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STUDIES ON THE ACCLIMATION OF COMMERCIALLY CULTURED SAROTHERODON SPECIES TO SEA WATER

Thesis submitted to the University of Stirling
for the degree of Doctor of Philosophy

by

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March 1982

Graduation: June 1982

PREFACE

The ability of euryhaline teleosts to acclimate to sea water at a young age enables such species to be raised in areas where fresh water is short. In fact in very arid regions like Saudi Arabia this is the only alternative available for developing aquaculture. Acclimation to sea water is invariably associated with environmental, physiological and other problems depending, among other variables, on the species and geographical area.

This thesis is concerned with the acclimation of Sarotherodon species to sea water and associated problems. A general introduction to the subject is followed by a description of the methodology employed in this study. There then follows a description of the gradual acclimation of the fish to sea water, its effect on fish survival and consequent adjustments in the plasma osmotic concentration. An assessment of the benefits of feeding high salt diets to the fish prior to transfer to salt water in alleviating the resulting osmotic stress forms the basis of the following chapter. The next two chapters investigate the effects of some stressful environmental factors such as suboptimal temperatures and poor water quality on the ability of the fish to transfer to salt water. This is followed by an evaluation of the effects of different salinities on growth rates of fish. Fish gills with their chloride cells are vital sites for controlling plasma osmotic concentration. The penultimate section describes light and electron microscopical examination of gill epithelia with their chloride cells both in fresh and sea water adapted fish. Finally some general conclusions emerging from this study and areas which need to be urgently investigated are discussed.

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ACKNOWLEDGEMENTS

My gratitude goes to my supervisor Professor R.J. Roberts for his constant encouragement and excellent supervision. Dr J. Muir, my joint supervisor, suggested this research project, showed great interest and provided valuable comments throughout the work. I am indebted to Dr C. Agius for his help with electron microscopy, his advice and critical comments on the manuscript.

Thanks are also due to all the academic staff at the Institute of Aquaculture, particularly Drs D. Macintosh and B. McAndrew for supplying fish and Dr K. Jauncey for advice and help with fish nutrition. Messrs I. MacGowan, J. Boyd, A. Porter and W. Hamilton helped with fish maintenance. Mr I.H. MacRae and his staff helped with the histology.

I would also like to thank Mr T. Forrest of the Biology Department and his staff for their help with the electron microscope. Special thanks are also due to Dr D.S. McLusky for his help with the osmolality determination.

The Ministry of Higher Education of the Kingdom of Saudi Arabia and the Saudi Arabian Cultural Attache in London organised my stay in Scotland with great efficiency.

My fellow research students, especially Mr H. Appler, helped in many ways, especially during difficult times.

Mrs A. Agius typed this thesis with great patience and care.

Sincere thanks to my wife Fareedah, my daughters Reem and Raniah for their patience, and the newcomer Fatimah for bringing much joy that helped alleviate the pressure of work. Finally I would like to dedicate this work to my son Ibrahim. May there be a successful and happy future for them all.

ABSTRACT

The initial aims of the present study were to investigate the problems associated with salt water transfer in Sarotherodon spp. with the objective of maintaining high rates of survival and satisfactory growth rates. Members of the euryhaline commercially cultured Sarotherodon species viz. Sarotherodon aureus, S. mossambicus, S. spilurus, S. niloticus and the hybrids of S. aureus/S. niloticus were used. The specific salinity tolerance and the capability of tolerating direct transfers to specified salinities and the comparative abilities of surviving gradual increases of salinity up to full strength sea water were investigated. The involvement of the plasma osmotic concentration in the osmoregulatory process, and the physiological changes following the direct transfer to salt water were examined with special reference to the possibility of using changes of plasma osmotic concentration as an indicator of fish transferability. S. aureus proved to be best able to withstand salinity changes, though in all cases gradual transfer was required to limit mortalities.

Feeding of high dietary sodium chloride diet was evaluated for S. mossambicus and S. aureus/S. niloticus hybrids as a method of stimulating osmoregulatory process prior to salt water transfer. This was found to alleviate only slightly the osmotic stress following direct transfer to a known lethal salinity.

Water deterioration and temperature fluctuation during fish transportation are inevitable, especially after long journeys. The

effects of water quality deterioration, temperature fluctuation and food deprivation, typical of transport conditions, were studied in combination with salinity transfer effects. Effects were significant in all cases though, of the species studied, S. niloticus appeared to show the highest resistance to the combined effects.

Following full acclimation to full strength sea water, the effects of prolonged exposure to sea water on the subsequent survival, growth rates and food conversion were investigated. S. niloticus and the hybrids of S. aureus/S. niloticus were found to be less tolerant to long-term exposure than the other candidates, which were selected for further detailed study.

Light and electron microscopic studies of the gills and chloride cells were carried out in S. mossambicus and S. spilurus. This study showed the modified role of the chloride cells in fresh and sea water environments. Increases in number and developments of the ultrastructure of these cells were observed in sea water adapted samples from both of the species. Consequently the significance of the chloride cells in the osmoregulatory process was discussed.

The significance of these results was considered in terms of aquaculture practice and suggestions are made for improved transfer methods in this context.

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

The Basis of the Study

The significance of imported food for the entire Arabian peninsula has been confirmed by all the recent national and international reports relating to food and nutrition (FAO, 1980). This of course is mainly due to the very rapid economic growth of the area, the increased value of oil exports, and to the concomitant major increase in population. Hopper and Peacock (1978) for instance stated that the Kingdom of Saudi Arabia imported 35,550 tonnes of fish in 1977 for human consumption, double the figure for three years earlier. There is little doubt that were statistics available for the succeeding three years, to 1981, a continuation of this trend would be demonstrated.

Since oil represents about 97% of the total annual income in almost all countries of the area, it seems obvious that if the oil wells were to be destroyed or worked out or if foreign sources of food were to be cut off for any political, economic or environmental reason, the expected results would, without massive international aid, be mass starvation for the Arabian peoples. In attempts to insure against this risk, all of the countries of the area have development programmes aimed at decreasing this major dependence on imported food both by greater exploitation of existing food sources and developments aimed at creating new sources of food production.

During a four year collaborative fisheries development project between the White Fish Authority of the United Kingdom and the Ministry of Agriculture and Water Resources of the Kingdom

of Saudi Arabia it was concluded that the demand for fish in Saudi Arabia could in no way be satisfied either by the present fishery or by any foreseeable development of that fishery (Peacock, 1979). This pointed to the only alternative approach, that of introducing marine culture as a radical new approach to the provision of indigenously produced fish. Among the local species considered for such culture were species of the familes Mugilidae, Serranidae, and Siganidae. Unfortunately, however, preliminary surveys showed that none of these indigenous species possessed very much potential, with present technology for commercial fish farming, in the short term (Neve, 1978), since it was obvious that none of these species had been sufficiently studied in an aquaculture situation to establish a commercially acceptable breeding technique. The exploitation of fresh water culture in such a very arid country would be unthinkable. Thus attention was directed towards species which could survive in the tropical marine environmental conditions of the area and for which commercially viable production techniques, particularly at the hatchery stage, were available.

The African Tilapia (and Sarotherodon) species combine high growth rate and hardiness, with an optimum temperature range that includes the spread of Red Sea values (between 25-30°C). Tilapias are essentially fresh water fish species although salt water living tilapia have been reported (Bayomi, 1969), and salt water ancestry has been postulated to account for the large number of euryhaline species among them (Kirk, 1972). However, the salinity tolerance of the various species, the physiological response of the osmoregulatory organs, and the growth rate response in sea water had not yet been adequately defined.

Since the commercially cultured Sarotherodon species have been reported to attain better growth rates and bigger sizes than their counterpart Tilapia species (Fryer and Iles, 1972; Chervinski and Zorn, 1974) and are thus commonly chosen as culture species in Africa, and since Sarotherodon species are already widely dispersed and more readily obtained, these were considered the most suitable individual species for studies on their feasibility for use in an arid environment, and since the scarcity of fresh water was likely to be the major limiting factor in any commercial development, the present study was defined in order to allow investigation of the physiological capacity of various Sarotherodon species to adjust at an early age to high salinities and to study the feasibility of using various husbandry manipulations such as acclimation and prestimulation with high salt diets to allow more rapid adaptation to a haline environment.

