Determination of salinity tolerance limits of tilapia, *Oreochromis mossambicus*, for use in tuna line fishery

Assignment presented in partial fulfillment of the requirements for the degree of MPhil. in Livestock Industry Management at the University of Stellenbosch

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Declaration

I the undersigned hereby declare that the work contained in this assignment is my own work and has not previously in its entirety or in part been submitted at any university for a degree.

Abstract

Many species of tilapia such as *Oreochromis mossambicus* are euryhaline, able to adapt to different salinity waters. Their ability to withstand high salinity levels has given rise to the possibility of using tilapia as baitfish for tuna line fishery. The purpose of the study was to determine the survival rate of tilapia *O. mossambicus* during direct transfer from freshwater to the salinity levels of 0, 15, 20, 22.5, 25, 27.5, 30, 32.5, and 35 ppt. The data was analysed through means of univariate ANOVA and regression analysis.

O. mossambicus showed no mortality to all salinity regimes up to 25 ppt. Mortality was observed at 27.5 ppt, with 100% mortality at 35 ppt. LC 50 and LC 90 were found to be 30.5 and 34.2 ppt, respectively. The results indicate that tilapia (*O. mossambicus*) will survive a direct transfer to salinities up to 25 ppt. acclimation will be required in the event of transfer to salinity levels above 25 ppt, in order to prevent significant levels of mortalities.

Opsomming

Meeste van die tilapia spesies soos *Oreochromis mossambicus* het die vermoë om by water van verskillende soutgehaltes aantepas. Dit is hierdie vermoë om hoë sout vlakke te weerstaan wat die moontlikheid vir gebruik as lewende aas in die tuna langlyn visvangbedryf moontlik maak. Die doel van hierdie studie was om die oorlewingsvlak van tilapia, *O. mossambicus* te bepaal by die oorplasing van varswater direk na soutwater by vlakke van 0, 15, 20, 22.5, 25, 27.5, 30, 32.5, en 35 dele per duisend. Die data is verwerk deur gebruik te maak van eenvariant ANOVA en regressie analises.

O. mossambicus het geen mortaliteite tot gevolg gehad by al die oorplasings van vlakke tot en met 25 dele per duisend sout nie. Mortaliteite is wel gevind vanaf 27.5 dele per duisend, met 100 % mortaliteite by 35 dele per duisend. LC 50 en LC 90 was gewees 30.5 en 34.2 dele per duisend onderskeidelik. Die resultate toon aan dat tilapia (*O. mossambicus)* sal oorleef by direkte oorplasing na soutwater by vlakke van tot en met 25 dele per duisend. Tilapia wat na hoër vlakke as 25 dele per duisend oorgeplaas wil word, sal eers geleidelik moet akklimatiseer om mortaliteite te beperk.

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CHAPTER 1 INTRODUCTION

Tilapia are important aquaculture species with high economic and social values. Tilapia are widely distributed in temperate and tropical countries throughout the world, although their original distribution was limited south central Africa northward to Syria (Popma and Lovshin, 1994).

Tilapia are tolerant to most of the environmental hardships that occur in their natural habitat. However, only few species are able to tolerate the wider variation in salinity, temperature, and other environmental parameters that exist in the culture conditions.

The tilapias used for culture are considered freshwater species but they all can tolerate brackishwater (Popma and Lovshin, 1994). Some tilapia species such as *Oreochromis mossambicus* (Peters, 1852) can however tolerate and grow, even reproduce in full strength seawater. Such euryhalinity in *O. mossambicus* has made it possible to use them for culture in seawater in areas with limited freshwater, particularly in arid and semi arid areas.

Tilapia *O mossambicus* is not commercially important species in the world. However, its hardiness to various environmental parameters is high that it can with stand high variations in environmental equilibrium within a short period of time. This robust species has the ability to withstand high salinity levels that it may be used as baitfish in tuna line fishery.

Increasing demand for top predators, such as tuna (Tidwell and Allan, 2001), requires a substantial quantity of live bait at attractive prices. In addition continual and regular supply of the bait fish has the advantage of reducing time and cost of collecting baitfish from the wild. In order to provide the necessary bait supplies for future large-scale commercial baitfish, aquacultural production of baitfish with production of tilapia, *O.*

mossambicus is suggested (Gopalakrishnan, 1976). *O. mossambicus* has the advantages of high environmental tolerance to suite the use of the species in tuna line fishery However, to acclimate the species in shorter possible time for use in holding tanks of the fishing vessels is important.

This study aims at evaluating the salinity tolerance of the species after direct transfer to different levels of salinity regimes. This investigation would result in identifying the critical point of salinity above which the fish starts to die. This salinity level would then be taken as a base for the acclimation of the species in the shortest time possible in the overall acclimation of the fish to full strength seawater. The second part of the experiment should have dealt with the determining the shortest time possible for the fish to first transfer to the critical level of salinity and than to the full strength seawater. However the high mortality of the fish in the control group, probably due to stress in transportation and/or disease, has led to the result that would be unreliable and the experiment is recommended to be tried at a later stage with new batch of fish.

Fortunately, the experiment in the first trial, with the different batch of fish, has a solid result that this paper is presented in this volume. Other experiments involving the transfer of fish to higher salinity levels in shorter time interval and that of identifying the difference in survival of the fish when having both sexes mixed up would need to be investigated in the future trials.

References

Gopalakrishnan, V., 1979. Status and problems of culture of baitfish for the skipjack fishery in the Pacific region. In: Pillay, T.V.R. & Dill, Wm.A. (ed.). Advances in Aquaculture. Papers presented at the FAO technical conference on aquaculture, Kyoto, Japan. pp. 58-62.

Popma, T.J. & Lovshin, L.L., 1994. Worldwide prospects of commercial production of tilapia, Department of Fisheries and Allied Aquaculture, Auburn University, Alabama. 43 pp.

Tidwell, J.H. & Allan, G.L., 2001. Fish as food: aquaculture's contribution. pp. 958-963 EMBO reports.

CHAPTER 2 LITERATURE REVIEW

INTRODUCTION

Tilapias belong to the Chichlid family which can be distinguished from other families of bony fish by the presence of an interrupted lateral line, running superior along the anterior part of the fish and inferior along the posterior portion (Pienaar, 1968, Pompa and Lovshin, 1994).

Table 2.1 Classification of tilapia:

Phylum Vertebrata
Subphylum Craniata
Superclass Gnathostomata
Series Pisces
Class Teleostomi
Suborder Percoidei
Family Cichlidae

Tilapias of economic importance are divided into three major taxonomic groups based primarily on reproductive behavior. These are substrate incubators, maternal mouthbrooders and paternal and bipaternal mouthbrooders (Pompa and Lovshin, 1994). The original distribution of tilapias was limited to south central Africa northward into Syria. *Oreochromis mossambicus* was apparently the first tilapia species to be exported from Africa (Pompa and Lovshin, 1994).

Tilapia as Baitfish

Evaluation of the natural bait resources of the Pacific Ocean indicated that baitfish populations are unevenly distributed and their abundance fluctuates significantly from year to year. In other areas, availability of live bait is a key factor governing exploitation of tuna stocks, even in existing fisheries (Gopalakrishnan, 1976). Urgent steps are therefore

required to provide the necessary bait supplies for future large scale commercial tuna fishing ventures. Several attempts have been suggested in the past to solve these problems by adopting different techniques (Tidwell and Allan, 2001). Of these, the feasibility of culturing baitfish has attracted considerable attention because of its relative stability and controlled production. Therefore, there is continuing interest in the potential of aquaculture to meet the demand for baitfish. Fish culture techniques developed in recent years are considered to have application in the baitfish industry.

The general qualities required of cultivated baitfish are well known. The species must be prolific and have an extended breeding season, good growth rate, and must be resistant to handling, transport and disease In addition, qualities such as survival, fishermen acceptance, availability, behavior, size, colour and body form must be considered.. It is also important that the species selected for culture in any particular area must be adapted to the prevailing environmental conditions (Gopalakrishnan, 1976). One of such species is tilapia *Oreochromis mossambicus*, that is an advanced and phylogenetically "modern" teleost species with the advantages of hardiness, adaptability of artificial conditions in bait tanks and possibility of holding for indefinite periods thriving in broad ranges of salinities (Vonck, *et al.*, 1998).

Oreochromis mossambicus is a robust fish which can withstand high water temperature as well as high salinity. The species has been introduced and cultivated in several countries in both freshwater and brackish water, even full strength seawater. The fish is omnivorous feeder, consuming wide variety of plants and animals. Its respiratory demand is relatively low and large numbers of juveniles can be contained easily in containers. The fish can spawn almost throughout the year in warm waters at 5-7 weeks intervals, with an average of 8 times/year. The reproductive potential is high, mainly due to parental care. Tilapia can

spawn even in salt water, though the activity may be inhibited with increasing salinity (Pienaar, 1968).

