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Trend in Ammonia Excretion during Acclimatization of Adult Freshwater Red Hybrid Tilapia *Oreochromis mossambicus* (Peters, 1852) X *Oreochromis niloticus* (Linnaeus, 1758) in Different Salinities

P.J. Pradeep, T.C. Srijaya, A. Hussain, S. Mithun, F. Shaharom and A. Chatterji

Institute of Tropical Aquaculture, University Malaysia Terengganu, Mengabang Telipot, 21030, Kuala Terengganu, Malaysia
E-mail: *legendofmortals@yahoo.com*

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Abstract
An attempt was made to study the trend in ammonia excretion by red hybrid tilapia during their acclimatization in seawater under slow and sudden conditions. Acclimatization of salinity 5 ppt showed an increase of ammonia level in water by 83.35% (7.69 mg/kg/l/day) as compared to the control group ($P<0.05$) whereas the value was 24.97% (10.25 mg/kg/l/day) at 10 ppt. The level of ammonia was dropped at salinity 15 ppt (5.12 mg/kg/l/day) by 50% and 25% at 20 ppt (3.84 mg/kg/l/day). The ammonia level with sudden transfer to different salinities were; 1.94, 1.38 and 2.77 mg/kg/l at 10, 20 and 30 ppt respectively, after 2 hrs of acclimatization whereas it reached to 11.11 mg/kg/l at 10 and 20 ppt within 24 hrs. All fishes were found dead within 3 hours of their sudden transfer to 30 ppt. The Results suggest that a gradual salinity increase is beneficial for acclimatizing marine tilapia.

Key words: Ammonia excretion, salinity, acclimatization, red hybrid tilapia, euryhaline fishes

Introduction

Tilapias, the native fish from Africa, are one of the world's most commercially important euryhaline species suitable for intensive mariculture. During their adaptation to sea water, tilapia is well known for their readjustment of several physiological and biochemical processes (Jurss *et al*., 1984). All teleostean fishes including tilapia maintain an internal hypo-osmotic environment to seawater and therefore, when they are acclimated to salt water they maintain this balance through osmotic water loss and diffusive ion gains (Payan *et al*., 1984; Sardella *et al*., 2008). However, such process has been found to show an increase in drinking rate, the Na^+ exchange and the net outward transport of NaCl at the level of the branchial mitochondrial-rich cells (Jurss *et al*., 1984; Payan *et al*., 1984; Marshall, 2002; Sardella *et al*., 2008). Accordingly in this process of more water intake, fishes normally excrete higher urea and ammonia contents along with their urine. In fact, the un-ionized form of ammonia is the most toxic form to aquatic organisms as it can readily diffuse through cell membrane which is highly soluble in water (Randall and Tsui, 2002). Moreover the excess amount of ammonia in water can cause impairment of cerebral energy metabolism, damage to gill, liver, kidney, spleen and thyroid tissue in fish, crustaceans and mollusks (Smart, 1978; Wilkie, 2002; El-Sherif and El-Feky, 2008). In fishes ammonia production mostly occurs in the liver from the transamination and subsequent deamination of excess of 1-amino acids (Wilkie, 2002). It may also originate in the muscle due to deamination of adenylates by exercising fish that causes decrease in dissolved oxygen concentrations.

The ionic regulation of freshwater invertebrates has been explained on a biochemical basis by studying the effects of salinity acclimation on nitrogen metabolism (Okuma and Abe, 1994; Huong *et al*., 2001; Sadok *et al.,* 2004). However, there is a paucity of information regarding the effects of salinity variations on nitrogen metabolism and excretion of ammonia in both fresh water and euryhaline fishes (Frick and Wright, 2002; Sadok *et al.,* 2004). Hence the present study was aimed to determine the level of ammonia excretion in red hybrid tilapia during their acclimatization from fresh water to marine water.

