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RESEARCH ARTICLE

Tissue damage in fish *Johnius dussumieri* infected with *Ichthyophthirius multifiliis* from Karachi coast Pakistan

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Abstract

Tissue damage observations are made on the skin infection of the fish *Johnius dussumieri* infected with *Ichthyophthirius multifiliis*. For this purpose histological sections were prepared by usual technique, stained with haematoxylin and eosin, dehydrated in graded series of alcohols, cleared in clove oil and xylene and mounted permanently in Canada balsam.Photographs were prepared with Nikon (Optiphot-2) photomicroscope using a Fuji colour film. This infection severely damaged the skin tissue, common findings were erosion of outer surface, dislocation of muscle fiber and shrinkage and atrophy which produced large spaces in between the muscles fibers. Hyaline degeneration of muscles fiber was also prominent in some sections. Muscle fibers were severely damaged and fragmented.

INTRODUCTION

Ciliary protozoans are found in large number of fishes. *Ichthyophthirius multifiliis* is one the most prevalent protozoan parasites and is an important pathogen of fresh water and marine fishes. It possess a serious threat for the rapidly growing aquaculture industry.

Ichthyophthiriasis (white spots) is a prevalent ectoparasitic disease of fresh water and marine water fishes, mostly affecting cultured and aquarium fishes. The morbidity rate due to this disease may reach up to 100 % causing great economic losses in fish farms. Ichthyophthiriasis has also been known as sand grain, gravel or ich disease. This dangerous ectoparasite mainly attacks skin, fins, gills and buccal cavity and characterized by the presence of white spots all over the external body surface. White nodules that look like white grains of salt or sugar of up to 1mm appear on the body, fins and gills. Each white spot is an encysted parasite.

Representatives of all major groups of parasitic protozoans use the external surface of the fish integument as an environment. The fish skin offers some protection against parasitic infestations. The protozoan parasites are harmful not only due to their own activity but also because of disseminating bacterial and other infections. Protozoan parasites in fishes damage the surface of the body causing wounds and ulcerations.

Fish skin is composed of two layers, an outer epidermis and inner dermis or corium. The epidermis is ectodermal in origin and consists of several layers of simple cells, of which the outer are being constantly worn away by wear and tear, and replaced by new ones which develops at its base. Layer of cells are composed of flattened cells which are known as stratified epithelium, of which the deepest layer are made up of columnar cells forming the stratum germinativum in which cells are always multiplying by mitotic division to replace the outer worn out cells. A superficial layer of dead horny cells, forming the stratum corneumnot present in fishes which is an adaptation to life in water (Khanna, 1993).

Ichthyophthirius multifiliis may be responsible for lethal disease in marine and fresh water (Fouquet,1876; Kamenev,1961; Calenius 1980 b; Noga, 1980; McCallum, 1982; Ewing et al.,1986; Bilqees,1991b; Bilqees and Haseeb,1995 ; Klesius and Roger,1995; Roberts, 2001; Maki et al. 2001; Kim et al., 2002; Naich and Bilqees, 1997; Ostrow, 2003; Piazz et al.,2006; Osman,2009; Majid et al.,2008; Aydogan et al.,2010; Bichi and Dawaki,2010; Al-Tayyar et al., 2011; Abowei et al., 2011; Poulin et al.,2012; Keremah and Inko-Tariah, 2013; Pirali-kheirabadi et al.,2014; Mhaisen et al.,2015; Baidoo et al.,2015; Adel et al.,2015).

There are several reports on fish skin histopathology of marine and fresh water fishes (Lucky, 1970; Reichenback – Klinke, 1973 ; Hines and Spira, 1973 a-b, 1974 a- b ; Paperna, 1980 ; Miyazaki et al., 1986; Ferraz, 1998 ; Awal, et al., 2001 ; Vogelbein et al. , 2001 ; Cruz – Lacierda and Erazo – Pagador , 2004 ; Zhang, 2004; Elsayed et al., 2006; Adeyemo and Agbede, 2008; Mohammadi et al.,2012).

Material and Methods

Several specimens of *Johnius dussumieri* (Sciaenidae) were collected from fish market of Moosa colony and from West wharf, Karachi. For parasites examination 135 slides were prepared by mucus smear scraping of 50 fishes.

For histological section pieces of infected skin of the selected fishes were fixed in Bouin's solution for 24 hours, then washed several times with 70% alcohol to remove as much as possible the yellow colour of the fixative. This material was dehydrated in graded series of ethyl alcohol (70%, 90% and 100%). The pieces of skin were then transferred to Cedar wood oil for 8 to 12 hours. Cedar wood oil was slowly removed by placing these in a mixture of equal amounts of xylol and cedar wood oil and then into pure xylol for 2 to 4 hours.

