

# Histopathological study of parasitic infestation of skin and gill on Oscar (*Astronotus ocellatus*) and discus (*Symphysodon discus*)

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**Abstract.** Histopathology provides a rapid method to detect effects of irritants and pathogens in different organs and it can be considered as the indicator for abnormal condition for fish environment. The present study was initiated to record the histopathological lesions of gill and skin associated with external parasites in two common aquarium fish, Oscar (*Astronotus ocellatus*) and Discus (*Symphysodon discus*) fish. Twenty Oscar and twenty Discus were obtained from aquarium shops and wet mount was prepared from skin and gill mucosa and histopathological study was performed on tissue samples of gills and skin on tissue sections which were stained with haematoxylin-eosin. Based on the results, *Dactylogyrus* sp. was the most prevalent parasites in Oscar and Discus fish. *Ichthyophthirius multifiliis*, *Trichodina* sp. and *Gyrodactylus* sp., *Epistylis* sp. and *Vorticella* sp. were seen in the skin and gill of fish. In histopathological examination, fusion of secondary lamella associated with hyperplasia, aneurysm, edema, purulent bronchitis were seen. Sections of *Ichthyobodo* sp. and purulent bronchitis are rare and in skin. Dermatitis was observed. Histopathological lesions in Oscar were in high rate in comparison with lesions which were seen in Discus and they are in relation to parasitic infestation.

**Key Words:** histopathology, Oscar fish, Discus fish, gill, skin.

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## Introduction

Ornamental fish culture has rapidly developed in different countries. Parasitic infestation is the most important disease affecting ornamental fish and it causes economical losses for this growing industry in intensive culture systems. Fish may be infected by the parasites as final or intermediate hosts in a parasitic life cycle (Hoffman 1999; Smith & Roberts 2010). Parasites of fish can either be external or internal. Parasitic infections often give an indication of the quality of water, since parasites generally increase in abundance and diversity in more polluted waters (Poulin 1992; Noga 2010). Parasites are capable to cause damage to the fish through injury to the tissues or organs. Fish parasites resulted in economic losses or mortality, treatment expenses, growth reduction during and after outbreak of disease and these interact with expanding of ornamental fish culture. There are no specific pathogonomic clinical signs for parasitic diseases in fish, although a group of clinical signs may be specific for some parasitic infestation (Reavill & Roberts 2007). Most external parasites can be readily identified on direct observation and wet mount preparations. But some parasitic infections need another paraclinical examination to be confirmed. Histopathology provides a rapid method to detect effects of irritants and pathogens in different organs (Johnson *et al* 1993) and it can be considered as the indicator for abnormal condition for fish environment (Roberts 2001). Oscar (*Astronotus ocellatus* (Agassiz,

1831)) and Discus (*Symphysodon discus* Heckel, 1840) belong to Cichlidae family and they have been kept in aquariums for many years and they are considered the “Kings and Queens” of the tank. The present study was initiated to record the histopathological lesions of gill and skin associated with external parasites in these two common aquarium fish, Discus and Oscar.

## Material and Methods

This research was conducted as from april 2010 to may 2011. Twenty Oscar and Twenty Discus were obtained from aquarium shops and transferred to the Fisheries laboratory. Biometry was performed and wet mount was prepared from skin and gill mucosa. For histopathological study, the fish were euthanized and tissue specimens of gills and skin were excised, rinsed in normal saline and fixed in formalin buffer 10% for 24 h. After fixation, the tissues were dehydrated in an alcohol series of ascending concentration (70%, 80%, 90% and 100%, respectively), embedded in paraffin and sectioned at 5 µm. The tissue sections were stained with haematoxylin-eosin (H&E) and were examined by light microscope.

## Results

Table 1 shows the frequency of parasites which were observed on wet mount in Oscar fish and Discus fish. Based on the results, *Dactylogyrus* sp. was the most prevalent in Oscar fish (Figure

1A). Infection with *Ichthyophthirius multifiliis* is approximately prevalent and it was seen in skin and gill of four Oscar fish (Figure 1B). *Trichodina* sp. and *Gyrodactylus* sp. were detected in gill and skin of two fish (Figure 1C). *Epistylis* sp. was observed in the skin and gill of one case (Figure 1D) and *Vorticella* sp. was seen in the skin and gill of one case.