The species studied were the following euryhaline commercially cultured Sarotherodon species:

1. Sarotherodon mossambicus (Peters)
2. Sarotherodon spilurus (Günther)
3. Sarotherodon aureus (Steindachner)
4. Sarotherodon niloticus (L.)
5. Sarotherodon aureus/S. niloticus hybrids (Figs. 1-5)

The first task was to investigate the salinity tolerance of the young stages of each species, the associated physiological changes and the effect of gradual acclimation on reducing fish mortalities associated with acclimation. Once the most reliable

18.6

Figure 1

Young male of Sarotherodon mossambicus 12 cm S.L.

The body of this species is slim and slightly dark at all sizes with caudal peduncle about as deep as it is long. The overall profile is long and sloping. This species is endemic to the warmer waters of Southern and South Eastern Africa.

Figure 2

Ripe male of Sarotherodon spilurus 14.7 cm S.L.

The body is elongated but deeper than that of S. mossambicus and with thicker caudal peduncle. Edges of dorsal and tail fin orange yellowish. Note the concave profile of the dorsum and upper head. This species is indigenous in an area north of the range of S. mossambicus in East Africa.

Figure 3

Male of Sarotherodon aureus 17.6 cm S.L.

This is an attractive species with its moderately elongated and rounded body. Note the bright reddish edges of the dorsal fin. The brown bars on the tail fin are reminiscent of S. niloticus, but these are discontinued and located only near root of the tail. Upper line of head profile running upwards from mouth at sharp angle. The natural distribution of this species is wide and extends from Middle and North Africa to the Middle East.

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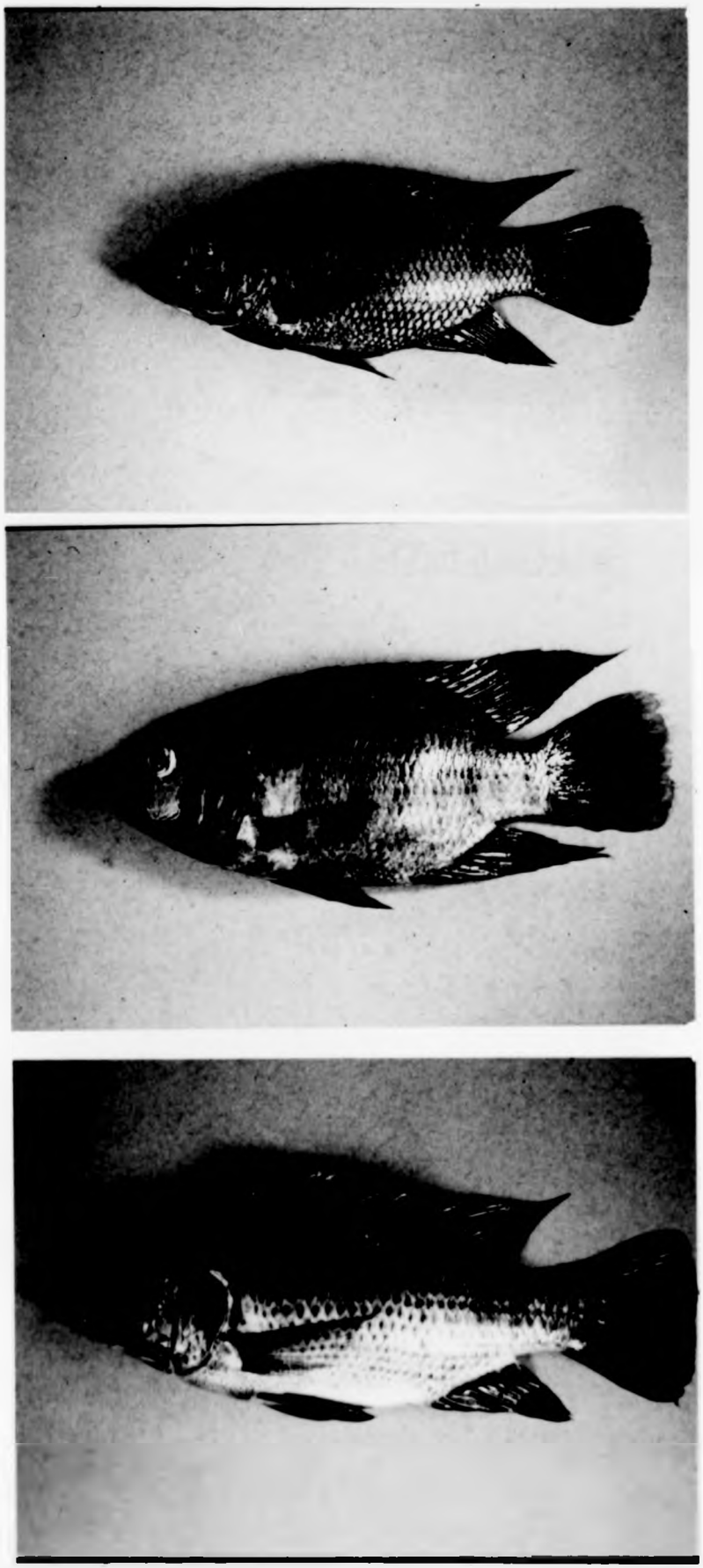


Figure 4

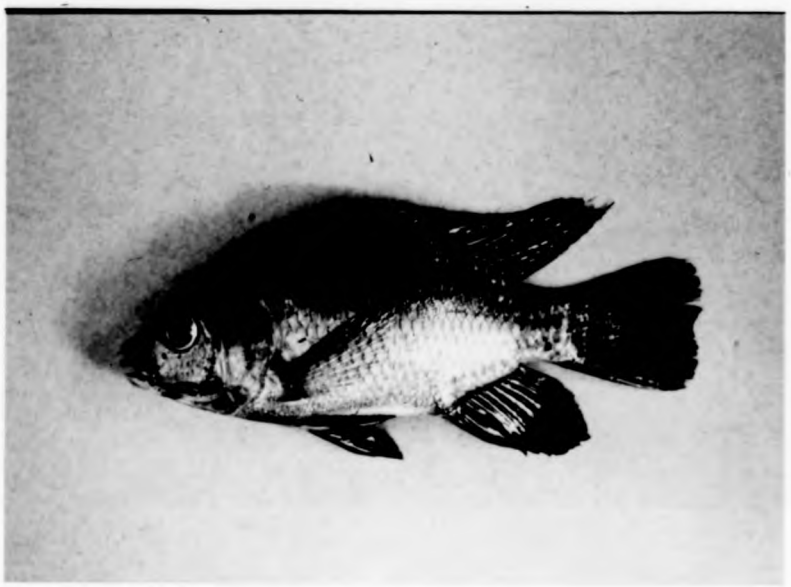
Young male of Sarotherodon niloticus 11.3 cm S.L.

The body shape of this species is generally deep with a slightly rounded profile. It is distinguished from the other species by the dark brown bars over the whole tail. The upper head profile is straight or slightly convex. This species is endemic to the same natural habitats of S. aureus

Figure 5

Young male of Sarotherodon aureus/S. niloticus hybrids 11.8 cm S.L.

The body is remarkably deep with slightly rounded profile. Generally this crossed species possesses morphological characteristics passed from both parents of S. aureus and S. niloticus



regime of sea water acclimation had been defined it was decided to investigate the possibility of reducing the osmotic stress of transfer by feeding of a high salt diet beforehand. Jackson (1979) had reported that feeding of a diet containing a high level of NaCl prior to the transfer to sea water had proved beneficial in reducing both the osmotic stress and the mortality in young rainbow trout (Salmo gairdneri) transferred to salt water. Water quality is always a major constraint to fish culture and especially in the conditions of fresh water shortage and poor fresh water quality which occur in Arabia it was considered important to have indications of the minimum acceptable levels of water quality parameters which could be correlated to reasonable transfer survivals. Finally the growth rate obtainable with the different species of Sarotherodon fully acclimated to sea water were studied to confirm the potential of these species for intensive culture in Arabian marine conditions.