Wax blocks were prepared by standard procedure as described by Bilqees (1995). Thin sections of 6-8µ were cut by rotary microtome. Sections were dewaxed, stained with haematoxylin and eosin and mounted permanently in Canda balsam by usual procedure .

Photographs of selected portions of the sections were prepared in support of observations. Agfa colour film was used and photographs were taken with a Nikon (Optiphot - 2) photomicroscope.

Observations

Ichthyophthirius sp. were observed on 40 slides of 9 fishes out of 135 slides of 50 fishes along with other protozoan parasites.

Diagnosis of *Ichthyophthirius multifiliis*

It is rounded or pear-shaped, having two nuclei, meganucleus and micronucleus. It is very dark in color due to the thick cilia covering the entire cell. The characteristic identification of *Ichthyophthirius* is the presence of basophilic horse shoe – shaped macronucleus with vacuolated cytoplasm and adult parasite have C-shaped nucleus. Micronucleus is not clearly visible in most of the parasites. The circular periphery is prominently seen.

Histopathological observations were made on the skin sections of the fish *Johnius dussumieri* infected with *Ichthyophthirius* sp. *Ichthyophthirius* sp. was found mostly in many sections of skin. Skin of *Johnius dussumieri* was damaged by this protozoan parasites. Skin has shown severe tissue damage. The aberrant condition of skin tissue of *Johnius dussumieri* include erosion of outer surface, destruction, separation, dislocation, shrinkage, atrophy and hyaline degeneration of muscles which were important findings. The section of skin has shown severe tissue damage.

Muscle layers were severely affected. Part of skin was in a deformed condition. Large spaces were formed inbetween the muscle fibres. Hyaline degeneration of muscle fibres was a common finding. Both the epidermis and dermis was affected. These layers were not in a normal condition. Shrinkage and atrophy of muscle fibres was obvious. Due to shrinkage muscles were separated from each other resulting into small spaces inbetween.Dissolution of tissue was clearly seen. *Ichthyophthirius* were present in the superficial layers as well as in the deeper layers. Aggregation of protozoans was observed in the form of cysts at the surface of section. Nuclei of muscle fibres were not seen (fig. 3).

The muscle layers were obviously affected loosing the normal morphology. Muscle fibres appeared as homogeneous masses. Large spaces were formed inbetween muscle fibres. A large part of skin was deformed showing penetration of protozoans from surface to deeper layers. The protozoans have destroyed the structure of skin. Normal morphology of skin was totally disturbed. The architecture of epidermis and dermis was lost and not identifiable. Hyaline degeneration and shrinkage of muscle fibres was a common finding with destruction and erosion of outer surface of skin. Muscle fibres were separated from each other with spaces of varying sizes. The infected skin showed severe damage to both epidermis and dermis (fig. 4).

Muscle layers appeared to be severely affected. In most sections nuclei of muscle fibers were not seen. Destruction and erosion of outer surface of skin was observed with large spaces between deeper tissue. In deeper tissue sections of protozoans and surrounding spaces were more obvious. The muscle layers were obviously affected loosing their normal morphology. These appeared as elongated masses of unidentified tissue without any trace of nuclei. Bundles of fibres were fused together leaving small spaces and exhibit hyaline degeneration which was a common finding (fig.5).

Discussion

During the present studies *Ichthyophthirius* was identified on the skin of *J. dussumieri*. Genus I*chthyophthirius* (Fouquet, 1876) was reported previously from various fishes of different countries, mostly on skin and gills of fishes. I*chthyophthirius* cause white spot disease of marine fishes. Body is oval, ciliation uniform, pellicle longitudinally striated, cytostome at anterior end, with a short cytopharynx with cilia. The nucleus is blackish and opaque except when the animal is full of food particles, horseshoe-shaped macronucleus, micronucleus adhering to macronucleus. *Ichthyophthirius* may bore into the body wall from outside and actually acquire the habit of feeding on tissue cells of the attached animal and damages the host tissues.

Ichthyophthirius diseases of minor carp was reported by Hines and Spira(1974).

Paperna (1972) reported infection by *Ichthyophthirius multifiliis* on fish in Uganda for the first time from tropical Africa.

Maki et al. (2001) reported *I. multifilliis* infected fresh water fish world wide caused an economically important disease as “Ich” or “White spot”

Majid et al. (2008) reported an outbreak of white spot disease (‘Ich’) caused by *I. multifiliis* in rainbow trout. Approximately 3, 000 fish died or were killed in a period of 5 weeks. Postmortem examinations were carried out on 12 fish, all of which showed thickened whitish foci on the skin.