Materials and methods

Live adult specimens of red hybrid tilapia *Oreochromis mossambicus* (Peters, 1852) X *Oreochromis niloticus* (Linnaeus, 1758) were procured from the cage culture farm of Tasik Kenyir, Malaysia. Fishes were conditioned for 1 week inside a cement holding tanks of 5000 liter and fed with feed consisting of 43% protein (ASEAN Marine Fish Feed, Ltd). Fishes were fed at the rate of 4% of the total biomass once in a day. Three aquarium tanks (capacity: 50 liter) with 5 ppt saline water were selected and in each tank one specimen of tilapia (weight~390±4 g) was transferred from the holding tank. The aquarium tanks were provided with adequate aeration. These fishes were slowly started acclimatizing in seawater and to increase the salinity from 5 ppt onward, commercial salt (Merck, Germany) was used. The salinity of water in the treatment group was increased by 5 ppt and fishes were allowed to acclimatize in that particular salinity for three days. Another group of triplicates maintained under normal fresh water (0 ppt) was considered as control groups. An attempt was also made to examine the direct exposure of fishes to different salinities (10, 20, 30 ppt) in three separate aquarium tanks without gradual acclimatization.

In all experimental tanks, 100% water exchange was given daily during the entire experimental periods. Before water exchange, the water samples were taken for measuring ammonia concentration following Phenate-hypochlorite method as described by Berthelot (1859) where ammonia was converted to indophenol blue. For development of color, 1 ml of water sample, phenopentacy non-itrosyloferrate solution (1 ml) and alkaline hypochlorite (1 ml) were added and samples were allowed for completion of reaction for 5 minute at 37°C. This was followed by an addition of 7 ml distilled water. Absorbance of the samples was measured at 625 nm using a spectrophotometer (PerkinElmer LAMBDA 35 UV/Vis Spectrophotometer, USA). The readings were compared with series of standard solutions of anhydrous NH_4CL as described by Berthelot (1859) for calculating ammonia levels and the values were expressed as mg/kg/l/day for all groups. All the experimental chemicals were obtained from the Sigma Aldrich of analytical grade.

Results and discussion

The trend in ammonia excretion with respect to increase in salinity is presented in figure 1. Salinity 5-10 ppt was found more critical during acclimatization of fishes in the present study. The ammonia level in water was 7.69 mg/kg/l/day at salinity 5 ppt that was further increased to 10.25 mg/kg/l/day when the salinity was increased gradually from 5 to 10 ppt. The ammonia level dropped down to 5.12 and then 3.84 mg/kg/l/day when the salinities were increased to 15 and then 20 ppt respectively (Fig. 1). Further, the ammonia level showed an increasing trend where the values were ranged from 6.41 and 8.97 mg/kg/l/day when the salinity was raised from 25 to 30 ppt respectively. It was clearly evident from results, that the ammonia level was higher by 83.35% as compared to control group ($P<0.05$) when the salinity was increased to 5 ppt. The ammonia level was subsequently increased by 24.97% higher when the salinity was raised from 5-10 ppt (Fig. 1). There was a decline in ammonia level by 50 and 25% at salinities 15 and 20 ppt respectively, whereas it was increased by 40.09 and 20.43% at 25 and 30 ppt salinities respectively.

A group of fishes were also directly exposed to salinities 10, 20 and 30 ppt without following their gradual acclimatization process (Fig. 2). The concentrations of ammonia were 1.94, 1.38 and 2.77 mg/kg/l at 10, 20 and 30 ppt salinities respectively within 2 hrs which further reached to 11.11 mg/kg/l within 24 hours at both 10 and 20 ppt salinities respectively ($P<0.05$). At 30 ppt all fishes were found dead within 3 hrs of exposure.