General Biological Characteristics of Tilapias

The tilapias were generally in the past considered to be one genus but recent study by Trewavas (1982) supported by genetic studies by McAndrew (1981) have shown that they are best classified as two genera with the nest brooders remaining as the members of the genus Tilapia and the mouth brooders having their own taxon as the genus Sarotherodon.

There are great differences in the feeding habits of the different tilapias. However, most of the commercially cultured

Sarotherodon species are omnivorous and the species used in the present study were all readily adapted to feeding on artificial pelleted diets.

Growth rates vary between species and conditions of culture. Males usually have a higher growth rate over females and therefore successful tilapia culture is very dependent on methods of sexing juvenile fish to take advantage of all-male culture. Maturation does not appear to have a major effect on the growth of male fish but females do not grow during spawning time and the reproductive energy requirement makes food conversion very much less efficient than in males.

The two "Tilapia" genera differ in their breeding characteristics. Tilapia species are "nest brooders" and lay large numbers of eggs. The Sarotherodon species are "mouth brooders". In this case the male makes the nest, the female deposits eggs in it, and then picks them up in her mouth after which the male deposits sperm in the nest and this is also picked up by the female. Each female lays 75-500 eggs and hatching occurs about 3-7 days after fertilisation.

Larvae remain in the mouth of the female until the yolk sac is absorbed. They may then leave, although the mouth is still used as a retreat for a period while the young fry are vulnerable to attack by predators. Mouth brooders produce up to 3000 eggs per year, whereas nest brooders can be expected to produced 6-7 times that amount.

Most tilapias are tolerant of brackish water but some are better adapted to it than others and may thrive and even breed in sea water (Bardach, 1972). They are essentially tropical lowland fish, though tolerance of extreme temperature ranges have been reported for most of them (Yashouv, 1958; McBay, 1961; Kirk, 1972).

Culture of Tilapias

In fresh water, tilapias are generally produced in extensive systems, but there has been a recent trend towards intensive culture in cages or ponds (Reynolds, 1976; Moav et al., 1977; Galbreath and McCoy, 1980; Balarin and Haller, 1982).

Two types of stocking systems are in common use - polyculture and monoculture. In the former system more than one species of Tilapia or Sarotherodon, often with other fish species, is usually used while in the monoculture only one species is used. Polyculture systems have been reported to be more productive than monoculture in terms of total pond productivity (Moav et al., 1977), but in order to eliminate competition precautions must be taken as the ecological niches of various tilapias are imperfectly known and there is great difficulty in such uncontrolled systems in maintaining monosex culture. However tilapias can tolerate a very high stocking density (up to 217 kg. fish per cu.m of water) and grow well (Otte and Rosenthal, 1979).

Depending on the latitude and objectives of culture, a variety of types of ponds has been used in different countries. These have ranged from subsistence and commercial fresh water

ponds in the Cameroon to experimental intensive culture in power station cooling ponds in Belgium (Jauncey, personal communication). Beside the traditional pond culture systems successful culture has also been developed in cages (Pagan, 1969 and 1970), raceways (Kloppfenstein and Kloppfenstein, 1977), tanks (Haller, 1974) and rice fields (Huet, 1972).

The major problem of tilapia culture is the difficulty of preventing them from spawning and thus wasting food and energy on reproduction and producing unbalanced populations of small fish. The young of these prolific fish mature at an age of 2-3 months at which time they are 6-10 cm long. From then on they can breed every 3-6 weeks for 18 months or more (Macintosh, 1982).

Several methods have been suggested to reduce this over-population in tilapia culture. These include separation of the parents and young, monosex culture, control by predators, cage culture, chemical castration and hormonal sex reversal. However, the most reliable technique has been monosex culture by hybridisation of interspecific or intraspecific crosses to produce all-male offspring (Chen, 1969; Pruginin et al., 1975; Lovshin and Da Silva, 1975), and provided parent stock are pure this works well.

Advantages of Sea Water Culture

In temperate regions it is already well recognised by trout farmers that an extra increment of growth can be obtained in winter by transferring rainbow trout to warmer sea water. Such

promotion of better growth in sea water was first described by Sverdrup et al. (1942) and this has led to an expansion of use of marine sites. In contrast to the limited number of suitable fresh water sites inland, sea water culture offers a considerable range of suitable unexploited areas in the temperate countries and has still to reach its full potential.

In tropical waters, however, temperature is uniformly high (about 28°C) and seasonal differences are less marked (about 8°C) (Bogoruv, 1960). Thus the advantages of using the marine environment are not based on temperature but on their availability as sources of reliable readily available pure water, which is particularly important in arid sub-tropical areas such as the Arabian peninsula. Exploiting sea water for fish culture purposes thus presents possibilities of great potential in areas such as this. As has been mentioned earlier, tilapias appear to have distinct advantages in this respect and the limited published work available relating to growth of tilapias in salt water suggests that provided transfer acclimation can be achieved, transfer of most of the known species to salt water does not appear to have any adverse effect on the growth of the fish (Zanaveld, 1959; Chervinski and Zorn, 1974).

The Red Sea is known as one of the warmest and purest semi-closed seas in the world. This is in part due to its geographical location and partly to the lack of human activity around its shores. Previous study has shown that its waters are of excellent quality with high levels of oxygenation (Howard et al., 1978). Equally

important tilapia is a known and well recognised fish in the area because of its availability from Palestine and from trade with East Africa, although it is not indigenous. This is a significant advantage because fish as a consumer product is notoriously difficult to market if not already accepted by the consumer and there is little or no consumption of indigenous fresh water fish in peninsular Arabia for obvious reasons. Fattening of tilapia in sea water also adds to its market appearance as large silvery fish caught in sea water are very much appreciated in the area, especially when they do not have large numbers of small bones and are of firm texture and white flesh.

Salt Secretion in Fish

The main object of the present study was to determine the feasibility of utilising the physiological mechanisms for euryhalinity for transferring tilapias at an early stage to salt water. It was therefore considered important to check that these species possessed the same morphological components for salt secretion as other teleosts and to observe their modifications under high salinity conditions. Thus it was considered of interest in the present study to include a brief description of the salt secretion cells in these fish and to determine how they altered both in number and form at high salinity.

CHAPTER 2

GENERAL MATERIALS AND METHODS

Fish Stocks

The fish used in the studies were pure stocks of *Sarotherodon mossambicus*, *S. aureus*, *S. spilurus*, *S. niloticus* and *S. aureus*/*S. niloticus* hybrids. All of the fish used were obtained from the tropical hatchery of the Institute of Aquaculture at the University of Stirling. These fish were tested electrophoretically (McAndrew, pers. comm.) for accurate identification and control of stock purity.

The numbers and the weights of fish used in each experiment will be indicated individually in the text relating to those experiments.

Holding Facilities

Glass tanks

Most of the fish used in the salinity test experiments were held in groups of glass tanks. Two different sizes of such glass tanks were used depending on the numbers of fish and their sizes in each case. The bigger tanks measured 100 x 40 x 30 cm and 100 L. capacity each. The big tanks were fitted with E-heim filters of 4 L. volume and 6.3 L./min. pumping capacity each. These filters were filled with gravel of about 1 cm diameter and cottonwool to act as biological filters. The small tanks measured 50 x 40 x 30 cm and 50 L. capacity each and were equipped with seeded undergravel filters (50 x 40 x 5 cm) of about 0.5 cm diameter gravel. In all cases the filters were left running for 2 weeks before use with the experimental fish to ensure full filter activity.

In all the tanks except those used for temperature studies, the water temperatures were maintained at 26-28°C throughout the experimental period by "Interpet" combined

heater/thermostat heating units (200 watts). Two heating units were used in each big tank and one in each of the small tanks.

Recycling systems

The recycling systems were mainly used for study of the effects of salinity on the fish growth rate. Thus a full description of these systems will be given in Chapter 7.

All of the glass tanks and the recycling system tanks were artificially aerated (one air stone in each tank) so as to provide a dissolved oxygen level of at least 90% saturation.The water quality was also monitored regularly (vide infra).

