METHODS

A total of 217 specimens of *Clarias gariepinus* were examined among which 117 were males and 100 were females during the current study. Collected *C. gariepinus* were transported to the laboratory in plastic bags. Before carrying out parasitological examination, morphometric measurements of the fishes were also carried out. All the collected fish specimens were subjected for the gross examination of the presence of ectoparasites.

Morphometric measurements. As soon as the live specimens were brought to the laboratory morphometric measurements which included Total Length (TL), Head Length (HL) and Body Weight (BW) were taken by using stainless steel centimetre scale and digital electronic balance respectively. The experimental fishes were then divided into three length groups and designated as small (<13cm), medium (14-18 cm) and large (>18cm) adopting the earlier methodology (Laboni *et al*., 2012; Khalil *et al*., 2013).

Examination, isolation, identification and classification of Ectoparasites. The external surface of the fishes like fins, gills and skin was examined for external parasites (Schäperclaus, 1991). Skin and fins were brushed and examined through a simple magnifying glass for the presence of ectoparasites. Fish gills were dissected out and each gill filament and arch was examined with a hand lens for the presence of parasites. Skin scrapings were taken and examined separately wherever necessary. Recovered parasites were mounted on slides and viewed using compound microscope under high power magnification (×40) and identified to species level using appropriate keys (Paperna, 1996; Manwell, 1968; Yamaguti, 1963; Yamaguti, 1959).

Effect of parasite on fish health. The effect of ectoparasites on the host health was studied in terms of loss of weight and percentage gain in head length. The measurement of these indices was done using the following formulae:

$$\text{Loss of fish weight (g)} = \frac{\text{Av. weight of non-infected fish} - \text{Av. weight of infested fish}}{\text{Av. weight of non-infested fish}} \times 100$$

$$\text{Percentage gain in head length (GHL)} = \frac{\text{Av. head length of non-infected fish} - \text{Av. head length of infested fish}}{\text{Av. weight of non-infested fish}} \times 100$$

Histopathological studies. Histopathology of skin and gills of helminth infested as well as healthy ones was carried as per the methodology of Hossain *et al.* (2014). After dissecting fish tissues, the samples were rapidly washed with physiological saline solution(0.75% NaCl) and instantly fixed in 10% formalin. The following protocol was carried for the preparation of histological slides. This was followed by dehydration and paraffin impregnation. Sections were cut on a microtome (Histo core multi Cut mecrotoma) fitted with a sharpened microtome knife. For stretching the cut out ribbons, the temperature of the water bath was maintained between 55-56°C. Sections of 6-8 thickness were cut. The ribbons were attached to the glass slides that were already brushed with Mayer's albumen. After that the slides were immersed in xylene I and xylene II for ten minutes each to remove the paraffin. After rehydration in graded alcohol, slides were then dipped in haematoxyline for 5 min. After washing them properly under running water, they were then dipped in 0.5% alcohol for 30 sec and again washed. Finally, they were dipped in eosin for 1-2 minutes. The slides were then again dehydrated The stained tissues were finally cleaned in xylene for 10 min, mounted on DPX and analysed.

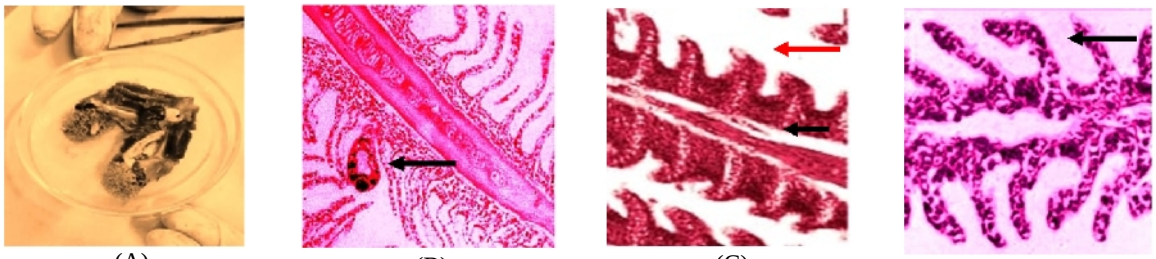
RESULTS

In the present study, three species of parasites were found which included *Gyrodactylus* sp., *Dactylogyrus* sp. and *Lernea*. Among 217 fishes of *Clarias gariepinus*, a total number of 91 fishes were found infested with parasites, thus exhibiting a prevalence of 41.93%.