The fresh water fish when introduced to saline waters, their metabolic activities was always altered and greater demand of salt excretion along with ammonia and urea was observed (Besra and Sharma, 2004). In the present study, control group where the salinity was maintained to 0 ppt, the ammonia level in the water was 1.28 mg/kg/l/day. However, when the salinities were increased to 5 and 10 ppt, the levels of ammonia increased to 7.69 and 10.25 mg/kg/l/day respectively. Similar trend of ammonia excretion was observed in *Anabas testudineus* fingerlings when they were acclimatized from fresh water to different salinities. The ammonia excretion was increased by 31.71% at 2.5 ppt sea water as compared to control groups whereas 127.83% at salinity 5 ppt. In the present study it was observed that when the salinity was increased from 0-5 ppt, the ammonia level increased by 83.35%. Similarly when the salinity was further increased from 5 to 10 ppt, the level of ammonia increased by 24.97%. The highest excretion of ammonia (313 mg/kg/day) was recorded at 5 ppt salinity in *A. testudinus* (Besra and Sharma,

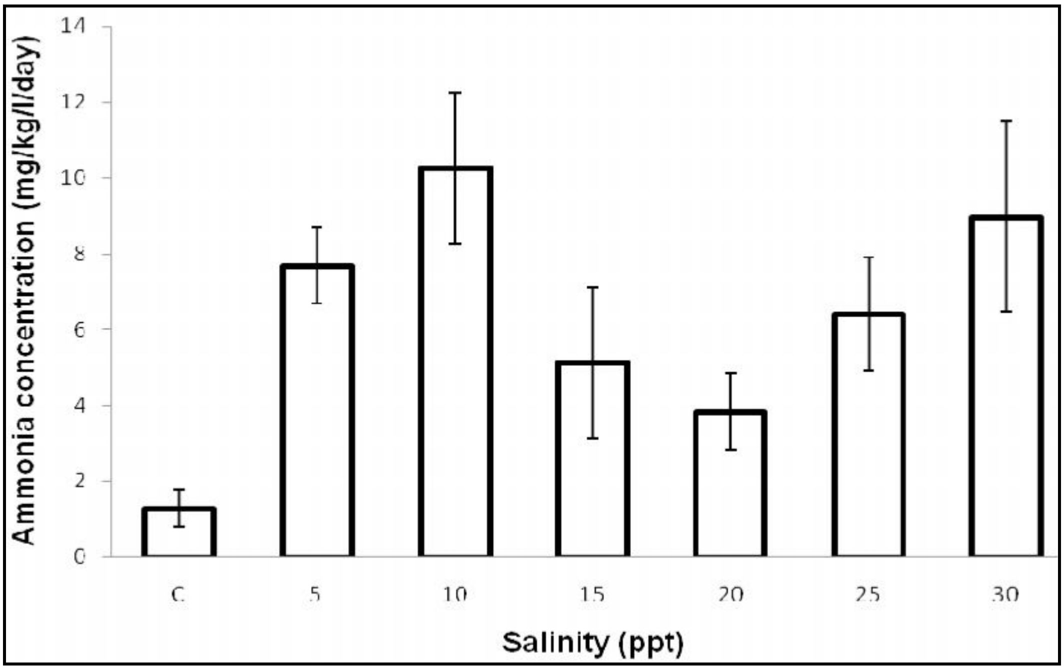

Figure 1. Trend in ammonia excretion with different salinities.

