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Histopathological and Behavioral Changes in *Oreochromis* sp. after Exposure to Different Salinities

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Abstract

A study was carried out in the laboratory on the adaptability and tolerance of the Tilapia *Oreochromis* sp. to different salinities. This data has provided important information on the possibility of its culture in marine environment or brackish water. We investigated the histopathological changes and behavioral changes of the challenged with four different salinity treatments including a control (0, 5, 20 and 35 ppt) for 96 hours. The Tilapia with the size 10-14 cm total length acclimated successfully to freshwater before introduced to hyper-saline environment. The results showed that all survive in 0 ppt and 5 ppt, while 75% death in 20 ppt and 100% death in 35 ppt. The mortality rate was increased with increased of salinity. Fish exposed to different salinities exhibited clinical signs agitated behavior, respiratory distress, abnormal nervous behavior and death were recorded. Degeneration, necrosis, hemorrhage and hyperplasia of kidney and gills were observed as major histopathological changes.

Keywords: *Oreochromis* sp.; Histopathology; Behavior; Salinity

Introduction

Tilapia, *Oreochromis* sp is an economically important fish cultured in Southeast Asia and is in high demand in Malaysia. To date, the consumption of saltwater tilapia fish has been increased because of their tasty flesh and not too strong fishy taste rather than freshwater tilapia. Tilapias are popular cultured species because of their high environmental tolerant characteristics. The rapid growth of tilapia, their resistance to poor quality, ability to grow under sub-optimal nutritional conditions, and high fecundity, all make them well suited for aquaculture Lawson [1]. Moreover, Tilapia is a good candidate for aquaculture especially in developing countries where there are high levels of animal protein deficiencies. The industry has a good potential to build up in Malaysia based on the local and Asia market. Besides, the saltwater Tilapia are capable to survive in the low salinity (<10 ppt) but the conditions may be stressful to the fish. The salinity stress responses are change behavior of the fish Claudia [2], growth rate of Tilapia Lawson [1] increases oxygen consumption and decreases specific growth rate El-Dahhar [3]. Baroiller et al. [4] suggested that the salinity 37 ppt to 40 ppt might not suitable for Tilapia culture. There are some species of Tilapia are potentially culture in brackish water farming such as *Oreochromis niloticus, Oreochromis mossambicus, Oreochromis aureus, Oreochromis spilurus, Oreochromis hornorum, Sarothherodon melanotheron* and hybrid red tilapia Dennis et al. [5].

The sudden salinity changes may impact the physiological condition of the fish and the tolerance limits of the fish will cause stress and lead to decrease the immune system level. Rearing Tilapia in saltwater could have no different between freshwater. The Tilapia appeared normal and healthy through the external observation in saltwater but the level of stress with the sudden introduced to different salinity level unknown. Hence, to get the better understanding of this situation, this present study was to elucidate effects of salinity on the behavior and histopathological changes of the fish. The severity of internal organs lesions were reflected with the behavior of the fish.

Materials and Methods

Fish

The Tilapia fish (*Oreochromis* sp.) were obtained from Terengganu and was examined live. They were transferred to the laboratory at University Malaysia Terengganu and kept in aerated aquaria containing freshwater. Sixteen fish of Tilapia with 10-14 cm total length (TL) were used for the experiment. The fish were acclimatized in laboratory conditions for a few weeks during which they were fed with commercial fish pellets.

Salinity tolerance experimental

For the salinity tolerance experiment, the tanks were set up by filling the tank (33.0 cm x 15.2 cm x 20.3 cm) with ½ of water. The different salinity of water were prepared include; 0 (control), 5 ppt, 20 ppt and 35 ppt. The observation was started by placing 4 tilapia fish randomly in each tank with the same range of size and 2 replicate in each aquarium containing different salinity as well as in the control. No water change and feeding throughout the experiment. There were four tilapia transferred from freshwater directly into salinities of 0 (control), 5 ppt, 10 ppt, 20 ppt and 35 ppt and hold for a period of seven days at room temperature. At the beginning, the test was checked hourly and the data were taken for the first 8 hours, then daily for the next 3 days. The death of fish were recorded when the opercula movement and the tail beat stopped and the fish no longer responded to mechanical stimulus. The observed dead fish were removed from the water in time to avoid the deterioration of the water quality.

