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Prateek Angadi
Department of Zoology, Rani Channamma University, Bhutaramanahatti, Belagavi, India

Corresponding Author:
Prateek Angadi
Department of Zoology, Rani Channamma University, Bhutaramanahatti, Belagavi, India

The physiological adaptations during salinity stress in Tilapia fish: A review

Prateek Angadi

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Abstract
Many review articles on salinity stress and fish ability to bring about physiological changes are available but review on recent studies on tilapia fishes physiological adaptations during stress are few. Salinity fluctuation is a common stress to fish which need to cope up by altering and reorganizing the physiological function to bring about homeostasis. Therefore, this small review focuses on newer research studies of salinity stress on osmoregulation, metabolism, endocrine, immune function and reproduction. Tilapia fish are known for their euryhaline capabilities by adjusting osmoregulation and energy metabolism, hence they are widely used in aquaculture throughout the world in different salinities.

Keywords: Salinity stress, tilapia fish, glucose metabolism, hormones, reproductive stress

Introduction
Fishes undergo through numerous stresses in this dynamic environment. Fluctuation in environmental parameters such as temperature, salinity, pH, or dissolved oxygen act as stress to the fishes which can alter their physiological balance. In such challenging conditions, homeostasis, an internal process brings about steady state balance. Among all the above mentioned environmental parameters, salinity is the front runner stress in fishes. This is especially more pronounced in migrating fishes as they undergo physiological changes to adapt to changing salinities which has been widely studied [10]. The fishes which can tolerate wide range of salinity changes are termed as Euryhaline fishes such as the migratory fishes and those fishes which cannot tolerate wide range of salinity that is, they have narrow range of salinity tolerance are termed as Stenohaline [19]. Among the euryhaline fishes of the world, Tilapia fish is known for its ability to tolerate wide range of salinity exposure. Though tilapia is a freshwater fish inhabiting ponds, lakes and rivers [17], they possess remarkable ability to hyper-regulate or hypo-regulate whenever they experience such salinity changes. Because of this characteristic ability to survive and grow in different salinities tilapia are widely used in aquaculture and is the second most farmed fish in the world after carps [17]. Due to their superior meat quality, rapid growth, ease of adjusting to confinement and omnivorous habits has made tilapia an excellent choice for aquaculture [25].
Numerous review articles on biology, physiology and scope of culturing tilapia in different conditions including various salinities are available [17]. However, updated review of tilapia focusing on different physiological function is lacking to my knowledge. Therefore, the current review will focus on osmoregulation, metabolism, immune function, endocrine and reproductive studies in recent times.

Methodology
In this review, information was collected using Internet search engines such as Google Scholar, PubMed and Research Gate. The suitable and relevant articles were selected for review while the irrelevant articles were rejected.

Salinity Stress on Osmoregulation
Osmoregulation is an active process to maintain osmotic pressure in different body fluids of fish. The main osmoregulatory organ in fish is gill. Among all the species of tilapia, Mozambique tilapia, *Oreochromis mossambicus* are more tolerant to salinity changes due to higher osmoregulatory capacity. They can regulate ionic concentration in different body

fluids to maintain osmoregulation through active transportation. When reared in different salinities, Tilapia experience increased inflow of ions or salts into their body fluids. This creates a condition called as hyperosmolarity, where plasma contains higher salt concentration than required. This results in osmotic stress where water is drawn out the cells and tissue leading to cellular damage [15]. To overcome hyperosmolarity, tilapia need to attain ionic homeostasis by secreting excess ions out of its body through active transportation using osmoregulatory enzymes. Ionocytes or Chloride cells are mitochondrial rich cells found in gill of teleost fish which provide energy to osmoregulatory enzymes such as Na^+/K^+-ATPase. Cell numbers of ionocytes of gill change during transfer from Freshwater fish to Seawater indicating that ionocytes are regularly replaced with newly differentiated cells due to changing salinity [15]. Histopathology is a vital tool used to recognize the histological changes in the gill induced by salinity stress which can be adaptive or harmful. Nile tilapia induced to salinity stress showed marked increase in number of mitochondrial rich cells in gill lamellae suggesting an adaptation to compensate increased active transport. But, liver displayed presence of hydropic degenerative lesions whereas kidney showed marked interstitial nephritis due osmotic stress [14].