The sea trials conducted with tilapia indicated that the fish served well as a supplementary bait to the popular bait fish *Stolephorus purpureus*. With respect to size, it has been observed that tilapia of 3.8-5.0 cm length is the optimum for skipjack pole-and-line fishing (Brock and Takata, 1955, King and Wilson, 1957, both cited in Gopalakrishnan, 1976). It was also reported that tilapia was a good bait for skipjack tuna weighing 8 to 11 kg (Gopalakrishnan, 1976). Although they may not be as good as a live bait as other popular and established species, they can serve as supplementary bait and also as bait to be used from small fishing boats.

Considering the distances involved and varying abundance and unpredictability of the natural populations of bait fish species, tilapia aquaculture can play important role in providing bait at specific locations as demanded by the industry.

Osmotic and Ionic Regulation in Fishes

Most aquatic vertebrates must osmo- and ionoregulate in order to maintain their plasmal salt content relatively consistent, which is required to regulate cell volume and intracellular salt content (Evans, 1993). The environments of fish range from freshwaters, which may have almost no dissolved solutes, through brackish waters to the sea, and hypersaline brine lakes. In such dynamic equilibrium of the surrounding environmental conditions of different solute and water availability, fish generally maintain a constancy of their internal body fluid composition via a process termed osmoregulation (Rankin *et al.*, 1983). Such a steady state is reached as a result of exchange between the fish and their surrounding environment (Prunet and Bornancin, 1989). To achieve this 'disequilibrium' between the ambient water and their extracellular fluids they employ a number of homeostatic organs that include gill, kidney,

urinary bladder, gut and, in elasmobranches, the rectal gland (Rankin *et al.*, 1983). These changes in external salinity affect the composition of the body fluids, which in turn, affects the metabolic processes (Spaargaren, 1995).

All fish, except the most primitive Myxinoids which have extracellular fluids resembling seawater in ionic composition, are descended from freshwater ancestors which, in the course of evolution, acquired reduced body fluid salt concentrations which alleviated the osmotic (water entry) and ionic (water loss) problems that is characteristic of life in a hypo-osmotic environment (Rankin *et al.*, 1983).

Most of the literatures dealing with fish osmotic and ionic regulation in fish are composed of studies on teleosts, probably because they are so common, relatively easy to collect, and have species that are easily maintained in the laboratory (Evans, 1993). According to Rankin *et al.* (1983) those bony fish and lampreys which re-evolved the ability to live in seawater maintained the reduced body fluid osmolarities that they were faced with the problem of osmotic loss of water, as well as diffusional entry of salts. The only way this loss could be replaced was by drinking seawater. Excess sodium and chloride ions are excreted by chloride cells in the gills, but magnesium and sulphate ions, which diffuse down their electrochemical gradients into the fish, are removed renally (Rankin *et al.*, 1983).

Most species of fish are stenohaline that, unlike to euryhaline species, cannot withstand large changes in environmental salinity (Rankin *et al.*, 1983). Since teleost plasma does not contain significant concentrations of organic solutes, these fishes are hyperosmotic to freshwater but hypo-osmotic to seawater (Evans, 1993). On the other hand, euryhaline species may face varying, and opposing, gradients that the fish are exposed to a complex suite of osmoregulatory problems over the time frame of hours or days (Evans, 1993, Hwang, 1987).

Osmoregulation in Freshwater Fishes

Kidney Control of Osmoregulation

Freshwater teleosts display well-developed glomeruli, proximal and distal tubules, as well as collecting tubules and ducts (Evans, 1993). The primary role of the kidney is excretion of divalent ions, particularly magnesium and sulphate, as a result of renal tubular secretory mechanisms (Rankin *et al.*, 1983). Renal adaptations to varying environmental salinities and their regulation in teleosts have long been studied. In some euryhaline species varying urine production rates may be achieved by regulation of tubular water reabsorption. In most species, however, varying urine production rates parallel changes in overall glomurular filtration rates. The overall glomerular filtration rate (GFR) is the sum of the filtration rates of all the filtering nephrones. Modifications in GFRs may therefore result from change in individual rates of filtration (single nephron glomerular filtration rates; SNGFRs) and/or modulation of the size of the filtering population; the latter is termed glomerular intermittency (Rankin *et al.*, 1983).

Control Mechanisms in Fish Osmoregulation

Control of teleostean kidney function is likely to involve both neural and endocrine factors (Rankin *et al.*, 1983). Our knowledge of the nerveous control of fish kidneys is almost non-existent (Evans, 1993). However, hormonal control of osmoregulation in teleost fish involve both rapidly-acting hormones such as catechlamines, somatostatin, glucagons, urotensins, and slowly acting hormones such as prolactin, cortisol, and thyroid hormones (Prunet and Bornancin, 1989).

Prolactin is especially important for adaptation of teleosts of freshwater. Its primary role is to prevent loss of ions and reduce water permeability in the kidney, urinary bladder and gill (Manzon, 2002). A number of

histological studies have shown that prolactin affects the structure of the renal tubule cells in both freshwater and seawater adapted teleosts (Prunet and Bornancin, 1989). The actions of prolactin seem to some extent dependent upon cortisol levels. Thus, although cortisol appears to be principally concerned with seawater adaptation it is important in salt conservation of freshwater teleosts where cooperative action of prolactin and cortisol activates sodium pumps in urinary bladder, kidney and gill. Cortisol's effects on membrane permeability are however antagonistic to those of prolactin. Cortisol markedly increases enzyme activities of osmoregulatory surfaces, in particular, that of sodium and potassium activated adenosinetriphosphatases (Na^+, K^+-ATPase) and also gill chloride cell differentiation (Dean *et al.*, 2003). Euryhaline fish transferred from fresh to sea water show a progressive increase in the activity of this enzyme in the gills and gut, alongside a reduced renal enzyme activity (Balm *et al.*, 1995, Rankin *et al.*, 1983).

Gill in Osmoregulation

Teleost fishes, both freshwater and seawater, maintain the osmolality of their body fluid at a relatively constant level. Chloride cells in the gill epithelium and opercular membrane are important osmoregulatory sites in maintaining ionic balance in fish. The cells are characterized by the presence of a rich population of mitochondria and an extensive tubular system in the cytoplasm (Uchida *et al.*, 2000). The tubular system is continuous with the basolateral membrane, resulting in a large surface area for the placement of ion transporting proteins such as Na^+, K^+-ATPase, a key enzyme for chloride cell activities. The chloride cells play an important role in ion secretion in seawater and possibly in ion uptake in freshwater. In general, when euryhaline teleosts are transferred from freshwater to seawater, chloride cells are increased in number and size, along with an increase in Na^+, K^+-ATPase activity (Hwang *et al.*, 1989, Uchida *et al.*, 2000, Hwang, 1987). Two types of chloride cells have been

identified: filament chloride cells activated in seawater are likely to play a central role in ion secretion in seawater, and lamellar chloride cells that disappear during seawater adaptation seem to be involved in ion uptake in freshwater (Uchida *et al.*, 2000).

Osmoregulation in tilapia

It has been speculated that tilapias evolved from a marine ancestors and secondarily invaded the freshwater environments (Myers, 1938; Steinitz, 1954; both cited in Stickney, 1986). Similarly, Evans (1993) and Rankin *et al.*, (1983) argued that teleosts invaded freshwater and maintained the lowest salinity characteristics of their body fluids as compared to seawater.

Various species of tilapia, most of which have not yet been cultured, occur in African lakes; others occur in Middle Eastern lakes where salinities may reach or exceed that of seawater during seasonal drought periods. *Oreochromis mossambicus* was the first tilapia to receive widespread distribution outside the native African and Middle Eastern range of the genus (Stickney, 1986).

Stickney (1986) has identified *O. aurea, O. niloticus, O. mossambicus* and *O. spirulus* as species of aquacultural interest. However, he concluded that only *Tilapia zilli* and *O. mossambicus* to be the only species of tilapia tolerant to the high salinity of seawater and brackish waters. Nevertheless, *T. zilli* was found to be of less interest to aquaculture because of its relatively slow growth and *O. mossambicus* has a disadvantage of reproducing at a very early stage, which result in overpopulation and stunting. Apart from the disadvantages of culturing *O. mossambicus*, it has less acceptability than other tilapias in the markets because of its dark colouration and black peritoneum. However, being a typical euryhaline fish, it has been widely used as a model in studies on endocrine aspects of stress and osmoregulation (Vonck *et al.*, 1998).