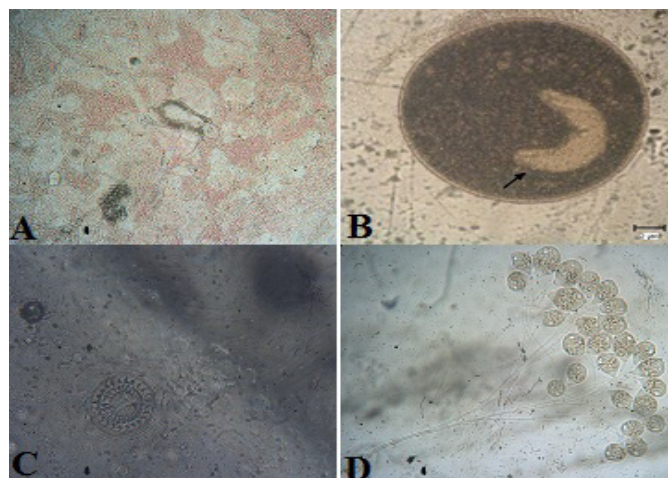


Figure 1: Wet Mount preparation: A) *Dactylogyrus* sp. on the gill, eye spots is clearly visible (Magnification  $\times 40$ ); B) *Ichthyophthirius multifiliis* on skin (Magnification  $\times 40$ ); C) *Trichodina* sp. on skin (Magnification  $\times 40$ ); D) *Epistylis* sp. on skin (Magnification  $\times 40$ )

Based on results which were shown in table 1, in the wet mount of skin and gills of Discus fish different parasites were found, such as *Dactylogyrus* sp., *Trichodina* sp. and *Vorticella* sp.; based on the results, *Dactylogyrus* sp. was the most prevalent parasite in Discus fish (frequency of 35%). *Trichodina* sp. was reported in gill and skin of 25% of Discus fish and *Vorticella* sp. which is a sessile ciliata was found in 10% of Discus fish. Also, there is not any observation for *I. multifiliis*, *Gyrodactylus* sp. and *Epistylis* sp. in Discus fish. In comparison, parasitic infestation in Oscar fish was more prevalent than in Discus fish. Histopathological examination of Oscar and Discus gills and skin revealed different lesions which are summarized in Table 2. Hyperplasia was observed in 95% and 35% of Oscar and Discus gill, respectively, and it was characterized by proliferation of epithelial, connective and inflammatory cells which were accumulated dorsal part of lamella (Figure 2). Aneurism in secondary lamella was detected in 90% and 30% of Oscar and Discus, respectively (Figure 3). Fusion of secondary lamella due to hyperplasia was seen in 55% and 25 % of Oscar and Discus, respectively (Figure 2). Edema was seen in 50% of Oscar and it was characterized by more free spaces between gill structures. Epitheliocystis was basophilic ball shape structures which was attached to endothelial of secondary lamella capillaries (Figure 4). These structures were seen in 35% of Oscar gill. *Ichthyobodo* sp. was observed in two shapes, free with flagella and attached to epithelial cells and without flagella and pear shape (Figure 5). This infestation was seen in 25% and 5% of Oscar and Discus, respectively. Sections of *Dactylogyrus* sp. were seen in 25% of Oscar gills. The attachment place of parasite showed necrosis of lamella (Figure 6). Sections of different stages of *I. multifiliis* have been seen between the lamella and

also penetrated to secondary lamella (Figure 7). These sections were detected in 25% and 10% of Oscar and Discus, respectively. Purulent bronchitis with accumulation of inflammatory cells was distinguished in 10% of both species. Histopathological study of fish skins revealed dermatitis in 15% of Discus fish and 35% of Oscar fish (Figure 8).