Behavior observation

Tilapia fish were exposed to the different salinity. The abnormal stress behaviors are observed by visual assesmently as suggested by Aysel [6]. Behavioral responses of fish such as convulsions, equilibrium status or imbalance, fin movement, hyperactivity and swimming rate were observed as suggested [6,7]. The behavioral response of the tilapia was conducted at 1–8 hours, and every 12 hours during the salinity stress test.

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Histopathological study

After examination of behavior, the organs such as gills and kidney from the dead fish were sampled and fixed in 10% formalin. After 96 hours, all the fish were killed and organs such as gills and kidney were taken and fixed in 10% formalin. The tissue samples were prepared by using the standard methods for histopathological techniques. After that, the tissue were stained with Haematoxylin and Eosin and observed under light microscope and histopathological was evaluated and described.

Results

Effects of salinity on the behavioral responses

The fish exhibited a normal response with no mortality when treated between 0 ppt and 5 ppt salinity. Various levels of response such as restlessness or hyper activeness or erratic behavior were displayed on salinities 20 ppt and 35 ppt. *Oreochromis* sp. exposed to high salinities exhibited signs of agitated behaviors, respiratory distress and abnormal nervous behaviors (Table 1-3). At first 3 hours throughout the test on 20 ppt and 35 ppt, the fish showed in initial frequent surface to bottom, aggression and sometime tried to jump out of the aquarium. They also expressed highly increased opercular movements which indicating respiratory distress and accompanied by excessive secretion of mucus Anur [8]. Abnormal nervous behaviors such as sudden darts, state of motionless and different postures were observed. The fish were become very weak, settle at bottom and died. After 96 hours, the behavior of the control fish were normal and no mortality was recorded (Figure 1). The exhibited signs and mortality rate increased with increased of salinity level. Fish exposed to the 20 ppt and 35 ppt showed 75% and 100% mortality respectively as shown in Table 4.

Clinical signs	Salinity (ppt)			
	0	5	20	35
Aggression	–	+	+	+++
Jumping	–	–	++	+++
Stunned posture	–	+	++	+++
FSBM	–	–	++	+++
Erratic swimming	–	+	++	+++

Frequent Surface to Bottom Movements (FSBM), None (-), Weak (+), Moderate (++) and Strong (+++)

Table 1: Agitated behaviors after exposure 96 hours.

Clinical signs	Salinity (ppt)			
	0	5	20	35
Opercula movement	–	–	+	+++
Air gulping	–	–	++	+++
VPES	–	–	++	+++
EMS	–	+	+++	+++

Vertical Posture with Exposed Snouts (VPES), Excessive Mucus Secretion (EMS), None (-), Weak (+), Moderate (++) and Strong (+++)

Table 2: Respiratory distress after exposure 96 hours.

Clinical signs	Salinity (ppt)			
	0	5	20	35
SSM	–	–	++	++
State of motionless	–	–	++	++
Sudden darts	–	–	+	+++
DP	–	–	+++	+++
Death	–	–	++	+++

Sluggish and Swirling Movements (SSM), Different Postures (DP), None (-), Weak (+), Moderate (++) and Strong (+++)

Table 3: Abnormal nervous behavior after exposure 96 hours.

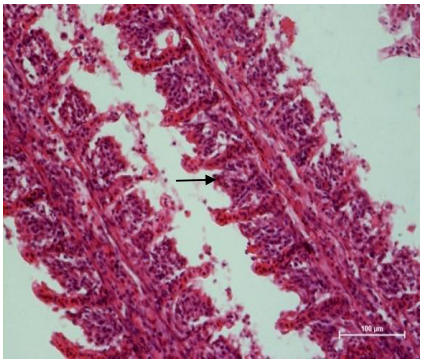

Figure 1: Gill structure of Tilapia, *Oreochromis* sp. exposed directly 20 ppt the gills showed lamellar fusion (arrow) after exposure 96 hours. (H & E stain, x40 objective).