Suresh and Lin (1992) identified the above species of commercially importance as being important for aquaculture in saline waters. However, he suggested that selection of tilapia for saline water aquaculture should consider environmental factors with special reference to salinity and temperature, as these factors can influence growth performance, reproduction and disease occurrence. Species such as *O. mossambicus* and *T. zilli* thrive in relatively high salinity waters, but they are not popular species for culture (Suresh and Lin, 1992). Short supply of freshwater for aquaculture uses in arid and semi-arid regions also has stimulated efforts to culture tilapia in saline waters (Watanabe *et al.*, 1988a).

Tilapia culture in saline waters is biologically sound. However, many species of tilapia tolerate varying limits of salinity with even some discrepancies in the reported salinity limits for a single species (Suresh and Lin, 1992). Such discrepancies could be attributed to differences in strains and other factors such as temperature, methods of acclimation and age and/or body size (Suresh and Lin, 1992; Villegas, 1990). Villegas (1990) also indicated race differences resulting in variations in the rate of acquisition of salt tolerance with size, which are associated with phylogenetic relationships to ancestral marine form (Cherviniski, 1961; as cited in Villegas, 1990). The ontogenic changes in salinity tolerance are known to be closely related to size than chronological age (Watanabe *et al.*, 1985a). Similar results were observed by Villegas (1990).

Increasing salinity tolerance with size has often been explained in terms of the body surface:volume relationship. Larger fish are subject to less osmotic stress than smaller ones since the ratio of gill area to body weight decreases as the body weight of the fish increases (Parry, 1960, as cited by Watanabe *et al.*, 1985a). It has also been explained as being related to the functional development of the osmoregulatory system (Ewing *et al.*, 1980, as cited in Villegas, 1990).

Temperature is a dominant factor controlling feeding, growth and survival of fish (Handeland *et al.*, 1998). (Tilapia species were shown to die at low temperature, Likongwe *et al.*, 1996). It has also been shown that temperature has a significant effect on the osmoregulatory process in fish larvae by increasing water permeability and drinking rates (Tyler and Ireland, 1994), which might be expected to lead to an increase in excretion of excess ions (Imsland *et al.*, 2002). Handeland *et al.* (2000) showed that Atlantic salmon, *Salmo salar*, smolts were transferred from freshwater to seawater at different temperatures, a rapid increase in plasma chloride levels and a gradual increase in gill Na^+, K^+-ATPase was observe at higher temperatures (18.9 °C), whereas these effects were delayed or absent at lower temperatures (4.6 °C and 9.1 °C). Similar results were also observed by Imsland (2002) that juvenile turbot, *Scophtalmus maximus*, showed positive correlation between temperature and Na^+, K^+-ATPase activity with the highest Na^+, K^+-ATPase activity at 22 ° C, whereas no difference or an inverse trend was seen at the other temperatures of 10, 14, and 18 °C.

Imsland *et al.* (2002) indicated an interactive effect of salinity and temperature on Na^+, K^+-ATPase activity in turbot (*S. maximus*). In contrast, Handeland *et al.* (1998) found no interactive effect of temperature and salinity on gill enzyme activity in Atlantic salmon. This difference in the interactive effect of temperature and salinity on gill enzyme activity between turbot and Atlantic salmon can possibly be explained by different osmoregulatory strategies by the two species, the salmon being anadromous and the turbot euryhaline.

Tolerance to salinity was assessed by correlated observations on gill structure, plasma sodium levels and gill Na^+, k^+-ATPase activity. In freshwater, tilapia presented a gill epithelium structure characteristic of freshwater stelohaline fish: no chloride cells on lamellae and few chloride cells on the filaments (Hwang, 1987). An increase in external salinity

induced the proliferation of chloride cell on filaments, a feature typical of seawater teleosts (Hwang, 1987, Avella *et al.*, 1993). This change in gill structure was accompanied by an increase of gill Na^+, K^+-ATPase activity (Hwang, 1987, Morgan *et al.*, 1997).

Conclusion

Assuming that salinity tolerance in the tilapias indicate osmoregulatory activity, then knowledge of maximum salinity tolerable of freshwater-spawned and reared stocks would provide a basis for the optimal time of transfer to seawater for grow-out or use in tuna line fishery. Thus this would be advantageous in use of tilapia as bait fish, when supply of bait fish is limiting, and the use of freshwater in seed production, especially when freshwater is limiting.

References

Avella, M., Berhaut, J. & Bornancin, M., 1993. Salinity tolerance of two tropical fishes, *Oreochromis aureus* and *O. niloticus*. I. Biochemical and morphological changes in the gill epithelium. J. Fish Biol., 42: 243-254.

Balm, P.H.M., Haenen, H.E.M.G. & Wendelaar Bonga, S.E., 1995. Regulation of interrenal function in freshwater and sea water adapted tilapia (*Oreochromis mossambicus*). Fish Physiol. Biochem. 14: 37-47.

Dean, D.B., Whitlow, Z.W. & Borski, R.J., 2003. Glucocorticoid receptorupregulation during seawater adaptation in a euryhaline teleost, the tilapia (*Oreochromis mossambicus*). Gen. Comp. Endoc., 132: 112-118.

Evans, D.H., 1993. Osmotic and ionic regulation. In: Evans, D.H. (ed). The physiology of fishes. CRC Press, Boca Raton, Florida. pp. 315-341.

Gopalakrishnan, V., 1979. Status and problems of culture of baitfish for the skipjack fishery in the Pacific region. In: Pillay, T.V.R. & Dill, Wm.A. (ed.). Advances in Aquaculture. Papers presented at the FAO technical conference on aquaculture, Kyoto, Japan. pp. 58-62.

Handeland, S.O., Berge, A., Bjornsson, B.T. & Stefansson, S.O., 1998. Effects of temperature and salinity on osmoregulation and growth of Atalantic salmon (*Salmo salar* L.) smolts in seawater. Aquaculture, 168: 289-302.

Hwang, P.P., 1987. Tolerance and ultrastructural responses of branchial chloride cells to salinity changes in euryhaline teleost *Oreochromis mossambicus*. Marine Biol. 94: 643-649.

Hwang, P.P., Sun, C.M. & Wu, S.M., 1989. Changes of plasma osmolality, chloride concentration and gill Na-K-ATPase activity in tilapia *Oreochromis mossambicus* during seawater acclimation. Marine Biol. 100: 295-299.

Imsland, A.K., Gunnarsson, S., Foss, A. & Stefansson, S.O., 2002. Gill Na+, K+-ATPase activity, plasma chloride and osmolality in juvenile turbot (*Scophthalmus maximus*) reared at different temperature and salinities. Aquaculture, 218: 671-683.

Likongwe, J.S., Stecko, T.D., Stauffer, J.R.,Jr. & Carline, R.F., 1996. Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia *Oreochromis niloticus* (Linneaus). Aquaculture, 146: 37-46.

Manzon, J.A., 2002. The role of prolactin in fish osmoregulation: a review. Gen. Comp. Endoc., 125: 291-310.

Morgan, J.D., Sakamoto, T., Grau, E.G. & Iwama, G.K., 1997. Physiological and respiratory responses of the Mozambique tilapia (*Oreochromis mossambicus*) to salinity acclimation. Comp. Biochem. Physiol. 117A: 391-398.

Pienaar, U. de V. 1968. The freshwater fishes of the Kruger National Park. The National Parks Board of Trustees of the Republic of South Africa.

Popma, T.J. & Lovshin, L.L., 1994. Worldwide prospects of commercial production of tilapia, Department of Fisheries and Allied Aquaculture, Auburn University, Alabama. 43 pp.

Prunet, P. & Bornancin, M., 1989. Physiology of salinity tolerance in tilapia: an update of basic and applied aspects. Aquat. Living Resour. 2: 91-97.

Rankin, J.C., Henderson, I.W. & Brown, J.A., 1983. Osmoregulation and the control of kidney function. In: Rankin, J.C., Pitcher, T.J. & Duggan, R.T. (ed). Control processes in fish physiology. Croom Helm Ltd, Australia. pp 66-88.

Spaargaren, D.H., 1995. A functional model for describing the responses to salinity stress in aquatic animals. Comp. Biochem. Physiol. 111A: 501-506.

Stickney, R.R., 1986. Tilapia tolerance of saline waters: a review. The progressive Fish-Culturist 48: 161-167.

Suresh, A.V. & Lin, C.K., 1992. Tilapia culture in saline waters: a review. Aquaculture 106: 201-226.

Tidwell, J.H. & Allan, G.L., 2001. Fish as food: aquaculture's contribution. pp. 958-963 EMBO reports.

Uchida, K., Kaneko, T., Miyazaki, H., Hasegawa, S. & Hirano, T., 2000. Excellent salinity tolerance of Mozambique tilapia (*Oreochromis mossambicus*): elevated chloride cell activity in the branchial and opercular epithelia of the fish adapted to concentrated seawater. Zool. Sci. 17: 149-160.