Histo-pathological Examination of *C. gariepinus* infested with Parasites

Effect of Parasites on Gills of C. gariepinus. Histological analysis of gills of *Clarias gariepinus* revealed the same pattern of changes due to the infection caused by helminth ecto-parasites (Fig. 1). One of the primary changes caused due to the infestation of *Lernea* was Telangiectasia in the secondary gill lamellae of both the fishes. Telangiectasia is a condition in which widened venules (tiny blood vessels) cause thread-like red lines or patterns on the skin. These patterns, or telangiectases, form gradually and often in clusters. They are sometimes known as "spider veins" because of their fine and web-like appearance. The respiratory epithelial wall was commonly found uplifted by the infestation of all parasites with damaged pillar cells. Overall hyperplasia was observed in the echo-pattern of gill

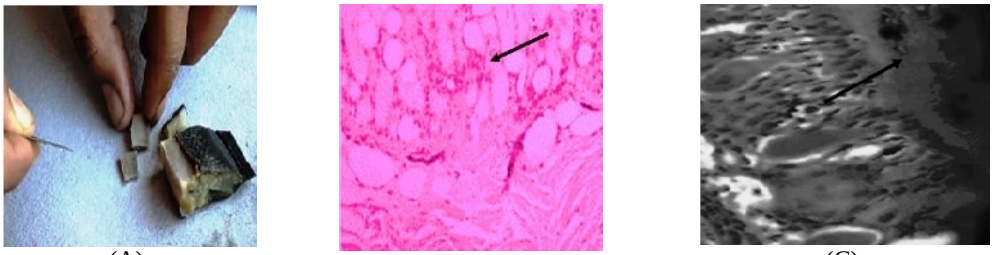
lamellae with the fusion of secondary lamella due to hyperplasic condition. Aneurism in the secondary lamella was rarely noticed indicating weakening of artery wall leading to the creation of a bulge, or distention of the artery. Edema, damaged pillar cells and the proliferation of epithelial, connective and inflammatory cells was also observed in infested fish species. Besides this, *Dactylogyrus* was seen to be attached to the primary gill lamellae by its haptor causing disruption of the primary gill lamellae and cartilaginous core. The frequency and types of lesions caused by the parasites in the gills of cat fishes are given in Table 1.



(A) (B) (C) (D)

Fig. 1. Histopathological changes in the gills of *C. gariepinus* infected with parasite; (A)Normal gills dissected out from fish (B) Aneurism (C) Hyperplasia (Red arrow) and blood congestion in primary lamellar (Black arrow), (D) Telangiectases.

Effect of Parasites on Skin of C. gariepinus. It was obvious to study the alterations in the pattern of skin of *Clariasgariepinus* fishes affected with parasites externally and compare that with the skin of non-infested fishes (Fig 2). During present study, the infected skin of both the fishes revealed eosinophilic infiltration in the integument with muscle fiber atrophy and necrosis in their connective tissue. A pronounced swelling of the subcutaneous and connective tissues with the overall loss muscle tissue was seen in the fishes infested with all the three parasites. A prominent formation of fibrous tissue was seen at the site of attachment of parasites in the skin with hemorrhages, congestion and erosion of epithelial tissue as response of host to the attack of parasites. Besides this, hypertrophy and hyperplasia of goblet cells, reduction of epithelium thickness and loss of goblet cells was also seen. Infested skin also revealed moderate score mono- and polymorphonuclear inflammation, leukocyte infiltration and vacation of blood at the site of parasitic attachment. The connective tissue in the skin was found disorganized with extreme epithelial necrosis, hemorrhage, and hypertrophy of the alarm cells also called claviform cells. The frequency and types of lesions caused by the parasites in the skin of cat fishes are given in Table 2.



(A) (B) (C)

Fig. 2. Histo-pathological changes in the skin of *C. gariepinus* infected with parasite; (A) Normal skin sections, (B) Eroded epithelial surface and (C) Haemorrhages in skin.

Table 1: Histological examination and frequency of lesions created by parasites in cat fish gills.

	Type of lesion	Frequency %
		Clarias gariepinus
Gill Histological Lesion	Hyperplasia	79
	Edema	41
	Fusion of secondary lamella	58
	Aneurism	69
	Damaged pillar cells	42

Table 2: Histological examination and frequency of lesions created by parasites in cat fish skin.