Concentration (ppt)	Numbers	12 h	24 h	48 h	72 h	96 h	Total of	Percentage %
0	4	0	0	0	0	0	0	0
5	4	0	0	0	0	0	0	0
20	4	0	0	1	2	0	3	75
35	4	4	-	-	-	-	4	100
Total of death	4		0	1	2	1	7	

Table 4: Mortality rate of *Oreochromis* sp. on exposure to salinity per treatment.

Result/Organs	Gills	Kidney
0ppt	Normal	Normal Slightly hydropic degeneration
5ppt	Hyperplasia of the epithelium	Hydropic degeneration Narrowing tubular lumen
20ppt	Hyperplasia Lamellar fusion	Tubular necrosis Edema in Bowman's capsule Hydropic degeneration Haemorrhage
35ppt	Hyperplasia Lamellar fusion Necrosis	Gross lesion-Kidney very fragile (sample not taken)-necrosis

Table 5: The histopathological responses of *Oreochromis* sp. to different salinity level for 96 hours.

Effects of salinity on the histopathological responses

The gills sections from the tilapia, *Oreochromis* sp. in the control group presented a normal appearance and not reveal any histopathological lesions in the tissues. The histological lesions were occurred severely with increased of the salinity (Table 5). Histopathological lesions observed in the gills of the fish exposed to the salinity showed different lesions which include from hyperplasia of the epithelium, fusion of secondary lamellae and necrosis. At 5 ppt salinity, the gills were start to change but with slightly changes. For the 20 ppt salinity, the fish was started showed the severely affecting of the gills which appearance on hyperplasia and fusion of lamellae while at 35 ppt salinity it showed a very severe lesion which necrosis of the tissue was appear (Figure 2).

The histopathology of control fish kidney showed normal appearance of glomerulus but slightly degeneration on the renal tubules. There were mild lesions with low salinity at the 5 ppt where the hydropic degeneration and narrowing tubular lumen was appearing.

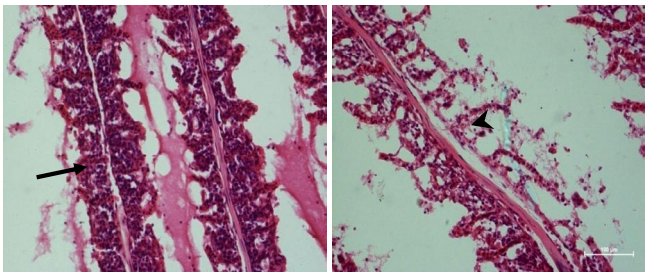

Figure 2: Gill structure of Tilapia, *Oreochromis* sp. exposed to 35 ppt salinity for 96 hours, showed severe lamellar hyperplasia, lamellar fusion (arrow) and necrosis (arrow head). (H & E stain, x40 objective).

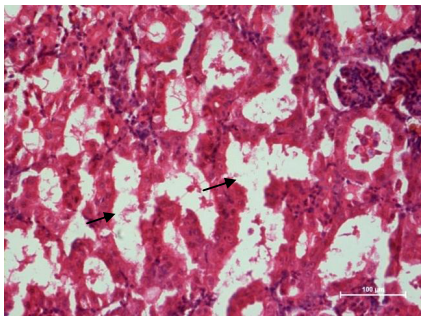

Figure 3: Histopathological appearance of the kidney tissue structure of Tilapia, *Oreochromis* sp., after exposure 96 hours directly in 20ppt the kidney showed tubular necrosis (arrow). (H & E stain, x40 objective).

The histopathological lesions were severely observed in high salinity between 20 ppt and 35 ppt such as hydropic degeneration, edema in Bowman's capsule, hemorrhage and necrosis into the surrounding tissue (Figure 3). At 35 ppt salinity it showed very severe lesions where the necrosis was observed in the kidney. Some of the kidney samples exposed to 35 ppt were not taken because it was very fragile and difficult to observe by histopathologicaly. Gross appearance from the dead fish, it was swollen, hemorrhage and soft.