Villegas, C.T., 1990. Evaluation of salinity tolerance of *Oreochromis mossambicus, O. niloticus* and their F1 hybrid. Aquaculture 85: 281-292.

Vonck, A.P.M.A., Wendelaar Bonga, S.E. & Flik, G., 1998. Sodium and

calcium balance in Mozambique tilapia, *Oreochromis mossambicus*, raised at different salinities. Comp. Biochem. Physiol. 119A: 441-449.

Watanabe, W.O., Ellingson, L.J., Wicklund, R.I. & Olla, B.L., 1988a. The effect of salinity in growth, food consumption and conversion in juvenile, monosex male florida red tilapia. pp. 623. *In:* T.B. R.S.V. Pullin, K. Tonguthai, J.L. Maclean (ed.) The second international symposium on tilapia in aquaculture, ICLARM Conference Proceedings, Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Watanabe, W.O., Kuo, C.-M. & Huang M-C., 1985a. The ontology of salinity tolerance in the tilapias *Oreochromis aureus, O. niloticus,*and an *O. mossambicus* x *O. niloticus* hybrid, spawned and reared in freshwater. Aquaculture 47: 353-367.

CHAPTER 3

Determination of survival rate of tilapia (*Oreochromis mossambicus*) during direct transfer from freshwater into a salinity range of 0 to 35 ppt

Abstract

Many species of tilapia such as *Oreochromis mossambicus* are euryhaline, able to adapt to different salinity waters. Their ability to withstand high salinity levels has given rise to the possibility of using tilapia as baitfish for tuna line fishery. The purpose of the study was to determine the survival rate of tilapia *O. mossambicus* during direct transfer from freshwater to the salinity levels of 0, 15, 20, 22.5, 25, 27.5, 30, 32.5, and 35 ppt. The data was analysed through means of univariate ANOVA and regression analysis.

***O. mossambicus* showed no mortality to all salinity regimes up to 25 ppt. Mortality was observed at 27.5 ppt, with 100% mortality at 35 ppt. LC 50 and LC 90 were found to be 30.5 and 34.2 ppt, respectively. The results indicate that tilapia (*O. mossambicus*) will survive a direct transfer to salinities up to 25 ppt. acclimation will be required in the event of transfer to salinity levels above 25 ppt, in order to prevent significant levels of mortalities.**

Introduction

Many species of tilapia are euryhaline (Suresh and Lin, 1992), surviving in both freshwater and seawater. Although considered a freshwater fish, different species of tilapia have been found to survive in different salinity waters with varying performance (Deguara and Agius, 1997; Popma and Lovshin, 1994). The varying tolerance limits of the

species depend on factors such as genetic strain, temperature, methods of acclimation and age and/or body size, the biggest factor being simply genetic ones as species-specific differences exists in salt tolerance of tilapia (Villegas, 1990; Suresh and Lin, 1992).

Their varying degrees of salt tolerance suggested the possibility of using tilapia as bait in tuna line fishery. Increasing consumer demand for top predators, such as tuna, in the developed world has caused sever pressure on existing stocks of bait fishes (Tidwell and Allan, 2001). In addition, consistent and continual supply of bait fish can only be assured through regular production of aquaculture fish stock with the potential for bait in line fishery. One of such a candidate for bait in tuna line fishery is tilapia *Oreochromis mossambicus* (Gopalakrishnan, 1976) with its high tolerance to wide range of environmental variables such as salinity (Villegas, 1990; Jonassen et al, 1997; Prunet and Bornancin, 1989; Perschbacher and McGeachin, 1988), unionized ammonia (Daud et al, 1988), temperature (Prunet and Bornancin, 1989) and probably pressure.

The objective of the study is to determine the maximum salinity level tolerable by *Oreochromis mossambicus* after direct transfer from freshwater to the respective salinity regimes. During this study the maximum salinity level tolerable is defined as the maximum salt concentration where there was 100% survival of directly transferred tilapia during the duration of the experiment. The experiment is part of series of experiments to use tilapia as bait in tuna line fishery around South Africa. *O. mossambicus* would be acclimated to seawater by first transferring them to the maximum salinity level tolerable and then to full strength seawater. For use as bait in tuna line fishery, tilapia are subjected to acclimation to different environmental parameters such as low temperature and high pressure that would be necessary to maintain them in the deep waters of the ocean.

Materials and Methods

This study was conducted at the division of Aquaculture, University of Stellenbosch (Stellenbosch, South Africa).

Male tilapia *Oreochromis mossambicus,* Kasinthula and Ndumu strains (Stander, Pers. Comm.), (measuring an average of 85g and 14.0 cm SL, N=289) were collected from a farm in Elsenburg, South Africa and were brought to the laboratory in 144 l glass aquaria (80x60x40) with 16 fish in each aquarium. They were kept in the aquarium for 2 weeks before salt solution was added, in order to alleviate the damage of handling and to familiarize them with the new environment. During this time they were fed trout pellets at *ad libitum.* The aquaria were supplied light by fluorescent lamp (40W AQUA-G20) for 16 hrs/day and aerators using air pump was used during the range of the study. Each aquarium was maintained at a constant temperature of 24±0.5 °C.

Nine salinity levels of 0 ppt, 15 ppt, 20 ppt, 22.5 ppt 25 ppt, 27.5 ppt, 30 ppt, 32.5 ppt and 35 ppt were chosen for the experiment to determine the critical salinity level where mortality occurs. Each salinity regime (salinity measured by HACH CO150 Conductivity Meter) had two replicates of independent aquaria with 16 fish in each aquarium. The desired salinity levels were attained by adding pre-prepared concentrated salt water (prepared using non-iodated coarse ROYAL SALT, from ROYAL SALT COMPANY, Parow) into the known volume of fresh water aquaria with the fish in it. The prepared solution of concentrated salt water was calculated to result in the desired salinity level in the aquaria. This procedure reduces the stress of handling during transfer.

Feeding stopped 24 hrs before the saltwater was added and the fish were not fed during the rest of the experiment. After adding salt of desired concentration the fish were held for 10 days and were monitored every 3 hrs interval for the first 4 days and every 6 hrs interval for the rest of the experiment.

Dead fish were removed from the aquaria and the time of death, body length and body weight were recorded. Cessation of operculum movements and failure to respond to gentle touch were used as criteria for death. Behavioral changes during addition of salt water were also observed.

Statistical Analysis

The analysis was subjected to univariate Analysis of Variance (ANOVA) and regression analysis. *STATISTICA* 6.0™ software was used for the analyses (StatSoft, Inc., 2001).

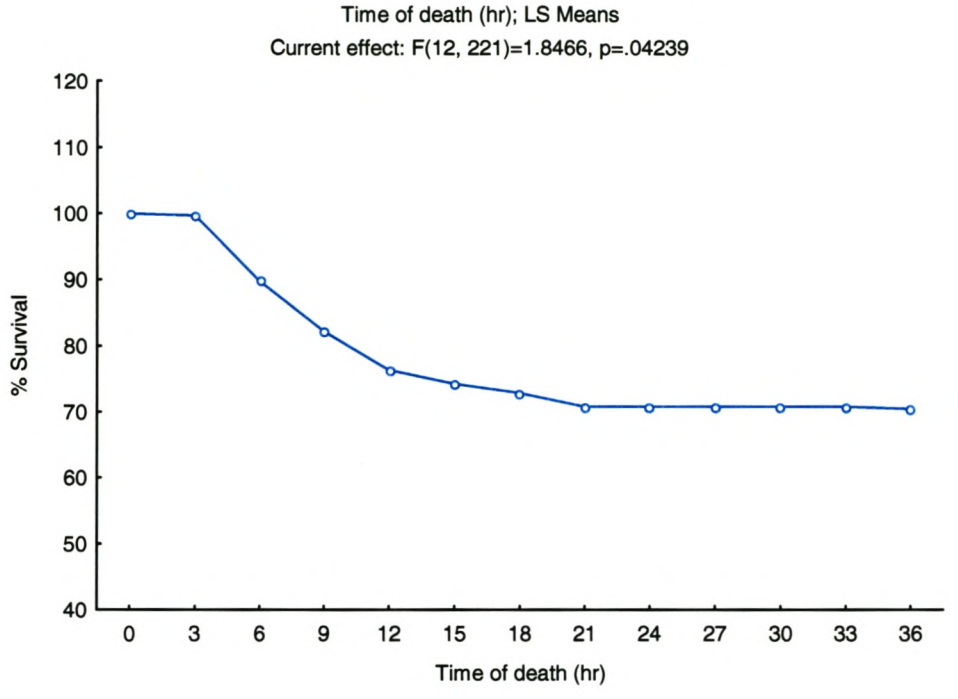
Results

The survival of *O. mossambicus* was explained by the salinity, time of exposure and the interaction of both salinity and time of exposure (r^2 = 0.99). The salinity levels in the aquaria and the percentages of the average mortality in these concentrations at different exposure times are summarized in Table 3.1