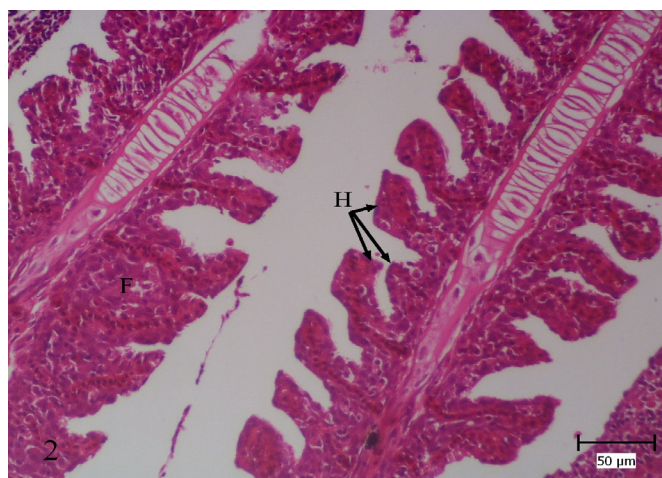


Figure 2. Hyperplasia (H) and fusion of secondary lamella (F) of Oscar fish is obvious (H&E, Bar 50 $\mu$ m).

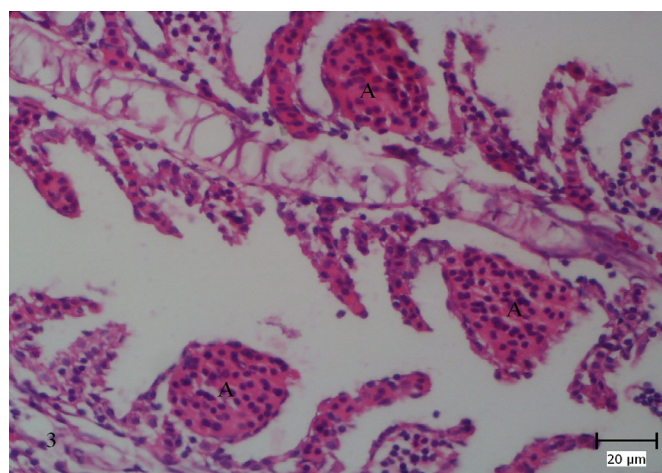


Figure 3. Aneurism (A) in secondary lamella of Oscar fish (H&E, Bar 20 $\mu$ m).

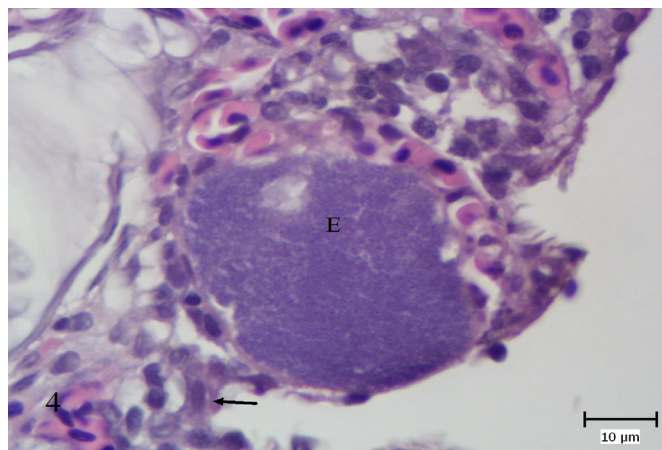


Figure 4. Epitheliocystis in secondary lamella. Note the basophilic ball shape attached to capillaries wall (E) and hyperplasia (arrow) (H&E, Bar 10 $\mu$ m).



Table 1. Frequency of parasitic infestation in gill and skin in Oscar and Discus (%)

Parasite infestation	Oscar gill	Oscar skin	Discus gill	Discus skin
<i>Dactylogyrus</i> sp.	50	20	35	35
<i>Ichthyophthirius multifiliis</i>	20	20	0	0
<i>Gyrodactylus</i> sp.	5	10	0	0
<i>Trichodina</i> sp.	5	10	25	25
<i>Epistylis</i> sp.	5	5	0	0
<i>Vorticella</i> sp.	5	5	10	10

Table 2. Frequency of histopathological effects on gill and skin in Oscar and Discus (%)

Gill and skin histopathological lesion	Oscar frequency	Discus frequency
Hyperplasia	95	35
Aneurism	90	30
Fusion of secondary lamella	55	25
Edema	50	0
Epitheliocystis	35	0
<i>Ichthyobodo</i> sp.	25	5
<i>Dactylogyrus</i> sp. section	25	0
<i>Ichthyophthirius multifiliis</i>	25	10
Purulent bronchitis	10	10
Dermatitis	35	15