Discussion

From the investigations of this study, it is proved Tilapia is tolerated with 0 ppt and 5 ppt salinity. In both salinity of 0 ppt and 5 ppt, normal behavior and no mortality were recorded. However, in 5 ppt salinity there was only a weak of excessive mucus secretion was observed on the fish. This is an indication that the fish were perfectly able to regulate their body physiology within this salinity. There were 100% mortality recorded in 35 ppt salinity, indicates that the fish has developed osmoregulatory failure. According to Deacon and Hecht [9] the mortality observed after 24 hours the fish exposed to 20 ppt salinity could be consequence of the progressive deterioration in the osmotic and ionic regulatory mechanisms including the fish inability to control the excessive water loss, leading to osmoregulatory exhaustion, collapse and finally death. In another study Lawson [1], the mortality was due to stress, duress and less resistance of the fish to this salinity. There was no mortality found between 0 ppt and 5 ppt which indicated the fish were able to withstand a wide salinity range. According to [10,11] the survival of the fish depends on the ability of the body fluids to function at least for short time in an abnormal range of abnormal internal osmotic and ionic concentrations. The fish can regulate the body fluid to restore the level of osmotic pressure to near normal. The migration or abruptly transferred of fish from freshwater to seawater will normally lead to increase osmotic concentration of fish blood serum and change in ionic contents [12,13].

The restlessness or erratic behavior in high salinities indicates the fish were approaching their tolerance limits and loss of water at fast rate to external medium from the fish [1]. This also may be due to biochemical body derangement including hepatic compromise Fadina [14]. The fish exposed to different salinity exhibited changes in behavior such as convulsions, equilibrium status or imbalance, fin movement, hyperactivity and swimming rate were observed in this study. From the present study, respiratory distress such as increase opercular activities, gulping of air, vertical movement and excessive mucus was recorded in 20 ppt and 35 ppt. For increased opercular frequency activities has been reported as an adaptive mechanism to hyper saline environments [15-18]. This sign is may be due to excessive mucus secretions because the mucus on the gills reduces respiratory activity in fishes and unable for fish to actively carry out gaseous exchange [8,19].

The progressive decrease in the opercular frequency led to swimming close to the water surface in order to increase the oxygen intake in the water surface Soares [20]. The increase of the salinity concentration interrupted the respiratory system in the fish and caused lamellar hyperplasia Reid [21]. The severe lesions in gill were observed in fish exposed to 35 ppt are hyperplasia, lamellar fusion and necrosis. This is in accord with Mallat [22] who concluded that the most common gill lesions which induced by toxic substances and other chemicals are necrosis, hyperplasia and lamellar fusion. A higher salinity in the water produce lamellar lesions such as necrosis which lead to death after exposed the fish to 35 ppt salinity. The degeneration of gills also causes a dysfunction of fish gas exchange ability causing an anoxic internal behavior Ajani [23].

Direct transfer from freshwater to higher salinity conditions cause the fish a strong respiratory distress. This can be showed by histopathological observation when the fish were exposed at 35 ppt salinity exhibited a severe lesion on the gills such as hyperplasia, lamellar fusion and necrosis. This indicates that the fish were approaching their tolerate limits and developed osmoregulatory failure [1]. A higher salinity and toxic in the water produced severe lesions on the gills and kidney such as severe hydropic degeneration, edema and necrosis which lead to mortality Yahona [24]. Several abnormal nervous behaviors such as sluggish and swirling movements with different postures, state of motionless, sudden darts and death may cause by the failure of the kidney function where the histopathological lesions were observed on the fish kidney Oti [25]. The lesions of the kidney tissue are including hydropic degeneration, edema in Bowman's capsule, hemorrhage and necrosis. The histopathology changes in kidney lead to mild of hydropic degeneration of renal tubules when the fish were exposed to 5 ppt salinity. In 20 ppt, the *Oreochromis* sp. started to show severe lesions of the kidney while in the 35 ppt salinity; gross necrosis was found on the fish. Histopathological changes in fish tissue and residue levels of test substances in fish are very important parameter for deriving the maximum acceptable concentration of chemicals in the context of fish culture requirements Svobodova [26].

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