Diet

Jauncey (1982) has shown that a diet of 37% protein was optimum for tilapias. Thus with the exception of the high salt diet experiment (Chapter 4), for all experiments of the present thesis a standard diet of 37.71% protein was used. In the experiments described in Chapter 4 the total amount of protein was slightly decreased to 36.51% as a result of adding 10% dietary sodium chloride. Appendices 1-4 provide the composition of the experimental diets, the mineral mix, the vitamin mix and the proximate analysis of the experimental diet used in the present thesis.

The weight of diet required for each experiment was first estimated according to the weight of the fish and the expected maximum growth rate. Between 15 and 20 per cent was added to

allow for losses during pelleting and drying. The dietary ingredients were then sieved to a particle size of less than 1 mm prior to weighing and pelleting to ensure that a homogenous mixture was obtained.

Diets were prepared by thoroughly mixing the dry ingredients in a domestic food mixer with the corn and fish oils. The requisite amount of water was slowly added to the diet which was continuously stirred until a stiff dough-like consistency was obtained. The moist diet was then extracted through a 2 mm die and the resultant pellets air dried at 30°C. Before use the pellets were broken into approximately 5 mm lengths. Further crumbling of the pellets into smaller sizes was carried out when feeding very young fry.

Water Quality

Since maintenance of good water quality is an essential factor in reducing extraneous environmental stress and in allowing the correct interpretation of experimental stress effects, routine water quality measurements were made. Thus in addition to measuring salinity, oxygen and pH levels, water samples were regularly collected from all the experimental tanks for monitoring of the ammonia and nitrite concentrations. Monitoring was done at least weekly and water samples taken at least every 2 weeks, though the schedule was changed, and samples taken more frequently during critical salinity transfers or periods of poorer water quality. Once the filters had established it was found that unless deliberately altered, water quality was maintained at satisfactory levels at all times.

Determination of ammonia

Full procedures for the preparation of chemicals for tests are given in Appendix 5, but briefly the method used for ammonia determination was a modified phenol hypochlorite method for fresh,

brackish and sea water samples (Muir, personal communication). The chemical reaction is that ammonia reacts with phenol and alkaline hypochlorite to give indophenol blue, intensified in colour by sodium nitroprusside.

In a 25 ml measuring flask, 10 ml of test sample was placed. 2.5 ml from each of citrate buffer, phenate reagent and alkaline hypochlorite solutions were added. The sample was then mixed well after each addition and made up to 25 ml with deionized water, stoppered and mixed well again. The sample was allowed to stand for 20 minutes at room temperature and then its absorption measured in a 1 cm cell at wave length of 700 nm.

Ammonia was determined as total ammonia-N (un-ionized + ionized ammonia as nitrogen). To obtain the concentration of unionized ammonia the following formula was used:

$$(NH_3 - N) = \frac{(\text{Ammonia} - N)}{1 + 10.0^{(pKa - pH)}}$$

where (Ammonia - N) = the measured concentration of total ammonia

pKa = the activity constant of the reaction

pH = the measured pH of the solution

The pKa value could be computed as a function of temperature from a comparative value scale provided by the work of Emerson et al (1975)(Appendix 6). At 27°C the pKa value is equal to 9.1839. Under normal pH and temperature conditions the percentage of un-ionized ammonia-N may range from 0.1 to 15 per cent.

Determination of nitrite

A standard method for nitrite determination was used (Strickland and Parsons, 1972). The principle of the reaction is that nitrite reacts with sulphanilamide in acid solution to form a diazo compound which combines with N-(1-naphthyl)-ethylene diamine (N.E.D) to produce a highly coloured compound. Colour produced is proportional to nitrite concentration.

To 50 ml of sample 1 ml of sulphanilamide was added and the sample left for 2-8 minutes. 1 ml of N.E.D. was then added and the sample mixed immediately. The absorption was measured at 543 nm within 10 min. to 2 hours. (see Appendix 7 for preparation of chemicals).

Artificial Sea Water

A variety of pre-packaged mixes of artificial sea water were used. The most common one was made by Tropicarium-Buchschlog Williborst of West Germany. In all cases however the salt mixture of the artificial sea water were approximately identical to those in natural sea water in ratios and concentrations.

Two big circular tanks of 100 gallons capacity each were used. One was for newly prepared artificial sea water and the other was equipped with subgravel biological filter and aeration, and was used for re-conditioning of old artificial sea water.

Artificial sea water was made by adding the pre-packaged salt mix to fresh water until the desired salinity concentration

was achieved. The solution of the trace elements was then added and the newly prepared artificial sea water was aerated for a minimum of 2 days before use. The desired salinity concentration was maintained by regular chemical analysis or by salinity probe (EIl MC5/2 oceanographic salinity and temperature measuring bridge). Regular testing of the quality of artificial sea water was also carried out.

The chemical method used for the determination of salinity concentration was the Harvey modification described by Martin (1968). Basically silver nitrate reacts and precipitates all the halogens in sea water, and the end points of the reaction indicated by potassium chromate.

Blood Sampling

The minimum amount of blood required from each fish for blood salinity assays was a full haematocrit tube. It was very difficult at times to collect this amount from some of the small juvenile (3-4g) fish. To obtain blood fish were first anaesthetised in a 1:10,000 solution of benzocaine (Ethyl-p-amino benzoate: Ross and Geddes, 1979). They were then blotted by wrapping in a paper towel. Blood was obtained through severance of the caudal peduncle. Blood flowing from the severed caudal artery was drawn straight into a heparinised haematocrit tube. The tube was then sealed in a bunsen burner flame. The sealed tubes were then placed in a Hawksley Micro-Haematocrit centrifuge and spun for 5 minutes. The haematocrit values were obtained using the Hawksley micro-haematocrit reader. Each haematocrit tube was then cut into two

halves, the half that contained the packed cells was discarded and the other half containing the remaining plasma was resealed, numbered, and deep frozen until required for analysis.

The plasma osmotic concentrations were obtained through the determination of the freezing point depression. The method used in the present thesis was first described by Ramsay and Brown (1955); and a detailed description of the method has been published by Coast (1973).

Essentially a small volume of sample enclosed in a capillary is placed in a bath of alcohol within the machine at a temperature below the expected melting point of the plasma sample. As the bath is slowly warmed the temperature is continuously recorded on a highly sensitive thermometer so that the exact temperature at which the plasma melts can be determined by observation.

The instrument used for the present work was more advanced than that originally described by Coast (1973), in that it had refinements involving both the cooling mechanisms and the highly sensitive electronic thermometer connected to it. The electronic thermometer was capable of accuracy to the fourth decimal place and had a reading frequency ranging from 10/sec to 1/10 sec, which allowed extremely accurate measurments of the freezing points. The osmolality of each sample was derived from its freezing point by the following formula:

$$\text{Osmolality} = \frac{\Delta^\circ C}{1.858} \times 1000 \text{ mOsm/kg water}$$

where $\Delta^\circ C$ = freezing point depression

Determination of Body Water Content of Fish

A standard method for moisture determination of small fish was used. The fish were killed and then carefully placed onto previously tared petri dishes and weighed accurately. The petri dishes with their samples were then placed in a drying oven at 105°C until constant weights were obtained indicating loss of all moisture. The weight of the resultant dry material was then obtained by subtraction of the tare of the petri dish.

CHAPTER 3

ACCLIMATION TO SEA WATER

INTRODUCTION

The first reports of the feasibility of culture of tilapias in sea water were by Vas and Hofsted (1952) who reported promising growth and regular breeding (see Chapter 7) of Sarotherodon mossambicus in sea water ponds of 30‰ salinity. Subsequent to these reports, the adaptability of various members of the genera Sarotherodon and Tilapia to sea water has been widely investigated (Lotan, 1960; Canagaratnam, 1966; Chervinski and Herring, 1973). Most of these workers have employed only one species in their studies and in some instances there was some doubt as to the true identity of the species used (Chervinski, 1966). Moreover, in most instances fish survival has been the sole criterion used to evaluate the tolerance of these species to various strengths of salt water (Fukusho, 1969; Chervinski and Hering, 1973; Maruyama, 1974).

In a limited number of studies the osmotic concentration of the plasma has been used as an indication of tolerance to increasing salinity for fish. This has been principally in studies of salmonids (Houston, 1959; Conte and Wagner, 1965; Jackson, 1981), but Lotan (1960) working with S. niloticus (suspected in fact to have been S. aureus) observed a rise of the plasma osmotic concentration with increasing salinity.