	Type of lesion	Frequency %
		Clarias gariepinus
Skin Histological Lesion	Reduction of epithelium thickness	63
	Hypertrophy and hyperplasia of goblet cells	56
	Loss of goblet cells	54

Effect of parasites on growth of catfishes. Effect of ectoparasites on the growth of catfishes was one of the objectives of this study. The objective was carried out by studying the effect of parasitic infestation on the morphometric indices of *Clarias gareipinus*. The main morphometric analysis on the basis of which fishes were categorised was length of fishes. The fishes were separated and categorized into three groups and classified into small (<13cm), medium (14-18cm) and large (>18cm). During this investigation, the parasitic infestation was found directly correlated with the size of fishes. The intensity and prevalence of parasites infection increased with increasing length.

Effect of Parasites on Head Length. Infestation of parasites was found to be associated with the gain in the head length of catfishes under study. The gain in the head length was measured in all the categorised fishes (small, medium and large) and was expressed as percentage gain of head length in all the cases and abbreviated as GHL. The percentage gain in head length (GHL) was found to vary in different length groups. In *Clarias gariepinus*, the higher % GHL was recorded in medium (14-18cm) sized fishes followed by large (>18cm) and then the small grouped (<13cm) fishes (Table 3).

In *Clarias gariepinus*, the percentage of mean head length was found highest in medium sized fishes (14-18cm) and was recorded 118.27%, followed by large small sized group (>18cm) and was recorded 109.47% followed by Small sized group (<13cm) and was recorded 99.20% shown in Table 1.

Table 3: GHL in non-infested and infested fishes of different length groups in *Clarias gariepinus.*

Length groups (cm)	Mean Head length (cm)		% Gain of mean head length
	Non-infested (Length cm)	Infested (Length cm)	
Small (<13)	2.53±0.23	2.51±0.17	99.20
Medium (14-18)	3.01±0.43	3.56±0.41	118.27
Large (>18)	4.12±0.47	4.51±0.47	109.47

Effect of Parasites on Body Weight. Infestation of parasites was found to be associated with the loss of body weight of the fishes under study. The loss in the body weight was measured in all the categorised fishes (small, medium, and large) and was expressed as percentage loss of body weight in all the cases and abbreviated as LBW. The percentage loss in body weight (LBW)was found to vary in different length groups. In *Clarias gariepinus*, the loss % LBW was recorded highest in large group (>18cm), followed by medium sized fishes (14-18cm) and then the small sized fishes (<13cm) shown in Table 4.

In *Clarias gariepinus*, the percentage loss of mean body weight was found highest in the large sized group (>18cm) and was recorded 93.31% followed by the medium sized fish group (14-18cm) and was recorded 90.54% and the small sized group (<13cm) with the overall percent loss of body weight of 89.20% shown in Table 2.

Table 4: LBW in non-infested and infested fishes of different length groups in *Clarias gariepinus.*

Length groups (cm)	Mean Weight (gm)		% Weight loss
	Non-infested (Weight gm)	Infested (Weight gm)	
Small (<13)	22.13±3.21	19.74±2.16	89.20
Medium (14-18)	32.87±3.32	29.76±4.23	90.54
Large (>18)	67.43±2.95	65.62±7.43	93.31

DISCUSSION

Parasites have a wide range of distribution in all groups of animals. They can infect fishes in different stages of their life, as well as different aquatic environmental conditions, and are also considered to be biological indicators of environmental pollution. Parasites interfere with the nutrition, metabolism, and secretory function of the alimentary canal, damage nervous system, and also upset the normal reproduction of the host. Most parasite species rarely cause problems in the natural environment but in aquaculture, parasites often cause serious outbreaks of disease (Roberts, 2012).

The effect of parasite infestation on the growth of fishes was evaluated by studying the percentage gain of head length and percentage loss of body weight. Percentage gain of mean head length (cm) due to parasite was calculated in different length groups of *Clarias gariepinus.* The observed percent gain of mean head length (cm) was highest in medium sized fish in both the species, followed by larger group and lastly by smaller ones. This is due to higher susceptibility of medium ones to parasites than the larger and smaller fishes. Earlier it was reported higher percent gain of mean head length (4.44 cm) in medium sized group (17-21cm) of *H. fossilis* during high level of infestation is also in agree with the present findings (Khalil *et al*., 2013). The percentage of weight loss in different length groups revealed highest percent loss in the large length group while lowest in smallest length groups. This suggests that more the infestation, more are weight loss effects.