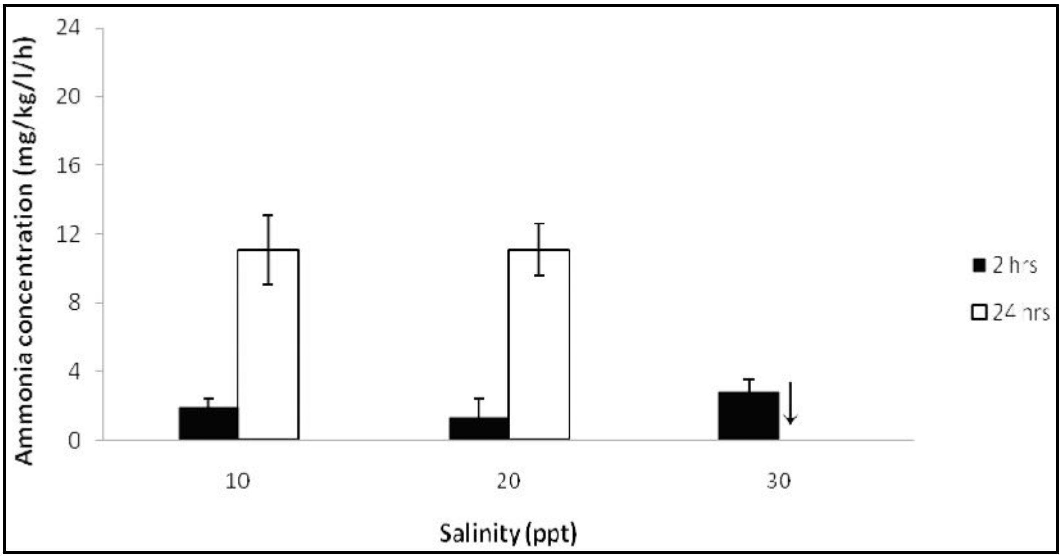

Figure 2. Ammonia excretion upon immediate exposure of fishes to different salinities (arrow indicates the mortality)

2004). Similarly, in *Cyprinus carpio*, the higher rate of ammonia excretion was reported at 10 ppt salinity as compared to 0 ppt salinity (De Boeck *et al*., 2000). The ammonia excretion in channel catfish, goldfish, rainbow trout and brown trout was higher at 9.0 ppt salinity as compared to other tested salinities whereas it has been found to be lowest in Gulf sturgeon and striped bass at the same salinity (Altinok and Grizzle, 2004). However, in hybrid sturgeons (*Huso huso×Acipenser ruthenus*) it has been observed that after the acclimatizing fish at 5 and 10% salinities for 1 week, the rate of ammonia excretion decreased, while urea excretion rate increased (Gershanovich and Pototskij, 1995).

Similarly in *A. testudineus* fingerlings, the excretion of ammonia has been reported to decrease at 7.5 ppt and 10 ppt salinity where only 44.06 and 92.43% respectively lower values were recorded (Besra and Sharma, 2004). Frick and Wright (2002) reported that the salinity of water had a pronounced effect on the nitrogen metabolism and excretion of *Rivulus marmoratus*. The mangrove killifish- *R. marmoratus* when exposed to freshwater (0 ppt) excreted less ammonia than in 30 ppt sea water on days 1st, 7th and 9th and fish at 45 ppt sea water on the day 7th.

Majority of the fish species cannot tolerate high environmental ammonia levels but some species are ammonia-tolerant and develop variety of strategies to avoid ammonia toxicity. Maintaining excretion and/or converting ammonia to other less toxic substances are some strategies used by fishes (Randall and Tsui, 2002). Salinity has been demonstrated to modulate NH_3-N and NO_2-N toxicity in several marine species (Weirich and Riche, 2006). In another study Sadok *et al*. (2004) reported that transfer of fish from freshwater to hyper-osmotic (6 and 12 ppt) media resulted in a marked decrease in ammonia efflux rates in freshwater pikeperch. Information available about the effects of salinity on ammonia excretion of fish is sometimes contradictory, probably because of species-related differences. Various studies indicated that the ammonia excretion is larger in proportion of the nitrogenous waste from marine species as compared to freshwater fishes (Wood, 1993; Jobling, 1994). In *Tridentiger obscurus* and *Tridentiger brevispinis* a significant decrease in ammonia excretion has been observed when they were transferred to sea water (Kakuta, 1988).

Active water drinking has been observed both in fresh water and sea water Atlantic salmon- *Salmo salar* where the drinking rates in this fish has been found to change from 0.1 to 3.88 ml/kg/1/h when the fishes were transferred from fresh water to sea water till 10th day and thereafter it reached to 7.94 ml/kg/1/h. (Fuentes and Eddy, 1997; Boeuf and Payan, 2001) During acclimatization process from lower salinity to higher salinity, it has been observed that fishes excrete considerable amount of ammonia and as such adequate exchange of water should be given to avoid toxicity in the culture system. It has also been strongly felt that the increment of salinity should not be more than 5 ppt at one time in tilapias that will also help in better survival of the fish.

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