In the present study the salinity tolerance of four Sarotherodon species is compared, viz. S. mossambicus, S. spilurus, S. aureus and S. niloticus. In addition, S. aureus/S. niloticus

hybrids, a cross which produces an all-male progeny given that the brood stock are genetically pure strains as in the present case, have also been studied. Fish survival and their plasma osmotic concentration were used as indices of fish adaptive ability and tolerance to direct transfer to salt water. Jackson (1981) observed that ability to control the plasma osmotic concentration is the best indicator of the ability of fish to adjust to changes in salinity.

The effect of direct transfer to salt water on body water content has been observed in salmonids (Houston,1959; Jackson, 1981) and in cyprinids (Maceina and Shireman, 1979). Maruyama (1974) observed a depression of 8.5% in the body water content of starved S. niloticus after transfer to salt water. Therefore it was also decided to evaluate the effect of direct transfer to salt water on the body water content of the fish. S. aureus/S. niloticus hybrids were used for this study.

MATERIALS AND METHODS

Fish

Fish fry of Sarotherodon aureus, S. mossambicus, S. spilurus, S. niloticus and S. aureus/S. niloticus hybrids were used in the present experiments. In all cases the fish were transferred from the hatchery of the Institute of Aquaculture to the holding fresh water tanks as described in Chapter 2. They were left in these tanks for a period of two weeks prior to being transferred to the acclimation tanks containing the various salinity concentrations. During this time the fish were fed on the standard diet. They were however always starved for 24 hours before a transfer was to be carried out.

Experimental Design

The studies comprised two groups. The first was a series of trials to establish the salinity levels which could be tolerated by the individual species, and the second group related to the physiological effects of such transfer on plasma osmolality and on total body water.

A. Establishment of salinity tolerance levels

Two different types of trials were carried out for these studies. The first was a broad spectrum approach to try to define the narrow range over which the second study, a precise analysis of salinity tolerance, should be carried out.

Experimental Trial 1

Comparison of direct transfer and gradual acclimation to sea water

The wide salinity range of 25, 50, 75 and 100 % sea water was used so as to determine the effect of direct transfer to these salinities on fish mortalities and to define the range over which the maximal tolerance level could be sought in subsequent experimental trials. Fry of 4 g. average weight of all the species of the study, viz. S. aureus, S. mossambicus, S. spilurus, S. niloticus and S. aureus/S. niloticus hybrids were used. These fry were either transferred sequentially through the range of the next higher salinities employed after holding for a period of 48 hours in the previous strength, or where very poor transferability was apparent they were held for longer periods in a particular level of salinity. For acute effects they were transferred directly from fresh to full strength sea water (36‰). The survival rates in different concentrations of salinity employed are shown in the tables of the results section. During the acclimation period fish were fed on astandard diet at approximately 5% of their body weight per day, but as indicated above they were deprived of food for 24 hours prior to each transfer.

Experimental Trial 2

Definition of precise critical salinity tolerance values

Precise critical salinity tolerance was determined by transferring thefish directly to a narrower range of salinities, ie. 60, 65, 70, 80, 85 % sea water on the basis of the information

derived from the first set of trials. S. mossambicus, S. spilurus, S. aureus/S. niloticus hybrids and S.niloticus, all of mean size 5g., were used in this trial.

The aim of this particular trial was to determine the precise salinity tolerance limits so as to allow later evaluation of the effects of alleviating osmotic stress by the feeding of high salt diet (Chapter 4) on enhancing tolerance or the deleterious effects of adding further stress on the fish prior to the transfer by exposing them to the poor water quality (Chapter 5).

The two trials were set up with triplicate groups of 20 fish, so that a total number of 400 and 350 fry from each of the species were used in the first and second trial respectively.

B. Study of the effect of salinity on plasma osmolality and body water content

1. Osmolality of Sarotherodon plasma in fresh and sea water

For the definition of physiological plasma osmolality levels in fresh and full sea water, fish of varying sizes were taken from various tanks. Usually 6 fish from fresh water and 6 from full sea water acclimated fish per species, viz. S. mossambicus, S. spilurus, S. niloticus and S. aureus/S. niloticus hybrids, were examined. The fish were examined for both the osmotic concentration of the plasma and for their haematocrit values.

2. Effects of sudden salinity change on plasma osmolality

For the study of the effects of the direct transfer to dilute sea water on the osmotic concentration of the plasma 50 fish (20 g. average size) were used from each of the species viz. S. mossambicus, S. spilurus, S. niloticus and S. aureus/S. niloticus hybrids. The normal 100 L. glass tanks were used.

The salinity concentration of 60% sea water was selected for this trial because it was a level which was stressful but tolerable for the four species of the present study (see salinity tolerance results section) and represented a salinity concentration well above the isosmotic point of the fish. All of the fish were fasted for 24 hours prior to direct transfer to this salinity. Three fish were sampled every 3 hours on the first day and every day thereafter for a period of 120 hours.

3. Body water content of S. aureus and S. niloticus hybrids in fresh water and in full sea water

Since only limited numbers of suitably sized fish were available for this aspect of the study, only one group of the hybrid S. aureus/S. niloticus was used and only 5 fish from fresh water stocks and four from fully sea water acclimated stocks were used.

4. Effect of salinity change on body water content

Sixty fish (mean weight 17.75 g.) of S. aureus/S. niloticus hybrids were used. The normal 100 L. glass tanks were prepared as in Chapter 2 and the fish were treated as in 2 above.

RESULTS

Comparison of Direct Transfer and Gradual Acclimation to Sea Water

All the fish species of this study viz. *S. aureus*, *S. mossambicus*, *S. spilurus*, *S. niloticus* and *S. aureus*/*S. niloticus* hybrids, were able to tolerate the direct transfer to 50% sea water (18‰) without mortalities, whereas the time for complete mortality in any given lethal concentration varied according to species. Thus in full strength sea water *S. aureus* were able to survive for 9 hours while *S. niloticus* did not survive for even 4 hours in the same salinity. They were also able to acclimate to full sea water (36‰) via gradual acclimation. Tables 1 and 2 summarise the results of the studies in relation to acclimation time, the gradual acclimation and the effect of the direct transfer to different concentrations of sea water on fish survivals.

The highest adaptability to salinity was demonstrated by *S. aureus*, *S. mossambicus* and *S. spilurus* in descending order. A large proportion of individuals of these fish species was capable of direct transfer to salinity of 75% sea water (27‰) with observed mortality varying from 20% after 48 hours in the case of *S. aureus* to 35% for *S. mossambicus* and 43.3% for *S. spilurus* (Table 1).

Moreover, the gradual acclimation regimes used to evaluate the acclimation time required for each of the specified species to enable it to be safely transferred to full strength sea water (36‰) show that the salt water adaptability was very species dependent. Thus 100% *S. aureus*, *S. mossambicus* and *S. spilurus* were able to

Treatment / Time (days)	Salinity ‰ (S) and Mortality % (M)																	
	S. aureus						*S. mossambicus*						*S. spilurus*					
	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M
1	18	0	27	18.3	36	100	18	0	27	30	36	100	18	0	27	30	36	100
2	18	-	27	20.0	-	-	18	0	27	35	-	-	18	-	27	43.3	-	-
3	27	0	36	23.3	-	-	27	0	36	35	-	-	27	0	36	48.3	-	-
4	27	-	-	23.3	-	-	27	0	36	-	-	-	27	-	36	-	-	-
5	36	-	-	-	-	-	36	3.3	-	-	-	-	36	-	-	-	-	-
6	36	-	-	-	-	-	36	3.3	-	-	-	-	36	-	-	-	-	-
Total Mortality %	0		23.3		100		3.3		35		100		0		48.3		100	

TABLE 1. Effect of direct transfer to different concentrations of sea water and the gradual acclimation of *S. aureus*, *S. mossambicus* and *S. spilurus*

Triplicates of 20 fish (4 g. average size) were used in each transfer. All fish tolerated the direct transfer to 50% sea water (18‰). No fish survived the direct transfer to full sea water (36‰). Four days were found to be adequate pre-acclimation time before the final transfer to full sea water.