Ostrow (2003) observed that *I. multifiliis* is one the most prevalent protozoan parasites of fish and is an important pathogen of ornamental and farm-raised food fish species. White nodules that look like white grains of salt or sugar of up to 1mm appear on the body, fins and gills. Each white spot is an encysted parasite.

Bilqees (1991 b) reported the ciliate *Ichthyophthirius multifiliis* from the skin of fishes *Pseudosciaena diacantus, Otolithus argenteus, Cybium gutatum, Arius serratus* and *Pomadasys olivaceus.*

Protozoans were penetrated between the muscle fibres. Penetration caused severe tissue damage. Noga (2010) observed most protozoan ectoparasites feed only on the epithelium’s surface, but a few such as Ichthyophthirius penetrate into the epithelium. Lesions produced by the parasites may also lead to secondary microbial infections

Cystic spaces were produced due to dissolution of tissues in *O.argenteus*. Zhang (2005) observed white filiform dermal cysts , varing in size (3-20 mm in length and 0.15- 0.35mm in width) in *Silurus meridionalis* by the protozoan sp. infection.

In sections of skin, muscle appear shrinked and exhibit atrophy. Atrophy of tissue resulted into shrinkage and loss of muscle fibers. Pathological changes were observed on the skin of fish *Silurus glanis* caused by *Ichthyophthirius* sp.(Lucky,1970). The parasite *Ichthyophthirius* localized between the epidermis and the corium. After massive infestations and after disintegration of a great part of the fish skin in which the parasite were mostly found to be

localize. Atrophy of mucous cells occurred especially with migration of the parasites through the skin or membrane of the host.

Atrophy of epidermal cells was observed of skin of fish *Carassius auratus* by infection of *I. multifiliis*. He noted that epidermal cells of skin of *Carassius auratus* around the parasite *I. multifiliis* appeared in atrophied condition (Elsayed et. al., 2006). Atrophy and shrinkage was obvious in all sections.

During the present observations some sections indicated dissolution and disintegrationof tissue. Disintegration was observed in the affected tissue of skin of grouper fish by the protozoan sp. infection (Cruz – Lacierda and Erazo – Pagador, 2004).

Mohammadi et al. (2012) observed the epithelial cell erosion and ulceration that has been resulted from the entrance of *I. multifiliis* parasites into skin and exit from the host are probably at least as damaging as its feeding activity while it is on the host.

Conclusion

It is concluded that protozoan infections in the skin of fish cause severe tissue damage. Both the epidermis and dermis is affected. Sloughing off epidermis may occur. Dissolution , atrophy and hyaline degeneration of muscle fibres may result due to protozoan infection.