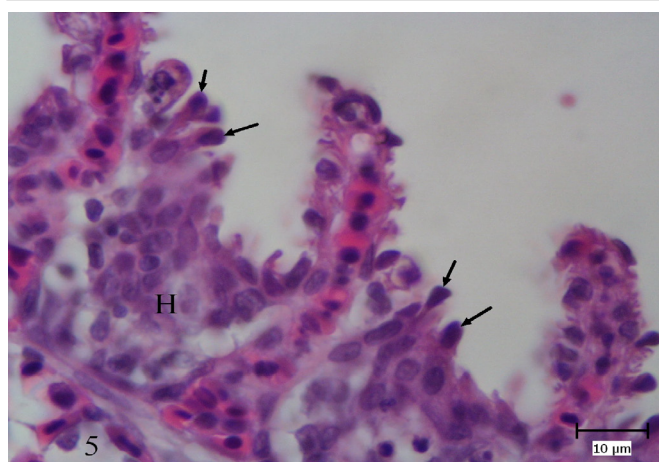
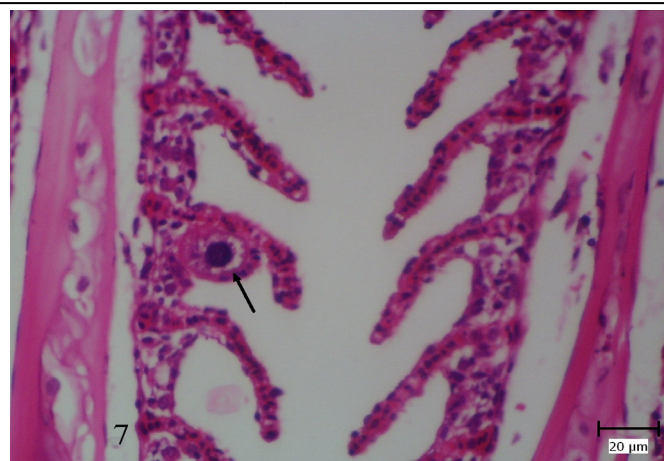
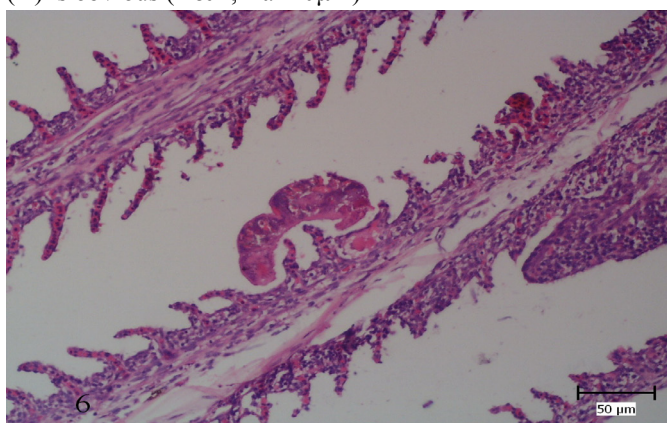
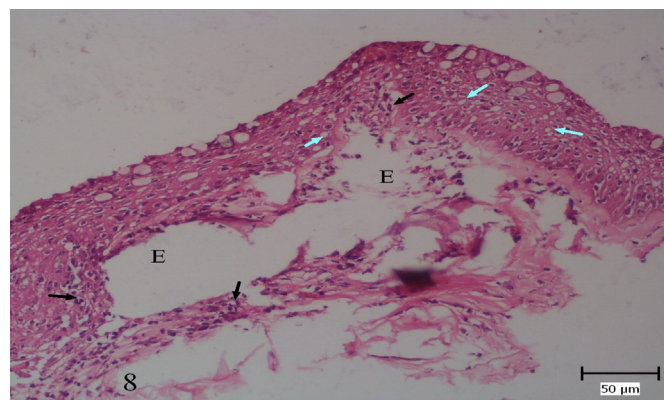
Figure 5. *Ichthyobodo* sp. attached to epithelial cells of secondary lamella (arrows) and they are pear shape. Also hyperplasia (H) is obvious (H&E, Bar 10µm)Figure 7. Trophont stage of *I. multifiliis* (arrows) in Oscar fish (H&E, Bar 20µm)Figure 6. Section of *Dactylogyrus* sp. in gill of Oscar. Note the necrosis of attachment place of parasite (H&E, Bar 50µm)