Treatment	Salinity ‰ (S) and Mortality % (M)											
	S. aureus/*S. niloticus* hybrids						*S. niloticus*					
Time (days)	S	M	S	M	S	M	S	M	S	M	S	M
1	18	0	18	0	27	100	18	0	18	0	27	100
2	18	0	18	-	27	100	18	0	18	-	27	100
3	27	23.3	18	-	-	-	27	20	-	-	-	-
4	27	31.6	18	-	-	-	27	28.3	-	-	-	-
5	36	35	27	0	-	-	36	46.6	27	0	-	-
6	36	35	27	-	-	-	36	46.6	27	-	-	-
7	-	-	-	-	-	-	-	46.6	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	36	0	-	-	-	-	36	0	-	-
10	-	-	36	-	-	-	-	-	36	-	-	-
Total Mortality %	35		0		100		46.6		0		100	

TABLE 2. Effects of direct transfer to different concentrations of sea water and the gradual acclimation of *S. aureus*/*S. niloticus* hybrids and *S. niloticus*

Three groups of 20 fish each (4 g. average size) were used in each transfer. The fish all tolerated the direct transfer to 50% sea water (18‰). No fish survived the direct transfer to 75% sea water (27‰). The fish were held for 4 days in 50% sea water, and for another 4 days in 75% sea water before the final transfer to full sea water.

transfer to full sea water in four days provided they were held in 50% sea water (18‰) for 2 days, and in 75% sea water for another 2 days before they were finally transferred to sea water, whereas when the same regime was used on the hybrids of S. aureus/S. niloticus and on S. niloticus there were 35% and 46.6% mortalities respectively (Table 2).

From these results it is possible to roughly classify the species of the study into two groups: the highly euryhaline, including S. aureus, S. mossambicus and S. spilurus, and the other less euryhaline including S. aureus/S. niloticus hybrids and S. niloticus. The difference in the euryhalinity was also marked by the evaluation of the total gradual acclimation time for the latter two species. Table 2 shows that the total gradual acclimation time required for both S. aureus/S. niloticus hybrids and S. niloticus was 8 days during which time the fish were able to transfer to full sea water (36‰). But in contrast with the highly euryhaline group this group were given 4 days in 50% sea water and another 4 days in 75% sea water, in order to enable them to achieve the final adaptability to full sea water.

Definition of Precise Critical Salinity Tolerance Values

The effects of transfer through a wide range of salinity concentrations ie. 50, 75 and 100% sea water on the ability to acclimate has been demonstrated above. Since it was obvious from these findings that the narrow range of 65-85% sea water was the range over which all species had difficulty acclimating, these were the levels used for definition of critical tolerance levels.

Treatment / Fish species	Fish mortalities at sea water concentration %						
	50% S.W. (18‰)*	60% S.W. (21.6‰)	65% S.W. (23.4‰)	70% S.W. (25.2‰)	75% S.W. (27‰)*	80% S.W. (28.8‰)	85% S.W. (30.6‰)
S. mossambicus	0	0	0	0	35	86.7	100
S. spilurus	0	0	0	0	48.3	100	100
S. niloticus	0	30	81.7	100	100	100	100
S. aureus/S. niloticus hybrids	0	0	56.7	96.6	100	100	100
S. aureus	0	0	0	0	20	-	-

TABLE 3. The precise critical salinity tolerance of the fish of the present study.

Three groups of 20 fish (5 g. average size) were used in each transfer. The fish were directly transferred from fresh water to each of the trial's salinities. They were held there for 2 days during which mortalities were counted. There were insufficient samples to carry out the rest of the trial with S. aureus.

* Quoted from the previous section (Tables 1 and 2)

Table 3 shows that at the size tested neither S. mossambicus nor S. spilurus was able to tolerate the direct transfer to 85% sea water (30.6‰), and salinity concentration of 70% sea water (25.2‰) was lethal to S. aureus/S. niloticus hybrids and S. niloticus. Although it was not possible for logistics reasons to complete the trial with S. aureus this fish species had already showed the best survival in 75% sea water. It can be seen from the table that the lowest salinity tolerance was again displayed by S. niloticus which could barely survive in 60% sea water.

The most important feature observed in this trial was that in most cases an increase of 5% sea water (1.8‰) concentration at the critical level could cause an increase of 50% higher mortality.

Osmolality of Sarotherodon Plasma in Fresh and Sea Water

In fresh water the plasma osmotic concentration values of the fish varied between 322 mOsm/kg for S. mossambicus and 341 mOsm/kg for S. niloticus. The values for the fish which were fully acclimated in sea water were between 341 mOsm/kg and 368 mOsm/kg for the same species respectively (Figs. 6-9).

Effect of Sudden Salinity Change on Plasma Osmolality

As can be seen in Figs. 6-9, the pattern of plasma osmotic concentration after transfer to salt water was similar for all species but with differences in time scale. Thus in S. spilurus, S. aureus/S. niloticus hybrids and S. niloticus the plasma osmotic concentration rapidly increased during the first 12 hours in salt

Figure 6

Effect of direct transfer to salinity of 60% sea water (21.6‰) on the plasma osmotic concentration of S. mossambicus.

The mean fish weight was 17.24 ± 2.35 g. and three fish were used in each sample. The high euryhalinity of this fish was indicated by the quick adjustment in this salinity and the quick return to the new physiological salt water level of plasma osmotic concentration.

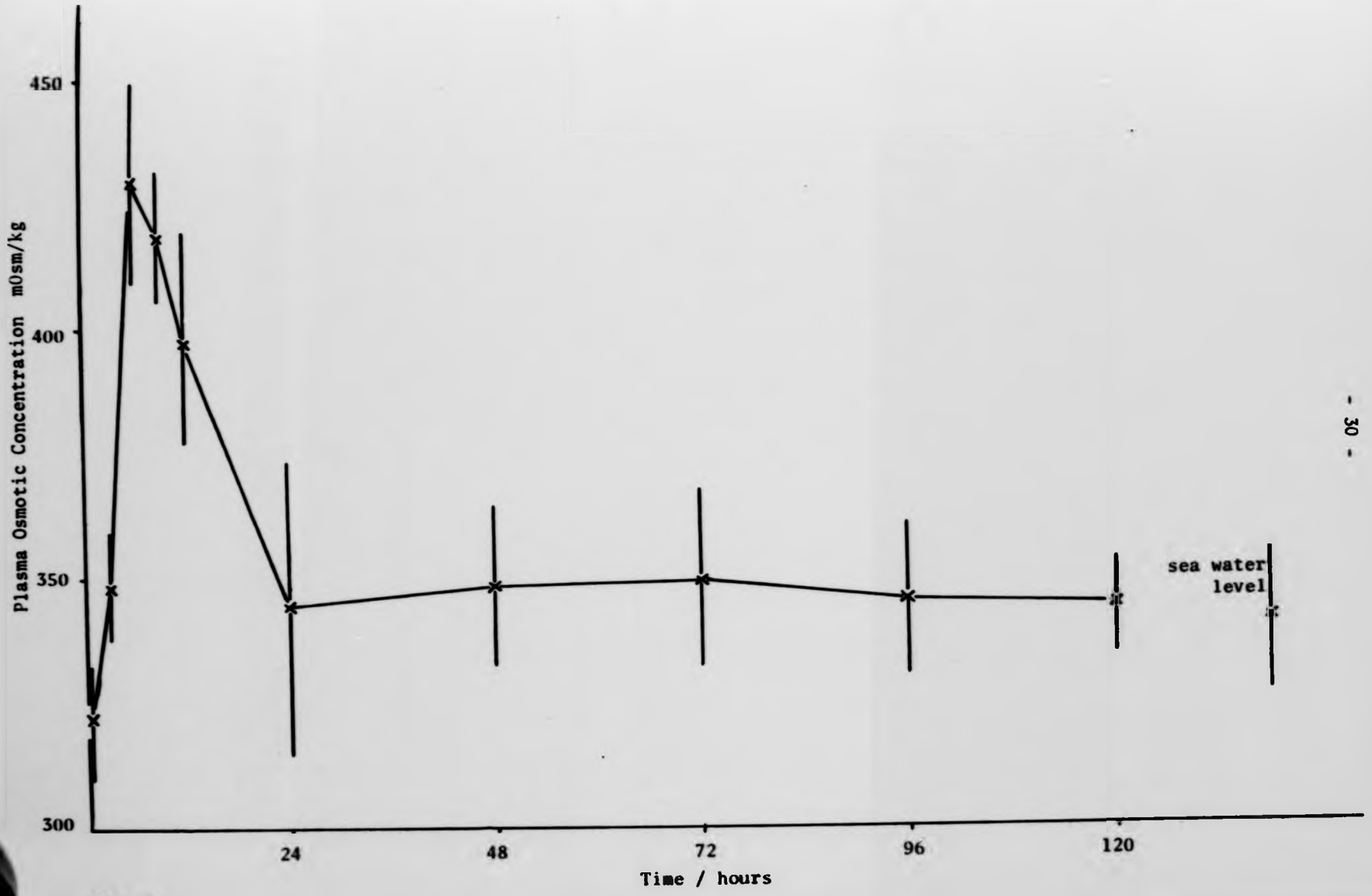

Plasma Osmotic Concentration mOsm/kg
450
400
350
300
24
48
72
96
120
Time / hours
sea water level