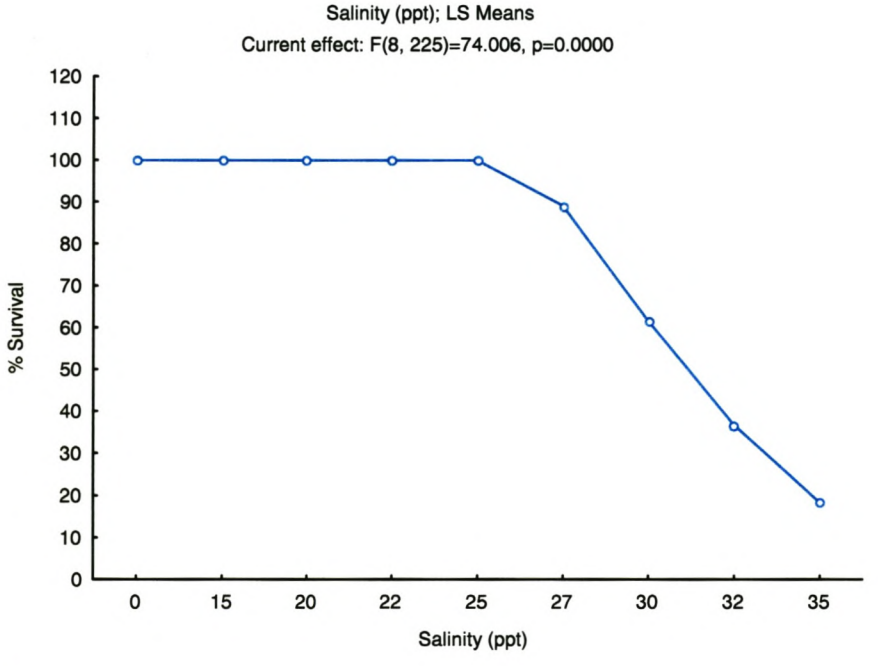
There was no mortality in all the salinity regimes from 0 to 25 ppt after direct exposure to the respective salinity levels from freshwater (fig 3.1 and fig 3.2). However, mortality was observed at 27.5 ppt with 100% mortality at 35 ppt after direct transfer from freshwater. There was no significant difference among replicates of the same salinity regimes ($p>0.05$). The regression fit to the data (fig 3.3) shows that the median lethal concentration (LC_{50}) of salinity (the salinity level where 50% of the fish dies) was 30.5 ppt. The graph also reveals that the lethal dose where 90% of the population dies (LD_{90}) to be 34.2 ppt. Therefore, the graph shows the quick shift in mortality in short intervals of the higher salinity levels after direct transfer to the respective levels. In addition, the time of exposure and salinity has a correlation that fish in higher salinity took shorter time to die than fish in lower salinity level after the critical point of salinity level (i.e. 27.5 ppt) where fish start to die (fig 3.2).

Table 3.1 The mortality level of *Oreochromis mossambicus*, expressed as percent average in relation to exposure time (hrs) at different salinity levels. The average mortality is based on two replicates of treatments, with 16 fish per treatment.

	Exposure time (hrs)											
	3	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**	**33**	**36**
Salinity												
0.0 ppt	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15.0 ppt	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20.0 ppt	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22.5 ppt	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25.0 ppt	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27.5 ppt	0.0	0.0	0.0	3.1	9.4	12.5	18.8	18.8	18.8	18.8	18.8	21.9
30.0 ppt	0.0	0.0	12.5	31.3	43.8	53.1	59.4	59.4	59.4	59.4	59.4	59.4
32.5 ppt	0.0	31.3	50.0	78.1	78.1	78.1	84.4	84.4	84.4	84.4	84.4	84.4
35.0 ppt	3.0	60.6	97.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0



a.



b.

Fig 3.1. **a.** Effect of time of exposure on % survival of *Oreochromis mossambicus* exposed to different salinity levels. **b.** Effect of salinity on the %survival of *O. mossambicus*. Note the point where mortality started to occur.

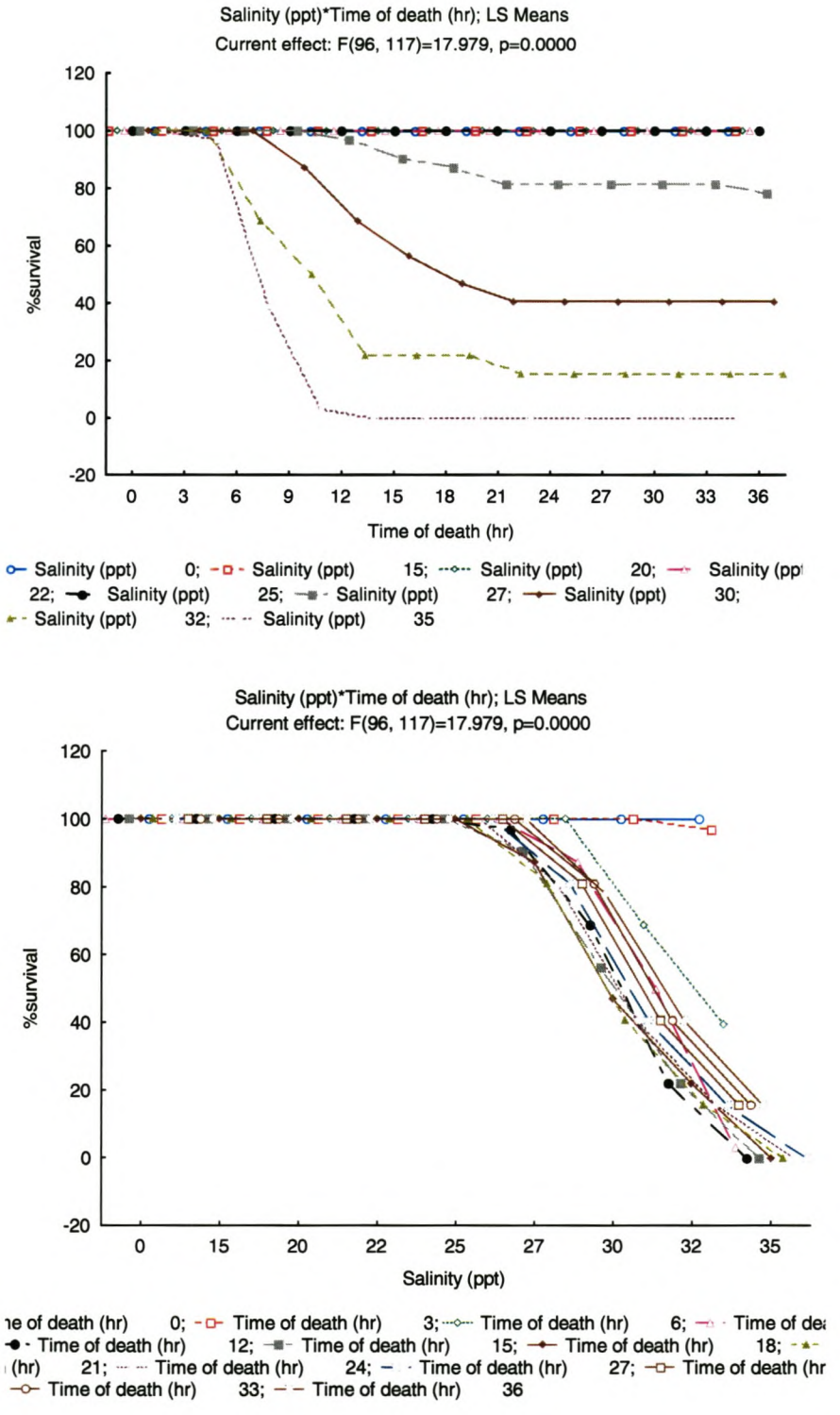


Figure 3.2 Effect of salinity, time of death and the interaction of both on the % survival of *Oreochromis mossambicus*.