Na^+/K^+-ATPase is serves as the principal regulator of salt or ion homeostasis in teleost fish. During hyper osmotic stress, Na^+/K^+-ATPase in the basolateral membranes of the Mitochondrial Rich cells removes excess of ions from body fluids allowing the fish to adapt to higher salinity environment. Increase in activities of Na^+/K^+-ATPase enzyme, expression of Na^+/K^+-ATPase α1a and α-1b mRNA expressions are reported in gill of Tilapia fish during different salinity exposures [1, 5, 8]. Because of their importance in salinity acclimatization and adaptations, Na^+/K^+-ATPase enzyme subunits are characterized to get insights of structure and conformational functions in Tilapia [22]. Intestinal Na^+/K^+-ATPase activity also increases during higher salinity. In Mozambique tilapia, highest activity was observed in Anterior Intestine, followed by Posterior Intestine and least change in activity was Middle Intestine. In contrast, Nile tilapia showed highest Na^+/K^+-ATPase activity in Middle Intestine, followed by Anterior and Posterior intestine. Similar trend was also observed in their mRNA expressions of Na^+/K^+-ATPase in both fish. This means capacity and routes to osmoregulate in hyper salinity is different in both species [4]. Na^+-Cl^- cotransporter (NCC) found in the basolateral membrane is an indicator of freshwater acclimation and its activity and expression decrease with increase in salinities whereas Na-K-Cl cotransporter enzyme (NKCC) is an indicator of seawater acclimation activity and expression increase with increase in water salinities [24]. Cystic fibrosis transmembrane conductance regulator (CFTR) works similar to NKCC but is found in apical membrane of gill epithelia cells. Aquaporin 3 is present on basolateral membrane of gill epithelia cells regulate water balance. Downregulation of aquaporin 3 mRNA expression is reported to maintain or conserve water balance and to reduce water loss in Tilapia reared in seawater. But in freshwater reared tilapia show increased expression of Aquaporin 3 mRNA for up taking water [15]. These active transport enzymes utilize energy in the form of ATP to drive the osmoregulation in Tilapia during salinity stress.

Salinity Stress on Metabolism

To compensate the enhanced osmoregulatory process during salinity stress, extra energy is required so, metabolic reorganization will occur to supplement this enhanced osmoregulation. Consumption of oxygen is an indicator of ongoing metabolism in fish and ratio of oxygen to nitrogen index (O:N) for different modes of substrate metabolism which is used in many stress physiology experiments including salinity. Nile tilapia when induced to different salinities displayed higher oxygen consumption and (O:N) during the early time exposure of 1 h and 72 h which indicates that more energy and different metabolic substrate was spent to maintain osmoregulation compared to later time exposures [9]. The authors also noted that Nile tilapia performs better in brackish water than sea water or freshwater. Salinity has also effect on lipid composition of Nile tilapia. Increasing salinity decreases whole body lipid suggesting that lipids and fatty acids serves energy through Beta-oxidation for osmoregulation [26] signifying that lipid metabolism is important for euryhaline fish during osmotic stress. *Oreochromis niloticus* decrease triglyceride production, reserve lysophosphatidic acid, monoacylglycerols and higher expression of Acyl-CoA synthetase to produce energy for osmoregulation [20]. Serum metabolites profile also reveal that haemoglobin content can decrease during salinity exposure. This decreases the oxygen carrying capacity of the fish thereby affecting aerobic glycolysis. To compensate this, lactate content decrease suggesting anaerobic glycolysis in muscles of Nile tilapia [14]. In protein metabolism, arginine and proline are two amino acids reported to be metabolised during osmotic stress serving as energy and act as regulators in osmoregulators [20].

Glucose is the most preferred mode of energy for all animals and utilize liver glycogen to convert to glucose during unfavourable conditions. When Mozambique Tilapia induced to higher salinities, reduction in hepatic glycogen and increase in serum glucose was observed. Hexokinase, phosphofructokinase, glucokinase, pyruvate kinase, and glycogen synthase mRNA expression upregulation has been reported indicating more enzyme proteins expression and higher enzyme activity is essential under stress [22]. This increase the regulation of glycogenolysis and glycolysis suggesting that higher glucose is needed as energy for osmoregulatory enzymes. But initially gill glycogen is depleted followed by liver glycogen in time-course experiments in *Oreochromis mossambicus* [1]. Here, gill glycogen might be used as immediate source of energy during early stages of salinity acclimation. Mozambique Tilapia is also able to regulate glucose homeostasis during salinity change by upregulating mRNA expression of Glucose Transporter protein, GLUT1 in gill and GLUT2 in liver [1]. This results in influx of glucose into the cells for catabolism in gill tissue for osmoregulation. These studies provide information that Tilapia may utilizes different energy metabolism during salinity stress.