Gill as well as the skin of *C. gariepinus* infected with ectoparasitichelminths revealed severe changes during the present study. The histological effects caused by monogenea mainly arise due to their morphological structures and specialized mode of attachment with

host. Fish gills are involved in many important functions such as respiration, osmoregulation and excretion and these remain in close contact with the external environment and are highly sensitive to changes in the quality of the water (Mazon *et al.*, 2002; Camargo and Martinez, 2007). Besides this, gills are generally considered as a good indicator of water quality and are used as a model for environmental impact studies. During the present study, infested skin showed hyperplasia, edema, aneurism and fusion of secondary lamella, pillar cell damage, telangiectasia at the tips of secondary gill lamella, uplifting of respiratory epithelial wall as well as proliferation in epithelial cells. The findings were in collaboration with earlier reports who reported proliferation of branchial tips, desquamation of primary and secondary gill lamellar epithelium, epithelium detachment and thinning and curling of secondary gill lamellae and damaged pillar cells as the major pathological effects induced in the gills of carps infected with monogenetic trematodes (Arafa *et al*., 2009; Tantawy, 2004; Mitchell *et al*., 2002; Hanna, 2001; Osman, 2001; Derwa, 1995). Rigorous alterations of gills might negatively affect the respiratory process of the fish. Monogenean gill parasites are pathogenic in enormous infestations as they damage the epithelia and excess mucus secretions which affect the normal respiration (Thurston *et al*., 1965). Daib *et al.,* while studying the gills of *O. niloticus* infected by *Clinostomum* sp. reported the sloughing of secondary gill lamellae, desquamation of epithelial cells and severe congestion of branchial blood vessels which might have occured due to the scraping and sucking activities of the parasites on host gills (Diab *et al*., 2006). Abnormal production of mucus and protective reaction of epithetical hyperplasia was seen in fish gills infected with *Chilodonellacyprini* and *Myxoboluskoi*. *Myxobolus* in gills can cause respiratory dysfunction and necrosis of gill leading to death (Kabata, 1985).

The skin of a fish serves important functions in providing physical protection, maintaining osmotic balance, and has an important role in respiration as well. The histological findings of our study revealed that the ectoparasite infested catfish shows eosinophilic infiltration in the integument, muscle fibre atrophy, necrosis in the connective tissue, swelling of the subcutaneous and connective tissues loss of muscle tissue, formation of fibrous tissue, hemorrhages, congestion and erosion of epithelial tissue, hypertrophy and hyperplasia of goblet cells, reduction of epithelium thickness and loss of goblet cells. Besides this, infested skin also revealed moderate score mono- and polymorphonuclear inflammation, leukocyte infiltration, vacation of blood, connective tissue disorganization, epithelial necrosis, hemorrhage, and hypertrophy of the alarm cells (claviform cells). Findings were in collaboration with Hossain *et al.* (2014) who reported hyperplasia in the histological sections of fish skin infested with parasite. As per them, the epithelial cells get irritated by the suction of the parasite, thus causing epithelial cellular growth and excess of mucus production (Hossain *et al*., 2014). They also reported haemorrhages, necrotic skin in the infected fish. This type of modification is characterized by the degradation of the tissue and the loss of its functions, caused by its transformation into an amorphous liquid mass, without specific structure (Zbesko *et al*., 2018). Similar findings were also reported in Japanese puffer fish infected by *Vibrio harveyi* (Mohi *et al*., 2010). Langer and Ahmad,2017 have reported an increase in the number and size of goblet cells present in the gut of *Labeorohita* fish, caused by infection with *Neoechinorhynchus* sp., and in the epidermis of *Oncorhynchus mykiss.*

CONCLUSION

The behaviour, morphology, and physiology of aquatic organisms are adversely affected by ectoparasites, which lower individual fitness. Parasitic infestations are usually accompanied with the loss in weight and percentage increase in head length of fish. Telangiectasia and aneurism in the secondary gill lamellae, hyperplasia and edema are the most common histological changes induced by those parasites in fish gill. The infected skin reveals eosinophilic infiltration in the integument with muscle fibre atrophy and necrosis in addition to haemorrhages.

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