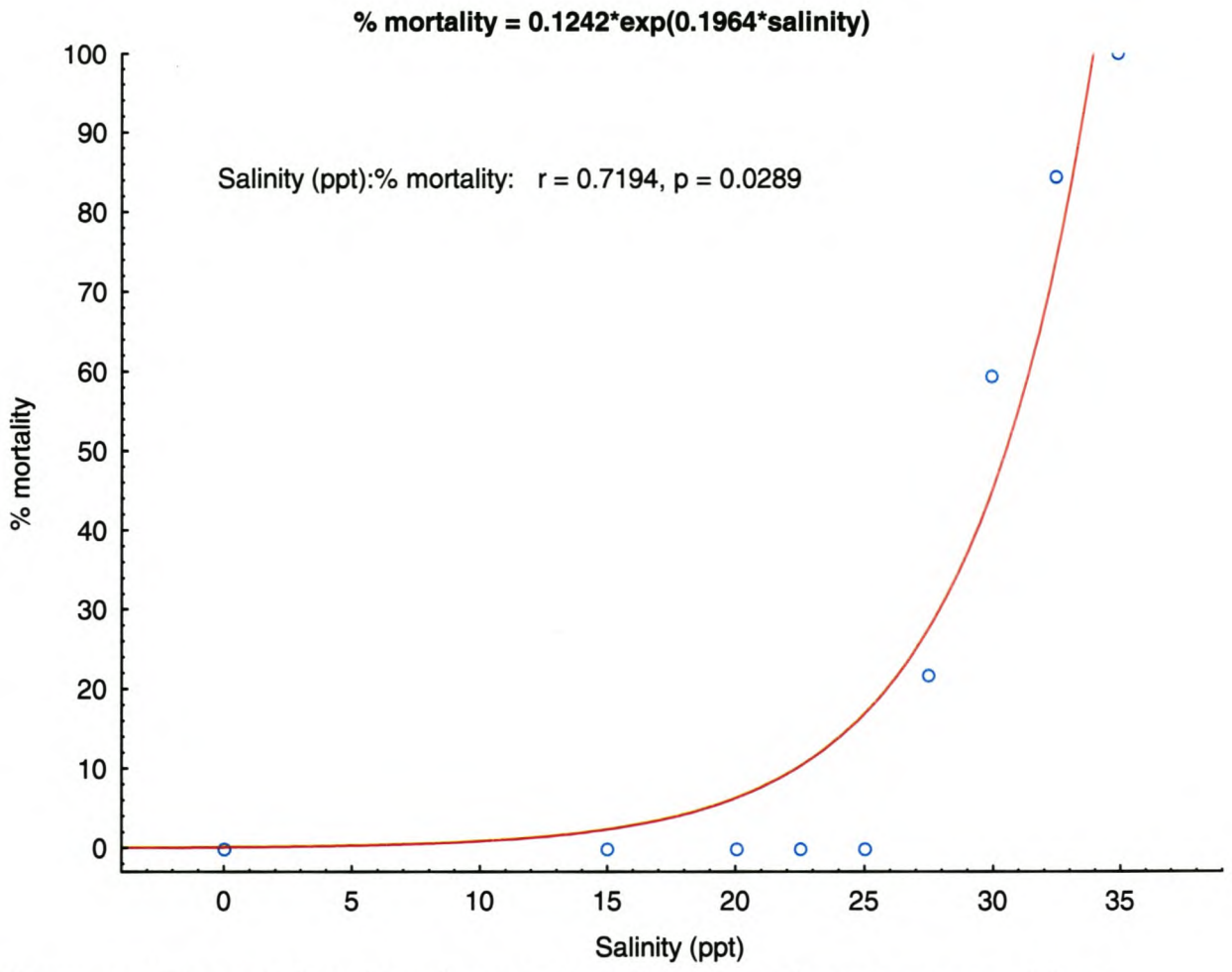


Figure 3.3 The relationship of mortality (%) of *Oreochromis mossambicus* at different treatments of salinity expressed as exponential regression.

Discussion

Survival of *Oreochromis mossambicus* in saltwater, after direct transfer, depends on the ability of the fish to efficiently control the osmoregulation of the body fluids and ions (Al-Amoudi, 1987b). *O. mossambicus* survived direct transfer from freshwater to 15, 20, 22.5 and 25 ppt with no mortality. It showed 22% mortality by direct transfer to 27.5 ppt. Similar result was also observed in another study on *O. mossambicus* to direct transfer to 27 ppt with 30% mortality (Al-Amoudi, 1987b). Direct transfer to 35 ppt killed all the fish as was observed in the study by Al-Amoudi (1987b) with 100% mortality to direct transfer to 36

ppt. The maximum salinity tolerable for direct transfer was, therefore, at 25 ppt with 100% survival. Similar result has been observed by Al-Amoudi (1987b) with 100% survival at 25.2 ppt. In another study, Perschbacher and McGeachin (1988) have shown that Florida red hybrid tilapia, *O. mossambicus-O. urolepis hornorum*, fully survived at 27 ppt. These differences could be because of different strains used in the various experiments (Suresh and Lin, 1992). On the other hand, Perschbacher and McGeachin, used all female tilapia which, according to Watanabe et al (1985a) have lower salinity tolerance as compared to all male tilapia used in this experiment. However, the physiological difference in salt tolerance between females and males has not yet been well established and further research is required.

Mortality to direct transfer to 30 ppt was 60% and to 32.5 ppt was 85%. Similar experiment on *O. mossambicus* transferred to 30.6 ppt showed 100% mortality (Al-Amoudi, 1987b). This variation in mortality could be due to the size differences used in these two different works. Watanabe et al (1985a) showed that size differences in *O. mossambicus* affects the ability of the fish to tolerate direct transfer to high levels of salinity.

The inability of *O. mossambicus* to survive direct transfer to full strength seawater had been explained by different authors (Hwang, 1987; Prunet and Bornancin, 1989; Uchida et al, 2000). According to these authors, the main element in the strong salinity tolerance of tilapia is attributed to the Chloride cells in the gill epithelium that has been considered responsible for controlling the osmoregulatory control of ions to the body when transferred to seawater (Hwang, 1987; Hwang et al, 1989). However, the functional modification of chloride cells do not occur immediately after transfer to full strength seawater; and pre-acclimation to a lower salinity level permits the fish to have sufficient time to adjust to dynamic changes in physiological, biochemical and morphological parameters (Hwang et al, 1989; Jonassen et al, 1997), the ultra

structural changes of chloride cells being of physiological significance (Hwang et al, 1989).

The time of exposure to the salinity level in which the fish was exposed has also been noted to have an effect on the mortality of the fish. Fish exposed to full strength seawater (35 ppt) has been observed to die quickly as compared to fish at 27.5, 30 and 32.5 ppt. This is because of the fact that as osmotic gradient in the external environment becomes higher, the time it takes the ions to move into the body fluids of the fish would be lower. The faster the movement of ions into the body fluids, the harder for the fish to regulate the lethal doses of ions in the body fluids (osmotic gradient in excess of 6 mOsm/kg per h in the first 24 hrs after direct transfer to salt water is lethal in *Oreochromis* spp, Al-Amoudi, 1987b) due to the dehydration of the fish (Hwang *et al.*, 1989). Hwang (1987) has found that the time it takes for chloride cells of the gill epithelium to change their shapes and adapt to ion regulations range from 12 to 24 hrs.

Conclusions

The survival rate of tilapia (*O. mossambicus*) in salt water was determined after direct transfer from freshwater, and it was found that tilapia *O. mossambicus* fully survived direct transfer from freshwater to a salinity levels from 0 to 25 ppt. Therefore, tilapia (*O. mossambicus*) can safely be transferred from freshwater to a maximum salinity level of 25 ppt directly without any acclimation. Transferring to higher salinity levels above 25 ppt would require acclimation in order to reduce high mortality of fish. This means that *O. mossambicus* can be used in tuna line fishery only after first transferring them to 25 ppt and then to higher salinity levels. It is therefore necessary to further investigate how to acclimate *O. mossambicus* to full strength seawater from the critical point of 25 ppt.

References

Al-Amoudi, M.M., 1987b. Acclimation of commercially cultured Oreochromis species to seawater – an experimental study. Aquaculture 65: 333-342.

Daud, S.K., Hasbollah, D. & Law, A.T., 1988. Effects of unionized ammonia on red tilapia (Oreochromis mossambicus/O. niloticus hybrid) fry. pp. 623p. In: T.B. R.S.V. Pullin, K. Tonguthai, J.L. Maclean (ed.) The second international symposium on tilapia in aquaculture, ICLARM Conference Proceedings, Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Deguara, S. & Agius C., 1997. Growth performance and survival of tilapia. Aquaculture Magazine: 43-54.

Gopalakrishnan, V., 1979. Status and problems of culture of baitfish for the skipjack fishery in the Pacific region. In: T.V.R. Pillay & Wm.A. Dill (ed.). Advances in Aquaculture. Papers presented at the FAO technical conference on aquaculture, Kyoto, Japan. pp. 58-62.

Hwang, P.P., 1987. Tolerance and ultrastructural responses of branchial chloride cells to salinity changes in euryhaline teleost Oreochromis mossambicus. Marine Biol. 94: 643-649.

Hwang, P.P., Sun, C.M. & Wu, S.M., 1989. Changes of plasma osmolality, chloride concentration and gill Na-K-ATPase activity in tilapia Oreochromis mossambicus during seawater acclimation. Marine Biol. 100: 295-299.