Salinity Stress on Immune Function

Salinity stress has bidirectional effects on immune response that is, it may suppress or enhance immune parameters. The extend of immune response depends on salinity range and duration of exposure. Overstimulation or lack of immune response can lead to inflammation and damage to tissues. Increase in salinity upregulates stress related genes HSP27, HSP70 (Heat Shock Proteins) and GST (Glutathione-s-transferase) in gill and liver tissues of Nile tilapia suggesting detoxification and repair of denatured proteins induced due to osmotic stress. Inflammatory-related gene expression of IL-1β, IL-8, and cc-chemokine were higher in liver but were downregulated in gill of Nile tilapia indicating a dysfunctional immune response during osmotic stress and a higher risk of infections in already stressed fish [5]. In brackish water, erythrocytes, macrophage and neutrophils cells showed higher accumulation in spleen and kidney of Nile tilapia as observed in histological studies indicating more accumulation is required to generate immune response and enhance the ability against pathogen infection [20]. Higher salinity acclimated fish have shown altered immune response but rapid decrease in salinity of acclimated tilapia is also reported of immune dysregulation. Lymphopenia, neutrophilia and monocytosis cells were observed higher in the peripheral blood. Phagocytosis, TGF-β and Interleukin-1β also increased in response to decreasing salinity [3].

Salinity Stress on Endocrine Function

Hormones are endocrine chemical molecules which act as a signal or messenger to communicate functions between different tissues and organs. Hormones play an important role in maintaining osmoregulation during salinity stress in fish. Prolactin promotes acclimation to freshwater in fish while growth hormone stimulates acclimation to seawater in fish and cortisol interacts with these two hormones, for their stimulation during for osmoregulatory function [13]. Cortisol stimulates differentiation of salt-secreting mitochondrion-rich cells and ion transport proteins in the gill to enhance the osmoregulatory function during salinity treatment. Plasma cortisol rise is observed in tilapia fish during such salinity treatment [23]. Cortisol has dual function on gill (osmoregulation) and liver (metabolism) as mineralocorticoids and glucocorticoids in Mozambique tilapia. The mRNA expression of corticosteroid receptors in gill and liver increased when transferred from freshwater to seawater. This helps facilitate breakdown of liver glycogen to glucose to provide sufficient energy to power the enhanced osmoregulation in gill [27]. Rise in mRNA expressions of Pro-opiomelanocortin in pituitary suggest that ACTH is synthesized by the pituitary which controls corticosteroid synthesis for seawater acclimation in Mozambique tilapia [27].

The growth hormone required for seawater acclimation works in synergy with cortisol in tilapia also affect the reproduction in tilapia. Immunohistochemical studies reveal changes in growth hormone, decrease in prolactin and somatolactin along with reduced mature follicles in female tilapia thereby decreasing reproductive function[16]. Plasma prolactin levels, mRNA expression of prolactin receptors in pituitary and gill decreased during seawater acclimation in tilapia but when transferred back to freshwater, levels of prolactin and its mRNA expression increased and return to stable levels [15]. Dopamine inhibits prolactin secretion. Tilapia acclimated to seawater displayed increase in expression of dopamine mRNA in pituitary indicating inhibition of prolactin secretion [2]. This provide evidence of prolactin role in regulating ion and water permeability in the gill during freshwater acclimation.

Salinity effect on Reproduction

Environmental stresses can disrupt reproductive cycle and functions in fish. Nile tilapia have been reported to attain first sexually maturity at 8–16 cm and 10–12 months of age and disturbance in water salinity can affect their reproductive functions [12]. Gonadosomatic index (GSI) is the measure of gonad mass to total body mass used as indicator of stage of testis and ovary maturation and sexual maturity of fish. GSI values of Nile tilapia were higher in freshwater and low salinity acclimated Nile tilapia but decreased in higher salinities indicating a testicular development retards with increase in salinity due to hampered metabolism thereby severely restricting reproduction, reducing spawning cycle, hatching success, and egg viability of tilapia [25]. Further, histological studies show higher lacunae or empty cyst without germ cells, delayed spermiogenic phase leading to decrease in spermatozoa count were observed in higher salinity suggesting negative effect on spermatogenesis and testicular function. In tilapia sperm activation and motility was hampered in seawater and sperm velocity was higher in freshwater compared to seawater suggesting that salinity influences sperm behaviour [11]. The oocytes density and fertilized eggs are important reproductive parameters for female fish that provide indications on success of fertilization and survival of eggs. Under hypersaline conditions, reduced oocyte density and fertilized eggs is reported in tilapia suggesting reduction in reproduction rate [8]. When freshly fertilised eggs from freshwater transferred to higher salinities displayed lower fertilisation rates compared to freshwater indicating eggs are the first to face salinity stress. But tilapia cultured in lower salinity to brackish water do appear to tolerate and show little changes in spawning and reproduction [6]. A comparative study on red tilapia and their hybrids cultured in salinity above 35-45‰ displayed reduced hatching rates and decreased yolk sac [18].

Conclusion

Recent research works on salinity stress in Tilapia have contributed to knowledge of understanding their physiological adaptations. Tilapia being the second largest farmed fish in the world is able to adjust to brackish water but higher salinities can induce stress in endocrine, immune and reproductive functions. To compensate for changed salinity, tilapia reorganize their metabolic pathways to maintain osmoregulation.

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