Fig. 6

Figure 7

The effect of the direct transfer to 60% sea water (salinity 21.6‰) on the plasma osmotic concentration of S. spilurus.

The mean fish weight is 17.71 ± 2.26 g. Three fish were used in each sample. The large standard deviation during the first 24 hours in salt water indicated that some fish were already past the adjustive phase while the others were still adjusting themselves.

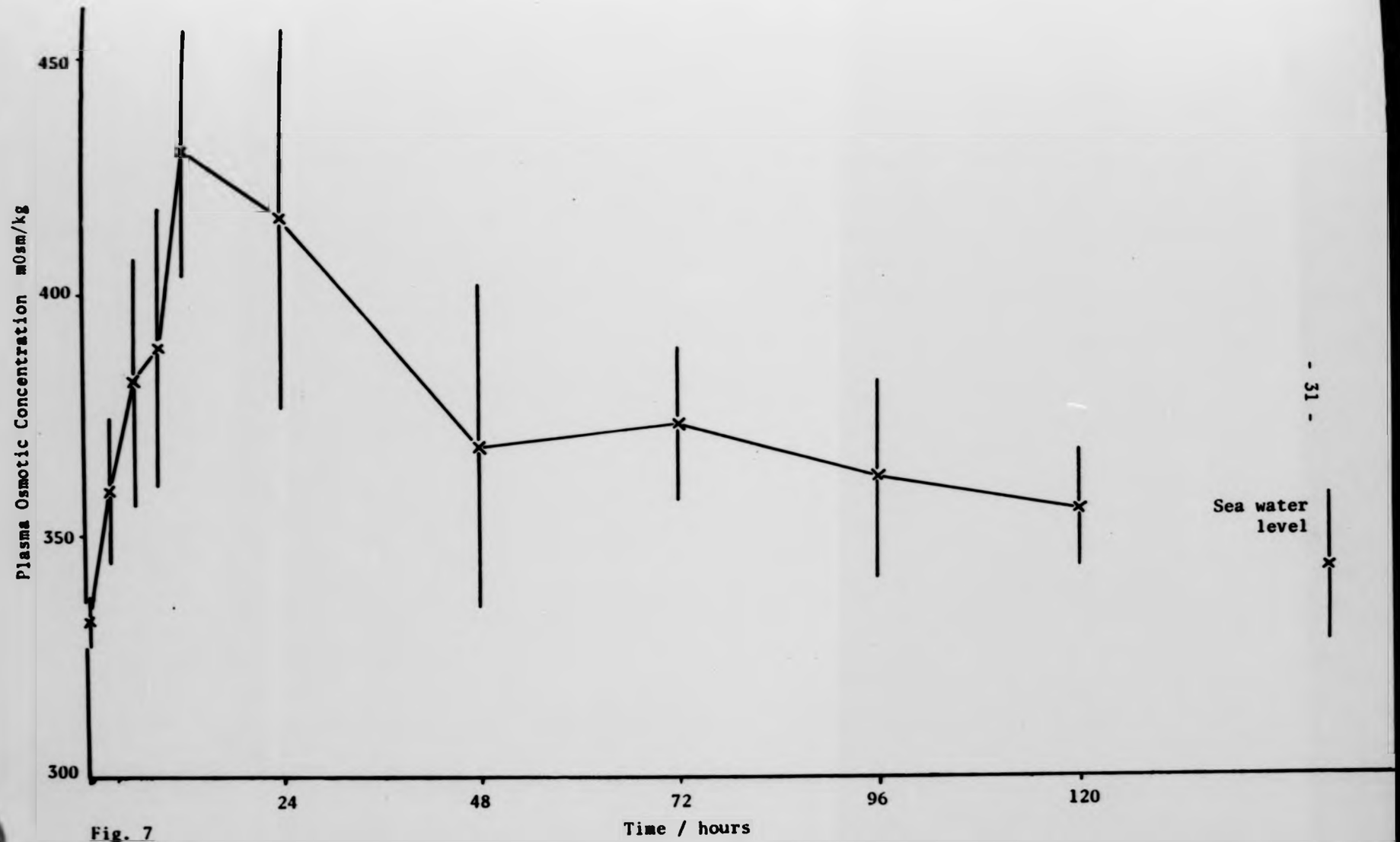

Plasma Osmotic Concentration mOsm/kg
450
400
350
300
24
48
72
96
120
Time / hours
Sea water level

Fig. 7

Figure 8

Effect of direct transfer to 60% sea water (21.6‰) on the plasma osmotic concentration of S. niloticus.

The mean fish weight was 16.35 ± 1.7 g. and three fish were used in each sample. This salinity is very close to the lethal limit of S. niloticus. Thus there were very high plasma osmotic concentration during the first 24 hours in salt water, but the fish that survived were able to regulate it thereafter.