Jonassen, T.M., Pittman, K. & Imsland, A.K., 1997. Seawater acclimation of tilapia, Oreochromis spilurus spilurus Gunter, fry and fingerlings. Aquaculture Research 28: 205-214.

Perschbacher, P.W. & McGeachin, R.B., 1988. Salinity tolerance of red hybrid tilapia fry, juveniles and adults. pp. 623. In: T.B. R.S.V. Pullin, K. Tonguthai, J.L. Maclean (ed.) The second international

symposium on tilapia in aquaculture, ICLARM Conference Proceedings, Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Popma, T.J. & Lovshin, L.L., 1994. Worldwide prospects of commercial production of tilapia, Department of Fisheries and Allied Aquaculture, Auburn University, Alabama. 43 pp.

Prunet, P. & Bornancin, M., 1989. Physiology of salinity tolerance in tilapia: an update of basic and applied aspects. Aquat. Living Resour. 2: 91-97.

Stander, H. University of Stellenbosch, Department of Genetics. Pers. Comm.

StatSoft, Inc. (2001). STATISTICA (data analysis software system), version 6. www.statsoft.com.

Suresh, A.V. & Lin, C.K., 1992. Tilapia culture in saline waters: a review. Aquaculture 106: 201-226.

Tidwell, J.H. & Allan, G.L., 2001. Fish as food: aquaculture's contribution. pp. 958-963 EMBO reports.

Uchida, K., Kaneko, T., Miyazaki, H., Hasegawa, S. & Hirano, T., 2000. Excellent salinity tolerance of Mozambique tilapia (Oreochromis mossambicus): elevated chloride cell activity in the branchial and opercular epithelia of the fish adapted to concentrated seawater. Zool. Sci. 17: 149-160.

Villegas, C.T., 1990. Evaluation of salinity tolerance of Oreochromis mossambicus, O. niloticus and their F1 hybrid. Aquaculture 85: 281-292.

Watanabe, W.O., Kuo, C.-M. & Huang M-C., 1985a. The ontology of salinity tolerance in the tilapias Oreochromis aureus, O. niloticus, and an O. mossambicus x O. niloticus hybrid, spawned and reared in freshwater. Aquaculture 47: 353-367.

Appendix A

A 1. Effect of time of exposure on %survival of *Oreochromis mossambicus* exposed to different salinity levels.

	Test of SS Whole Model vs. SS Residual (Sheet1 in Imported)										
Dependnt Variable	Multiple R	Multiple R^2	Adjusted R^2	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	F	p
%survival	0.30187	0.09113	0.04178	26343.0	12	2195.25	262726.3	221	1188.807	1.84660	0.04239

A 2. Effect of salinity on the %survival of *O. mossambicus*.

	Test of SS Whole Model vs. SS Residual (Sheet1 in Imported)										
Dependnt Variable	Multiple R	Multiple R^2	Adjusted R^2	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	F	p
%survival	0.85124	0.72461	0.71482	209464.8	8	26183.11	79604.52	225	353.7979	74.00583	0.00

A 3. Effect of salinity, time of death and the interaction of both on the %survival of *O. mossambicus*.

	Test of SS Whole Model vs. SS Residual (Sheet1)										
Dependnt Variable	Multiple R	Multiple R^2	Adjusted R^2	SS Model	df Model	MS Model	SS Residual	df Residual	MS Residual	F	p
%survival	0.994134	0.988303	0.976706	285688.2	116	2462.829	3381.204	117	28.89918	85.22141	0.00

A 4. ANOVA showing the significant effect of salinity, time of death and the interaction of the two on survival.

	Univariate Results for Each DV (Sheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
GENERAL Effect	Degr. of Freedom	%survival SS	%survival MS	%survival F	%survival p
Intercept	1	1440542	1440542	49847.17	0.00
Salinity (ppt)	8	209465	26183	906.02	0.00
Time of death (hr)	12	26343	2195	75.96	0.00
Salinity (ppt)*Time of death (hr)	96	49880	520	17.98	0.00
Error	117	3381	29		
Total	233	289069			

APPENDIX B RAW DATA

Salinity Treatment	Exposure Time Treatment (hrs)	Replicate No.	Number dead	Number survived
freshwater	0	1	0	16
	3	1	0	16
	6	1	0	16
	9	1	0	16
	12	1	0	16
	15	1	0	16
	18	1	0	16
	21	1	0	16
	24	1	0	16
	27	1	0	16
	30	1	0	16
	33	1	0	16
	36	1	0	16
	0	2	0	16
	3	2	0	16
	6	2	0	16
	9	2	0	16
	12	2	0	16
	15	2	0	16
	18	2	0	16
	21	2	0	16
	24	2	0	16
	27	2	0	16
	30	2	0	16
	33	2	0	16
	36	2	0	16

Continued

Salinity Treatment	Exposure Time Treatment (hrs)	Replicate No.	Number dead	Number survived
15 ppt	0	1	0	16
	3	1	0	16
	6	1	0	16
	9	1	0	16
	12	1	0	16
	15	1	0	16
	18	1	0	16
	21	1	0	16
	24	1	0	16
	27	1	0	16
	30	1	0	16
	33	1	0	16
	36	1	0	16
	0	2	0	16
	3	2	0	16
	6	2	0	16
	9	2	0	16
	12	2	0	16
	15	2	0	16
	18	2	0	16
	21	2	0	16
	24	2	0	16
	27	2	0	16
	30	2	0	16
	33	2	0	16
	36	2	0	16

Continued

Salinity Treatment	Exposure Time Treatment (hrs)	Replicate No.	Number dead	Number survived
20ppt	0	1	0	16
	3	1	0	16
	6	1	0	16
	9	1	0	16
	12	1	0	16
	15	1	0	16
	18	1	0	16
	21	1	0	16
	24	1	0	16
	27	1	0	16
	30	1	0	16
	33	1	0	16
	36	1	0	16
	0	2	0	16
	3	2	0	16
	6	2	0	16
	9	2	0	16
	12	2	0	16
	15	2	0	16
	18	2	0	16
	21	2	0	16
	24	2	0	16
	27	2	0	16
	30	2	0	16
	33	2	0	16
	36	2	0	16

Continued

Salinity Treatment	Exposure Time Treatment (hrs)	Replicate No.	Number dead	Number survived
22.5ppt	0	1	0	16
	3	1	0	16
	6	1	0	16
	9	1	0	16
	12	1	0	16
	15	1	0	16
	18	1	0	16
	21	1	0	16
	24	1	0	16
	27	1	0	16
	30	1	0	16
	33	1	0	16
	36	1	0	16
	0	2	0	16
	3	2	0	16
	6	2	0	16
	9	2	0	16
	12	2	0	16
	15	2	0	16
	18	2	0	16
	21	2	0	16
	24	2	0	16
	27	2	0	16
	30	2	0	16
	33	2	0	16
	36	2	0	16

Continued

Salinity Treatment	Exposure Time Treatment (hrs)	Replicate No.	Number dead	Number survived
27.5 ppt	0	1	0	16
	3	1	0	16
	6	1	0	16
	9	1	0	16
	12	1	0	16
	15	1	0	16
	18	1	0	16
	21	1	1	15
	24	1	0	15
	27	1	0	15
	30	1	0	15
	33	1	0	15
	36	1	0	15
	0	2	0	16
	3	2	0	16
	6	2	0	16
	9	2	0	16
	12	2	1	15
	15	2	2	13
	18	2	1	12
	21	2	1	11
	24	2	0	11
	27	2	0	11
	30	2	0	11
	33	2	0	11
	36	2	1	10

Continued

Salinity Treatment	Exposure Time Treatment (hrs)	Replicate No.	Number dead	Number survived
25 ppt	0	1	0	16
	3	1	0	16
	6	1	0	16
	9	1	0	16
	12	1	0	16
	15	1	0	16
	18	1	0	16
	21	1	0	16
	24	1	0	16
	27	1	0	16
	30	1	0	16
	33	1	0	16
	36	1	0	16
	0	2	0	16
	3	2	0	16
	6	2	0	16
	9	2	0	16
	12	2	0	16
	15	2	0	16
	18	2	0	16
	21	2	0	16
	24	2	0	16
	27	2	0	16
	30	2	0	16
	33	2	0	16
	36	2	0	16

Continued

Salinity Treatment	Exposure Time Treatment (hrs)	Replicate No.	Number dead	Number survived
32.5 ppt	0	1	0	16
	3	1	0	16
	6	1	5	11
	9	1	2	9
	12	1	5	4
	15	1	0	4
	18	1	0	4
	21	1	2	2
	24	1	0	2
	27	1	0	2
	30	1	0	2
	33	1	0	2
	36	1	0	2
	0	2	0	16
	3	2	0	16
	6	2	5	11
	9	2	4	7
	12	2	4	3
	15	2	0	3
	18	2	0	3
	21	2	0	3
	24	2	0	3
	27	2	0	3
	30	2	0	3
	33	2	0	3
	36	2	0	3