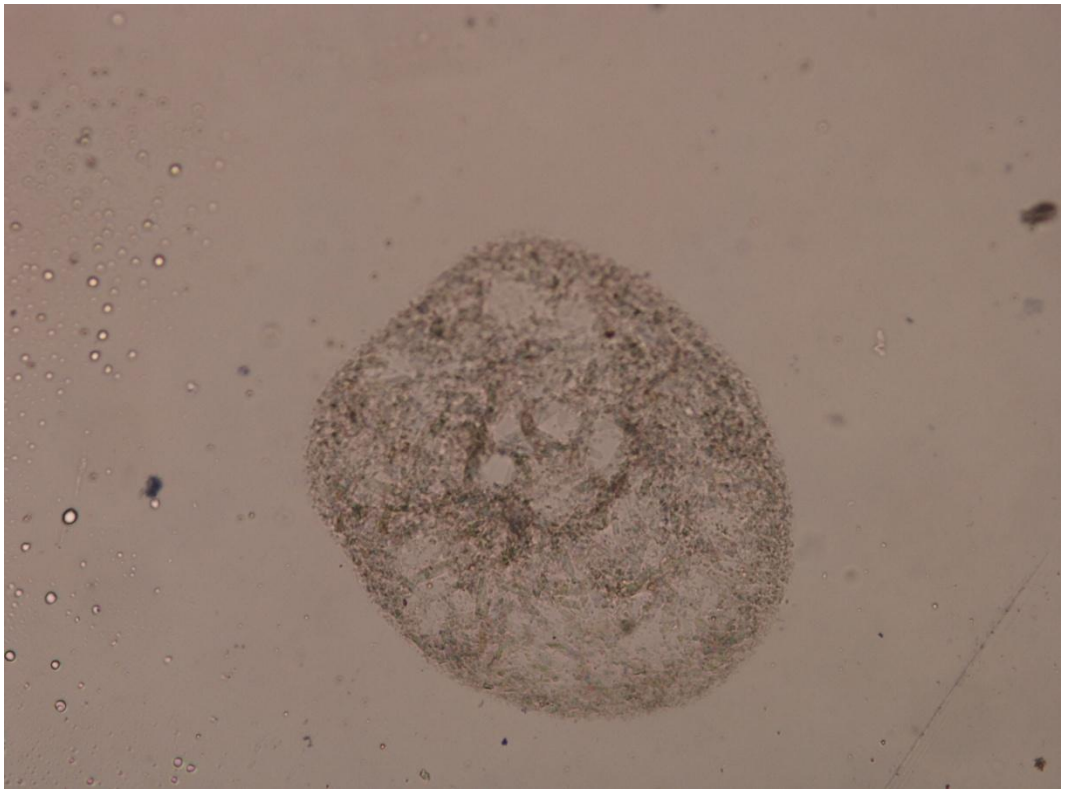

Fig. 1. Smear from skin of *J. dussumieri* showing infection of *Ichthyophthirius* cyst at early stage (10 X 10).

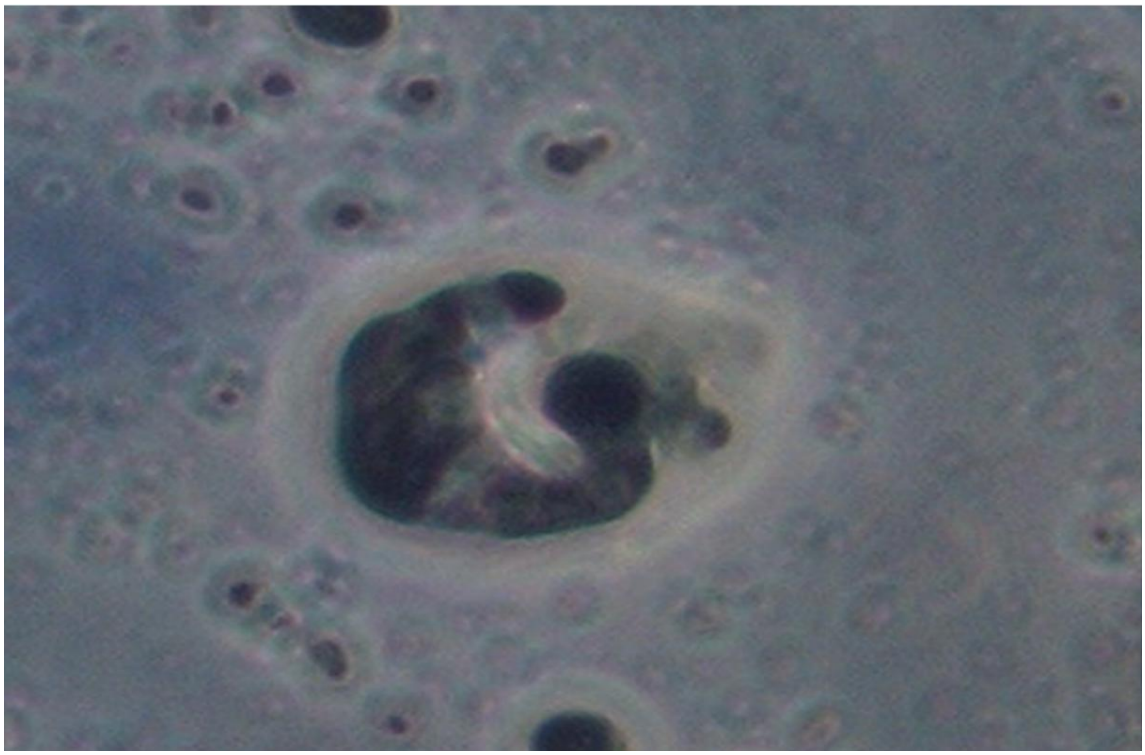

Fig. 2. Smear of skin *J. dussumieri* showing infection of *Ichthyophthirius* species (10 X 10).