Figure 8. Dermatitis in Oscar. Note to dermal edema and vacuoles in epidermal cells (blue arrows) and penetration of inflammatory cells between epithelial and dermal cells (black arrows) (H&amp;E, Bar 50µm)

## Discussions

Fish gills participate in many important functions such as respiration, osmoregulation and excretion and remain in close contact with external environment and it is particularly sensitive to changes in the quality of the water (Mazon *et al* 2003; Camargo & Martinez 2007). Gills are generally considered as a good indicator for water quality and it will be a model for studies of environmental impacts.

In the current study, *Dactylogyrus* sp. was found as the most common parasite in Oscar and Discus fish. Jalali & Barzegar (2005) reported that this parasite is commonly seen in fresh water fish in intensive culture. *Dactylogyrus* sp. was found in two Cyprinidae fish (*Alburnus alburnus* and *Carassius carassius*) in freshwaters from Turkey (Koyun 2011). The oviparous dactylogyrids are primarily gill parasites of freshwater fish but they may be seen in skin of freshwater fish (Noga 2010). The fish infected with dactylogyrus showed clinical symptoms including the lethargy, unilateral swimming and erosion on gill filament and scale loss (Longshaw & Feist 2001; Baker *et al* 2007). Gill filament fusion, secondary filament hyperplasia and aneurism were reported in fishes which were infected by *Dactylogyrus* sp. after Jalali & Barzegar (2005), which is in agreement with results obtained from this study. Infection with *Gyrodactylus* sp. was only seen in Oscar fish. The viviparous gyrodactylids are skin and gill parasites of both freshwater and marine fish. Various species of *Gyrodactylus* are pathogenic to Eels, Salmonids, Cyprinids, Ictalurids, Cichlids and Pleuronectids (Noga 2010). Histopathological lesions of *Gyrodactylus* sp. on gill and skin are the same as *Dactylogyrus* sp. (Jalali & Barzegar 2005; Noga 2010).

*I. multifiliis* was observed in 20% of Oscar fish but was not found in Discus fish. Ich is one of the most common diseases of freshwater fish and virtually all freshwater fish are susceptible to infection and up to 100% mortality may occur (Noga 2010). Stress plays a major role in Ich epidemics. Outbreaks are also more severe at high fish density. In current study, all infected fish showed white nodules on body and some of them showed lethargy, increased mucus production and gill filaments hyperemia which is in agreement to results of Smith & Roberts (2010). The epithelial cell erosion and ulceration that has been resulted from the entrance of parasites into skin and exit from the host are probably at least as damaging as its feeding activity while it is on the host. Lesions produced by the parasites may also lead to secondary microbial infections (Noga 2010). Dermatitis and purulent bronchitis which were seen in this study may be affected by Ich infection in Oscar and Discus.

*Trichodina* sp. was also found in Oscar and Discus fish. Clinical signs due to infestation by *Trichodina* sp. were scale loss and lethargy. Noga (2010) and Baker *et al* (2007) found lethargy in fishes that were infected with *Trichodina* sp.. Many trichodinid species infest marine and freshwater fish (Noga 2010, Thilakarante 2003). They have a similar morphology which includes cilia, a circular shape and a ring of hook-like denticles. The parasites are commonly found on skin and gills of fish from waters with high organic loads. In a heavily infected fish, their adherence and suction on the epithelium may cause enough damage to produce the clinical signs of anorexia and weight loss (Noga 2010).