Continued

Salinity Treatment	Exposure Time Treatment (hrs)	Replicate No.	Number dead	Number survived
30 ppt	0	1	0	16
	3	1	0	16
	6	1	0	16
	9	1	4	12
	12	1	2	10
	15	1	1	9
	18	1	1	8
	21	1	2	6
	24	1	0	6
	27	1	0	6
	30	1	0	6
	33	1	0	6
	36	1	0	6
	0	2	0	16
	3	2	0	16
	6	2	0	16
	9	2	0	16
	12	2	4	12
	15	2	3	9
	18	2	2	7
	21	2	0	7
	24	2	0	7
	27	2	0	7
	30	2	0	7
	33	2	0	7
	36	2	0	7

Continued

Salinity Treatment	Exposure Time Treatment (hrs)	Replicate No.	Number dead	Number survived
35 ppt	0	1	0	17
	3	1	1	16
	6	1	9	7
	9	1	6	1
	12	1	1	0
	15	1	0	0
	18	1	0	0
	21	1	0	0
	24	1	0	0
	27	1	0	0
	30	1	0	0
	33	1	0	0
	36	1	0	0
	0	2	0	16
	3	2	0	16
	6	2	10	6
	9	2	6	0
	12	2	0	0
	15	2	0	0
	18	2	0	0
	21	2	0	0
	24	2	0	0
	27	2	0	0
	30	2	0	0
	33	2	0	0
	36	2	0	0

COMPLETE REFERENCES

Al-Amoudi, M.M., 1987b. Acclimation of commercially cultured Oreochromis species to seawater – an experimental study. Aquaculture 65: 333-342.

Avella, M., Berhaut, J. & Bornancin, M., 1993. Salinity tolerance of two tropical fishes, *Oreochromis aureus* and *O. niloticus*. I. Biochemical and morphological changes in the gill epithelium. J. Fish Biol., 42: 243-254.

Balm, P.H.M., Haenen, H.E.M.G. & Wendelaar Bonga, S.E., 1995. Regulation of interrenal function in freshwater and sea water adapted tilapia (*Oreochromis mossambicus*). Fish Physiol. Biochem. 14: 37-47.

Daud, S.K., Hasbollah, D. & Law, A.T., 1988. Effects of unionized ammonia on red tilapia (Oreochromis mossambicus/O. niloticus hybrid) fry. pp. 623p. In: T.B. R.S.V. Pullin, K. Tonguthai, J.L. Maclean (ed.) The second international symposium on tilapia in aquaculture, ICLARM Conference Proceedings, Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Dean, D.B., Whitlow, Z.W. & Borski, R.J., 2003. Glucocorticoid receptorupregulation during seawater adaptation in a euryhaline teleost, the tilapia (*Oreochromis mossambicus*). Gen. Comp. Endoc., 132: 112-118.

Deguara, S. & Agius C., 1997. Growth performance and survival of tilapia. Aquaculture Magazine: 43-54.

Evans, D.H., 1993. Osmotic and ionic regulation. In: Evans, D.H. (ed). The physiology of fishes. CRC Press, Boca Raton, Florida. pp. 315-341.

Gopalakrishnan, V., 1979. Status and problems of culture of baitfish for the skipjack fishery in the Pacific region. In: T.V.R. Pillay & Wm.A. Dill (ed.). Advances in Aquaculture. Papers presented at the FAO technical conference on aquaculture, Kyoto, Japan. pp. 58-62.

Handeland, S.O., Berge, A., Bjornsson, B.T. & Stefansson, S.O., 1998. Effects of temperature and salinity on osmoregulation and growth of Atalantic salmon (*Salmo salar* L.) smolts in seawater. Aquaculture, 168: 289-302.

Hwang, P.P., 1987. Tolerance and ultrastructural responses of branchial chloride cells to salinity changes in euryhaline teleost *Oreochromis mossambicus*. Marine Biol. 94: 643-649.

Hwang, P.P., Sun, C.M. & Wu, S.M., 1989. Changes of plasma osmolality, chloride concentration and gill Na-K-ATPase activity in tilapia *Oreochromis mossambicus* during seawater acclimation. Marine Biol. 100: 295-299.

Imsland, A.K., Gunnarsson, S., Foss, A. & Stefansson, S.O., 2002. Gill Na+, K+-ATPase activity, plasma chloride and osmolality in juvenile turbot (*Scophthalmus maximus*) reared at different temperature and salinities. Aquaculture, 218: 671-683.

Jonassen, T.M., Pittman, K. & Imsland, A.K., 1997. Seawater acclimation of tilapia, Oreochromis spilurus spilurus Gunter, fry and fingerlings. Aquaculture Research 28: 205-214.

Likongwe, J.S., Stecko, T.D., Stauffer, J.R.,Jr. & Carline, R.F., 1996. Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia *Oreochromis niloticus* (Linneaus). Aquaculture, 146: 37-46.

Manzon, J.A., 2002. The role of prolactin in fish osmoregulation: a review. Gen. Comp. Endoc., 125: 291-310.

Morgan, J.D., Sakamoto, T., Grau, E.G. & Iwama, G.K., 1997. Physiological and respiratory responses of the Mozambique tilapia

(*Oreochromis mossambicus*) to salinity acclimation. Comp. Biochem. Physiol. 117A: 391-398.

Perschbacher, P.W. & McGeachin, R.B., 1988. Salinity tolerance of red hybrid tilapia fry, juveniles and adults. pp. 623. In: T.B. R.S.V. Pullin, K. Tonguthai, J.L. Maclean (ed.) The second international symposium on tilapia in aquaculture, ICLARM Conference Proceedings, Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Pienaar, U. de V. 1968. The freshwater fishes of the Kruger National Park. The National Parks Board of Trustees of the Republic of South Africa.

Popma, T.J. & Lovshin, L.L., 1994. Worldwide prospects of commercial production of tilapia, Department of Fisheries and Allied Aquaculture, Auburn University, Alabama. 43 pp.

Prunet, P. & Bornancin, M., 1989. Physiology of salinity tolerance in tilapia: an update of basic and applied aspects. Aquat. Living Resour. 2: 91-97.

Rankin, J.C., Henderson, I.W. & Brown, J.A., 1983. Osmoregulation and the control of kidney function. In: Rankin, J.C., Pitcher, T.J. & Duggan, R.T. (ed). Control processes in fish physiology. Croom Helm Ltd, Australia. pp 66-88.

Spaargaren, D.H., 1995. A functional model for describing the responses to salinity stress in aquatic animals. Comp. Biochem. Physiol. 111A: 501-506.

Stander, H. University of Stellenbosch, Department of Genetics. Pers. Comm.

StatSoft, Inc. (2001). STATISTICA (data analysis software system), version 6. www.statsoft.com.

Stickney, R.R., 1986. Tilapia tolerance of saline waters: a review. The progressive Fish-Culturist 48: 161-167.

Suresh, A.V. & Lin, C.K., 1992. Tilapia culture in saline waters: a review. Aquaculture 106: 201-226.

Tidwell, J.H. & Allan, G.L., 2001. Fish as food: aquaculture's contribution. pp. 958-963 EMBO reports.

Uchida, K., Kaneko, T., Miyazaki, H., Hasegawa, S. & Hirano, T., 2000. Excellent salinity tolerance of Mozambique tilapia (*Oreochromis mossambicus*): elevated chloride cell activity in the branchial and opercular epithelia of the fish adapted to concentrated seawater. Zool. Sci. 17: 149-160.

Villegas, C.T., 1990. Evaluation of salinity tolerance of *Oreochromis mossambicus, O. niloticus* and their F1 hybrid. Aquaculture 85: 281-292.

Vonck, A.P.M.A., Wendelaar Bonga, S.E. & Flik, G., 1998. Sodium and calcium balance in Mozambique tilapia, *Oreochromis mossambicus*, raised at different salinities. Comp. Biochem. Physiol. 119A: 441-449.

Watanabe, W.O., Ellingson, L.J., Wicklund, R.I. & Olla, B.L., 1988a. The effect of salinity in growth, food consumption and conversion in juvenile, monosex male florida red tilapia. pp. 623. *In:* T.B. R.S.V. Pullin, K. Tonguthai, J.L. Maclean (ed.) The second international symposium on tilapia in aquaculture, ICLARM Conference Proceedings, Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Watanabe, W.O., Kuo, C.-M. & Huang M-C., 1985a. The ontology of salinity tolerance in the tilapias Oreochromis aureus, O. niloticus, and an O. mossambicus x O. niloticus hybrid, spawned and reared in freshwater. Aquaculture 47: 353-367.