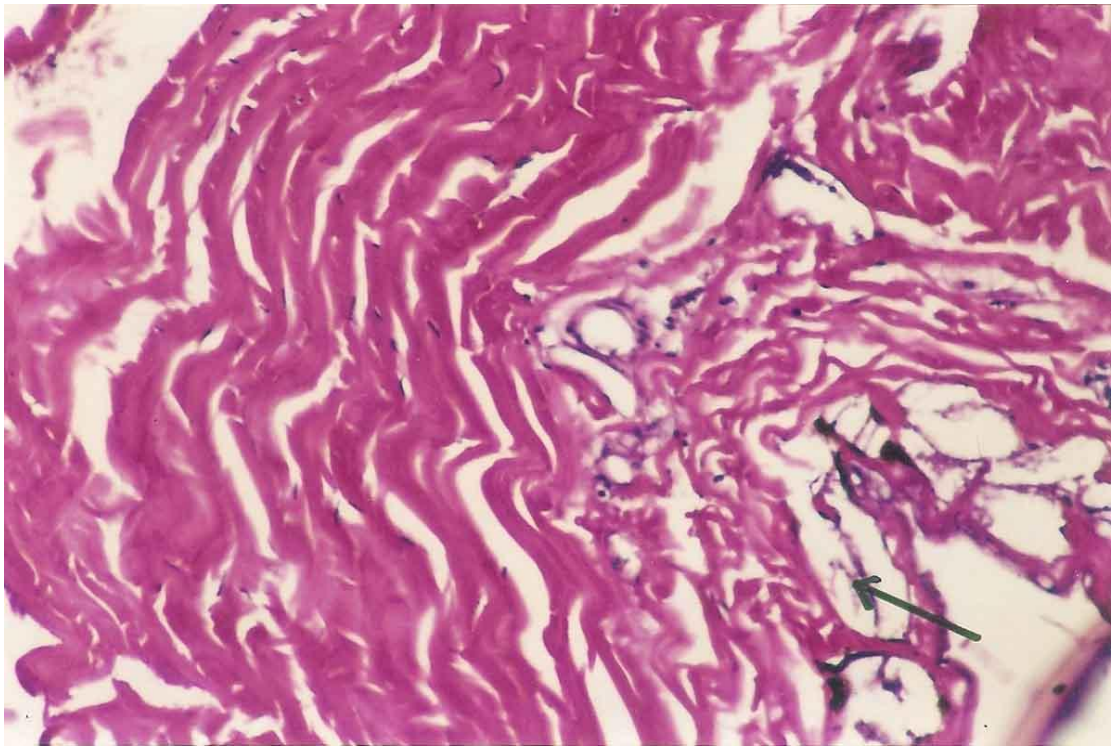

Fig.3.Section of infected skin showing severe tissue damage (on the right) and hyaline degeneration (on the left). A large part of the section is severely affected with breakage of muscle fibers and atrophy. Cystic spaces are produced due to dissolution of tissues (arrow) (X 100).