*Epistylis* sp. and *Vorticella* sp. were found in both Oscar and Discus fish. Saglam & Sarieyyupoglu (2002) reported *Epistylis* on

fresh water leech. *Epistylis* sp. and the related species *Vorticella* sp., are sessile and stalked ciliated protozoans generally found attached to vegetation or crustaceans (Saglam & Sarieyyupoglu 2002). In high organic water, they proliferate and attach to fish and eggs. They frequently affect goldfish and many species of bottom-dwelling freshwater fish. *Epistylis* sp. first appeared on the tips of both dorsal and pectoral fin spines. Then, *Epistylis* colonies spread down the spines and eventually covered much of the anterior region of the body. Epithelial tissue and underlying bone are eroded away at the site of attachment. Both have been isolated from ulcerated areas in association with *Aeromonas* bacteria (Noga 2010). The infected fish showed lethargy and erosion on gill filament. The infected fish were seen with other parasites in this study, so it is suggested that the symptoms appeared in this fish, are mainly caused by other parasites. However this ciliophora is potentially harmful if it is in large number especially to gill tissue where gas exchange may be impeded by the large numbers of parasites physically covering the gills. It is not known to kill or damage fishes, but may slightly reduce their productivity (Bunkley Williams & Williams 1994).

The histopathological lesions which were observed in this study include lamellar fusion, hyperplasia, purulent bronchitis and aneurysm. Some alternations in blood vessel may occur when fishes suffer from severe types of stress. In this case, damaged pillar cells can result in an increased blood flow inside the lamellae and cause blood congestion or even an aneurysm (Rosety-Rodriguez *et al* 2002). Hyperplasia of epithelial cells and fusion of some secondary lamella are examples of defense mechanisms of gills.

Purulent bronchitis was found in 10% of Oscar and Discus fish. This is a chronic alteration and in this case, a lot of inflammatory cells (lymphocytes and macrophages) can be found among lamella (Ferguson *et al* 2006). Especially, eosinophilic granular cells were seen. These granular cells were similar to submucosal layer cells of intestine. These types of cells increase in chronic diseases and maybe are inflammatory cells. Secretion of these cells in the gills justified that these are destruction and corruption response to different chemicals and pathogens (Kantham & Richards 1995).

Edema was found in 50% of Oscar fish. This alteration usually occurs when the environment is not normal (Roberts 2001). By removing of fish pathogens from gill and supplying better environmental condition, severe lamellar edema may be treated (Nero *et al* 2006; Schlacher *et al* 2007).

In this study, epitheliocystis was found in 35% of Oscar fish. Hyperplasia and aneurism were observed in the gill tissue of all of these infected cases. Epitheliocystis disease is a common condition that has been observed in various teleosts as an incidental finding (Bradley *et al* 1988; Turnbull 1993; Groff *et al* 1996; Polkinghorne *et al* 2010). This incidental form of the disease has been characterized as a nonlethal or chronic condition because of the apparent mild infection and associated host response that is typically absent or limited to a mild epithelial hyperplasia (Roberts 2001; Noga 2010; Polkinghorne *et al* 2010). A severe form of the disease has also been documented and characterized as a hyperinfection because of severe infection and the associated host response, which has been described as a diffuse, severe, proliferative epithelial hyperplasia often with branchial lamellar fusion and excessive mucus production (Bradley *et al* 1988; Groff *et al* 1996). Progression from the chronic to the proliferative form of the disease may

be secondary to stressful conditions, such as low temperature (Crespo et al 1990).

Epitheliocystis histologically affected chloride and epithelial cells and caused the single cell swelling (Bradley et al 1988). In the most samples that infected with Epitheliocystis, eosinophilic granular cells were observed, that is compatible with results reported by Groff et al (1996) on *Acipenser transmontanus* Richardson, 1836.

Infestation with *Ichthyobodo* sp. was 25% in Oscar fish and 5% in Discus fish. Infestation with *Ichthyobodo* sp. caused hyperplasia of epithelial cells and fusion of secondary lamella. In almost all of infected fish with *Ichthyobodo* sp., hyperplasia, fusion and aneurism were observed (Molnar & Baska 1993; Noga 2010). Dermatitis was the histopathological alterations found in skin of fish. The skin lesions are common in fish and the fish skin is constantly exposed to the water environment and external environment, is susceptible to the toxins, environmental pollution and pathogens (Abalaka et al 2010).

## Conclusions

Histopathological lesions in Oscar were in high rate in comparison with lesions which were seen in Discus and they are in relation to parasitic infestation.

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