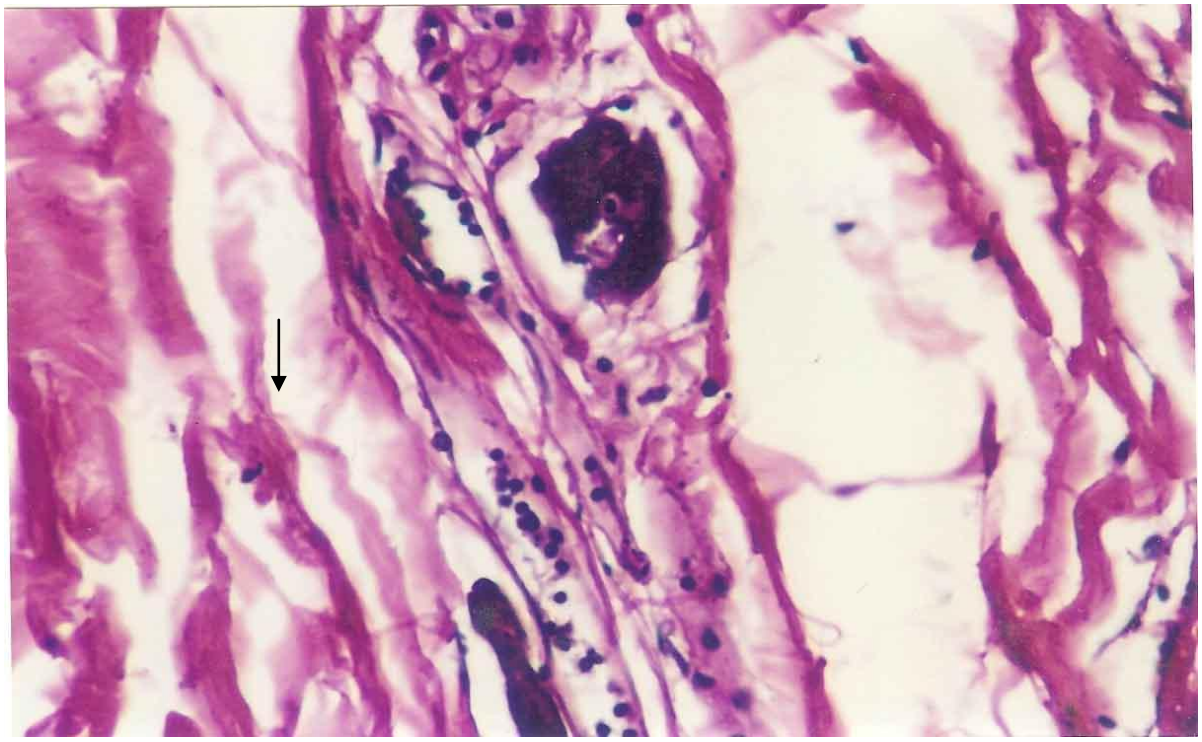

Fig. 4. Section of skin at higher magnification showing severe infection and damage to the skin. Muscle fibres are separated from each other, hyaline degeneration (arrow) and dissolution of muscle fibres is (X 200).

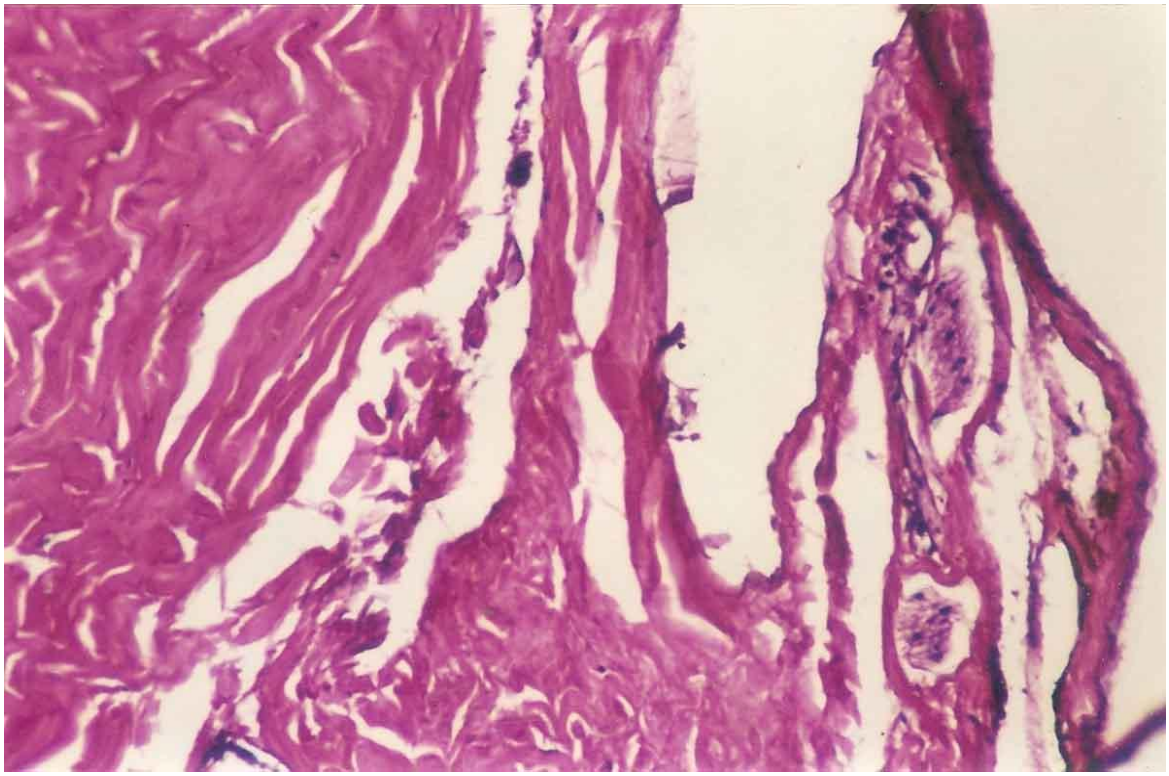

Fig. 5. Infected skin showing destruction of outer surface. Large spaces indicate tissue damage. Muscles appear as homogeneous material (X 200).

References

Abowei. J. F. N., Briyai. O. F.and Bass.S. E.(2011):A review of some basic parasitic diseases in culture fisheries flagellids, dinoflagellids and ichthyophthriasis, coccidiosis, trichodiniasis, helminthesis, hirudinea infestation, crustacean parasites and ciliates. Br. J. Pharmacol. Toxicol., 2(5): 213-226

Adeyemo, A. O. and Agbede, S. A. (2008): Histopathology of *Tilapia* tissues harbouring *Clinostomum tilapiae* parasites. African J. Biomed. Res., 11: 115-118

Adel, M ., Ghasempour, F., Azizi, H. R., Shateri, M. H. and Safian, A. R.(2015): Survey of parasitic fauna of different ornamental freshwater fish species in Iran.VRF, Iran. J. 6(1):75-78.

Al-Tayyar. O. B. A., Abdul Jabaar. W., Raof S.A.and Shather. M. M. (2011):Isolation of *Ichthyophthirius multifiliis*, *Gyrodactylus* and *Copepoda:Lernaeidae* species from six ornamental fish species for the firsttime in Iraq. Al-Anbar J. Vet. Sci. 4(2): 82-87

Awal, M. A., Begum, K. J., Chandra, K. J., Ahmad, G. U. and Kurohmaru, M.(2001): Myxosporidian infection of gills and skin among carp from nursery ponds in Bangladesh: histopathlogy.Vert. Arhiv 71(5): 265-276.

Aydogan, A., Avci, H., and Birincioglu, S. S. (2010): *Ichthyophthirius multifiliis* infection in black tetra (*Gymnocorymbus ternetzi*). Kafkas, Univ.Vet. Fak. Derg. 16(1): 135-137.

Baidoo,K., AbobiBaidoo, S. M ., Agbeko, E.(2015): Ecto-parasites Infestation of Nile Tilapia (Oreochromis niloticus) in Concrete Ponds in Tamale, Ghana, Inter. J. Aquacul., 5(3):1-5

Bichi, A. H. and Dawaki, S. S.(2010): A survey of ectoparasites on the gills, skin and fins of Oreochromis niloticus at Bagauda fish farm,Kano, Nigeria. Bayero J. Pure.Appl. Scien. 3(1):83-86.

Bilqees, F. M. (1991b): Icthyophthiriasis in fishes of Karachi coast Proc. Pakistan Congr. Zoo., 11: 219-223.

Bilqees, F.M. (1995): Histopathology of the liver of *Hilsa ilisha* (Ham.) Proc. Parasitol., 19: 1-20.

Bilqees, F.M. and Haseeb, M. F. (1995): Studies on protozoan parasite of fishes of the family Sciaenidae Proc. Parasitol., 19: 21-28.

Calenius, G. (1980 b): Parasites in fish in finland. V. *Trichophyra, Chilodonella* and *Icthyophthirius* . Acta Acad. Aboensis, Ser. B., 40 (5): 1-8.

Cruz –Lacierda, E.R. and Erazo – Pagdor, G . E. (2004): Parasitic disease. Diseases of cultured Grouper. Southern Asian Fisheries Development Center Aquaculture Department, IIoili, Philippines. 201: 211-217.

Ewing, M. S., Kocan, K. M.and Ewing,. S A.(1986): *Ichthyophthirus multifiliis* Mikhailov, T.K. 1975. Parasites of fish of the Azerbaidzhan water bodies. Akademii Nauk, Izdatelstvo ELM, Baku, USSR. 297 pp.

Ferraz, E. and Sommerville, C. (1998): Pathology of *Piscinoodinium sp*. (Protozoa : Dinoflagellidae), Parasites of the ornamental fresh water cat fishes *Corydoras spp*. and *Brochis splendens* (Pisces : Callichthyidae) Dis. Aqut. Org., 33: 43-49

Fouquet. (1876): Ichthyophiriasis is caused by *I. multifiliis* belong to family Orphryogaenidea. Arch. Zool. Exp., 5: 159-165.

Elsayed, E. E., Ezz El- Dien. N. and Mahmoud, A.M. (2006): Ichthyophthiriasis: various fish susceptibility or presence more than one strain of parasite. Nature and Science. 4(3): 5-13

Hines, R.S. and Spira D.T.(1973a.): Ichthyophthiriasis in the mirror carp *Cyprinus carpio* (L).I. causes of infection. J. Fish Biol., 5: 385-392

Hines, R.S. and Spira D.T.(1973b.): Ichthyophthiriasis in the mirror carp.11. Leucocyte response. J. Fish Biol., 5: 527-534.

Hines, R.S. and Spira D.T.(1974a.): Ichthyophthiriasis in the mirror Carp *Cyprinus Carpio* (L.). 111. Pathology. J. Fish Biol., 6: 189-196.

Hines, R.S. and Spira D.T.(1974b.): Ichthyophthiriasis in the mirror carp *Cyprinus Carpio* (L.). 1V. Physiological dysfunction. J. Fish Biol., 6: 365-371.

Kamenev, V.P. (1961): Parasites of the most important commercial fish in the esturies of the Sea of AZOV.Trudy Sooveshchanii Ikhtiol Komis. Akad. Naur. SSR., 9: 158-162.

Keremah, R. I. and Inko-Tariah, M. B.(2013):Comparative study of ectoparasites on Nile tilapia (Oreochromis niliticus) cultured under integrated and unintegrated pond systems. Afr. J. Biotechno. 12 (19): 2711-2714.

Khanna, S. S. (1993): An introduction to fishes. Central Book Depot. Allahbad, India. 531 pp

Kim, J. H., Hayward, C. J., Jon, S. J., and Heo, G. J. (2002): Parasitic infections in live fresh water tropical fishes imported to Korea. Dis. Aquat. Org. 52, 169-173.

Klesius, P. and Rogers, W. (1995): Parasitisms of catfish and other farm-raised food fish. J. Am. Vet. Med. Assoc. 207: 1473-1478.

Lucky, Z. (1970): Localization of parasites and pathological changes with ichthyophthiriasis of the sheath fish (*Silurus glanis).* Acta. Vet. Brno., Suppl., 1: 39-49

McCallum, H. I. 1982. Infection dynamic of *Ichthyophthirius multifiliis*. Parasitology, 85: 475-488.

Majid, S. E., Gopinath, C. and Jolly, D. W. (2008): Anoutbreak of white spot disease (*Ichthyophthirius multifiliis*) in young fingerling rainbow trout (*Salmo gairdneri* Richardson). J. small Ani. Pract.2008; 25(8): 517-523.

Maki, J. L., Brown, C. C., and Dickerson, H. W. (2001): Occurrence of *Ichthyophthirius multifiliis* within the peritoneal cavities of infected channel catfish *Ictalurus punctatus.* Dis. Aquat. Org., 44: 41-45.

Mhaisen, F. T., Al-Rubaie Abdul-Razzak, L. and Al-Sa'adi, B. A.H. (2015): Ciliophoran and myxozoan parasites of fishes from the Euphrates River at Al-Musaib City, Babylon Province, Mid Iraq. Amer. J. Biol. Life Scien. 3(1): 12-16

Miyazaki, T., Roger, W.A. and Plumb, A. (1986): Histopathological studies on parasitic protozoan diseases of the channel cat fish in the United States. Bull. Fac. Fish., Mie Univ., 13: 1- 9

Mohammadi. F., Mousavi. S. M. and Rezaie .A. (2012):Histopathological study of parasitic infection of skin and gill on Oscar (Astronotus ocellatus) and discus (Symphysodon discus).AACL Bioflux, 5(2): 88-93

Naich, M. and Bilqees, F. M. (1997): Influence of *Ichthyophthirius multifiliis* mucus infection on the growth of rainbow trout *Salmo*

gairdneri (Richardson). Pakistan J. Zool. (4): 411-413.

Noga, E.J. (2010.): Fish disease, diagnosis and treatment. 2th ed. Ames, USA: Wiley Blackwell Publishing. 536 pp.

Osman, H. A. M., El-Bana, L. F., Noor El Deen, A. E. and Abd El-Hady, O. K. (2009): Investigations on white spots disease (Ichthyophthriasis) in cat fish (*Clarias gariepinus*) with special reference to the immune response. Global Veterinaria. 3(2): 113-119.

Ostrow, M. E. (2003): Goldfish. Barron's Educational Series. 79pp.

Paperna, I. (1972): Infectiion by Ichthyophthirius multifiliis of fish in Uganda. Progue Fish, cult., 34(3): 162-164.

Piazz, R. S., Martins, M. L., Guiraldell, L and Yamashita, M. M. (2006): Parasitic diseases of fresh water ornamental fishes commercialized in Florianopolis, Santra Catarina. Brazil. B. Inst. Pesca. Sao. Paulo., 32 (1): 51-57.

Pirali-, E. and Hosseini-Shekarabi, S. P. (2014):Protozoan parasites of some endemic fishes in Bazoft river, Iran. Annu. Biol. Res., 5 (5):11-14

Poulin, S., Mood Sara, M. and Javad, S. (2012): A survey on ectoparasite fauna of cold water fish farms in Mazandaran province, Iran. Global Veterinaria 8(1):101-104

Reichenbach – Klinke, H. H. (1973): Fish pathology, T.H.F. publication, N.J. U.S.A., 512 pp.

Roberts, R. J.(2001): Fish pathology. Third ed, W. B. Saunders. 268-207.

Vogelbein, W. K. , Shicklds, J. D., Hass, L.W. , Reece, K .S. and Zwerner, D. E. (2001): Skin ulcer in Esturine fishes : A comparative pathological evalution of wild and laboratory – exposed fish. Enviromental Health Perspectives Supplements 5, 109 : 1-8

Zhang, Q. and Wang, Z. (2005): *Dermocystium* sp. infection in cultured juvenile southern cat fish *Silurus meridionalis* in China. Dis. Aquat. Org. 65: 245-250