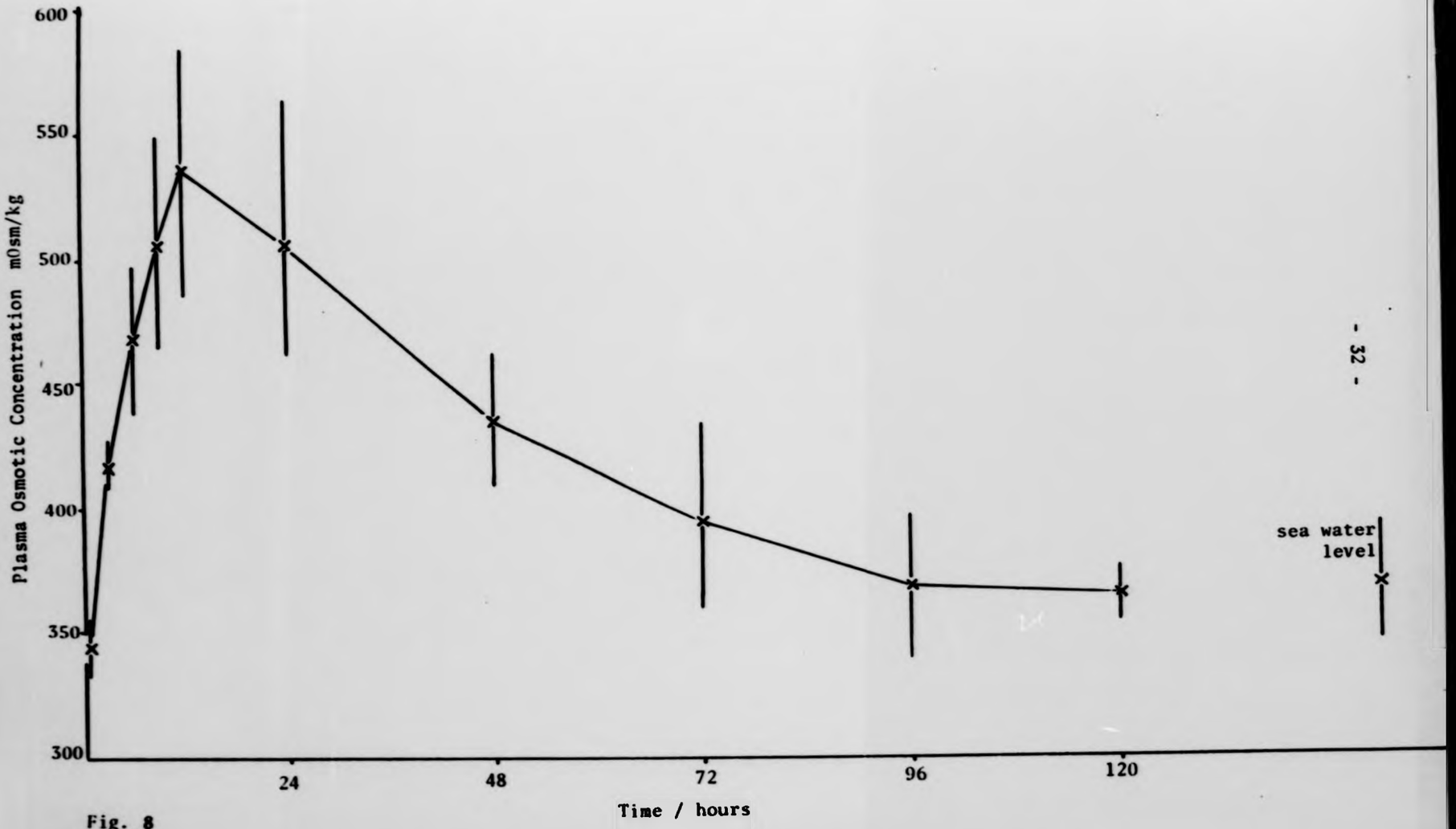

Plasma Osmotic Concentration mOsm/kg
600
550
500
450
400
350
300
24
48
72
96
120
Time / hours
sea water level

Fig. 8

Figure 9

The effect of the direct transfer to 60% sea water (21.6‰) on the plasma osmotic concentration in S. aureus/S. niloticus hybrids.

The mean fish weight is 23.6 ± 3.8 g., and three fish were used in each sample. After 12 hours from the transfer all of the fish were starting to regulate the osmotic concentration of the plasma, and after 96 hours all of the fish had almost attained the new physiological level in salt water.

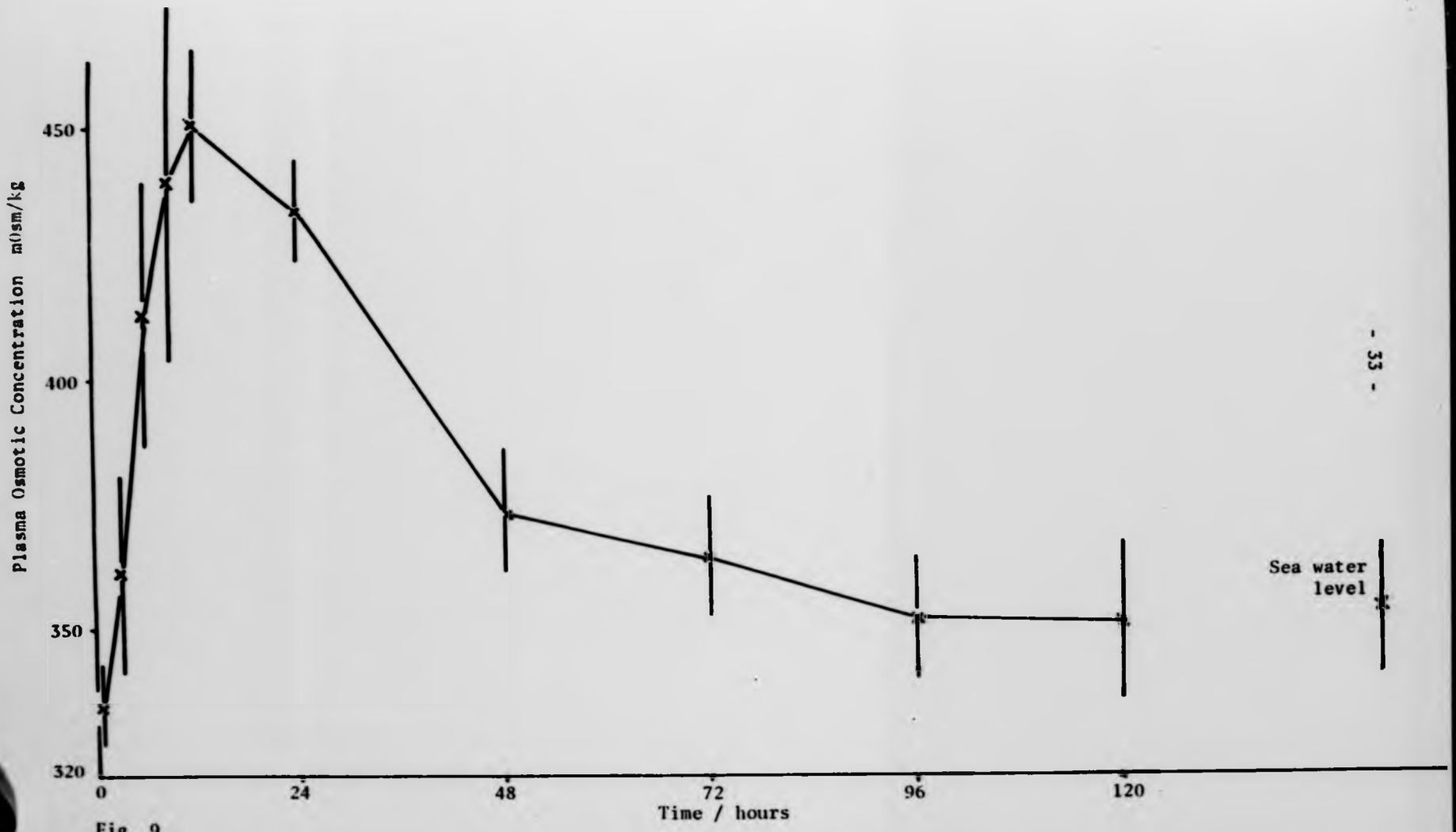

Plasma Osmotic Concentration mOsm/kg
450
400
350
320
0
24
48
72
96
120
Time / hours
Sea water level

Fig. 9

water while in S. mossambicus the peak was reached within 6 hours of transfer. The return to new equilibrium points was also relatively rapid and species dependent (i.e. circa 24 hours in S. mossambicus and 96 hours for S. aureus/S. niloticus hybrids).

A general clinical observation with all fish showing stress following a transfer to a lethal salinity was that for the first 2 hours the fish were very calm. Their heads generally pointed towards the bottom of the tank and they showed very slow opercular rhythms. Then they moved upwards close to the water surface and attempted to gulp air. They gradually became dark in colour, the colour change commencing as a dark band round the trunk. Some of them also showed skin haemorrhages. Later they lost their co-ordination and showed a variety of behaviour patterns. Generally they lay on the bottom or hung vertically with the tail down but occasionally some of them propelled themselves in a very fast motion across the surface. Eventually they lay down on the bottom again and the breathing activity completely ceased. Plasma osmotic concentration in moribund S. mossambicus could be as high as 627 mOsm/kg.

Body Water Content of S. aureus/S. niloticus Hybrids in Fresh Water and in Full Sea Water

The physiological water content of fish in fresh water was 77.866 ± 2.17% and in fully sea water adapted fish it was slightly lower (75.92 ± 0.99%).

Effect of Salinity Change on Body Water Content

Transferring fish from fresh water to salt water (i.e. a salinity concentration higher than the isosmotic point of that particular fish) caused rapid dehydration due to the effects of the hyperosmotic medium. Fig. 10 gives a good demonstration of this dehydration effect. As in the effect of the salt water on the plasma osmotic concentration of S. aureus/S. niloticus hybrid, the dehydration reached its maximum by about 12 hours after the transfer.

Figure 10

Effect of the direct transfer to 60% sea water (21.6‰) on the body water content of *S. aureus*/*S. niloticus* hybrids.

The mean fish weight was 11.51 ± 1.53 g., and three fish were used in each sample. The standard deviation of each sample is to be found in Appendix 9. The maximum body water content loss occurred after 12 hours from the transfer, and between 48 and 72 hours a new physiological level was achieved.

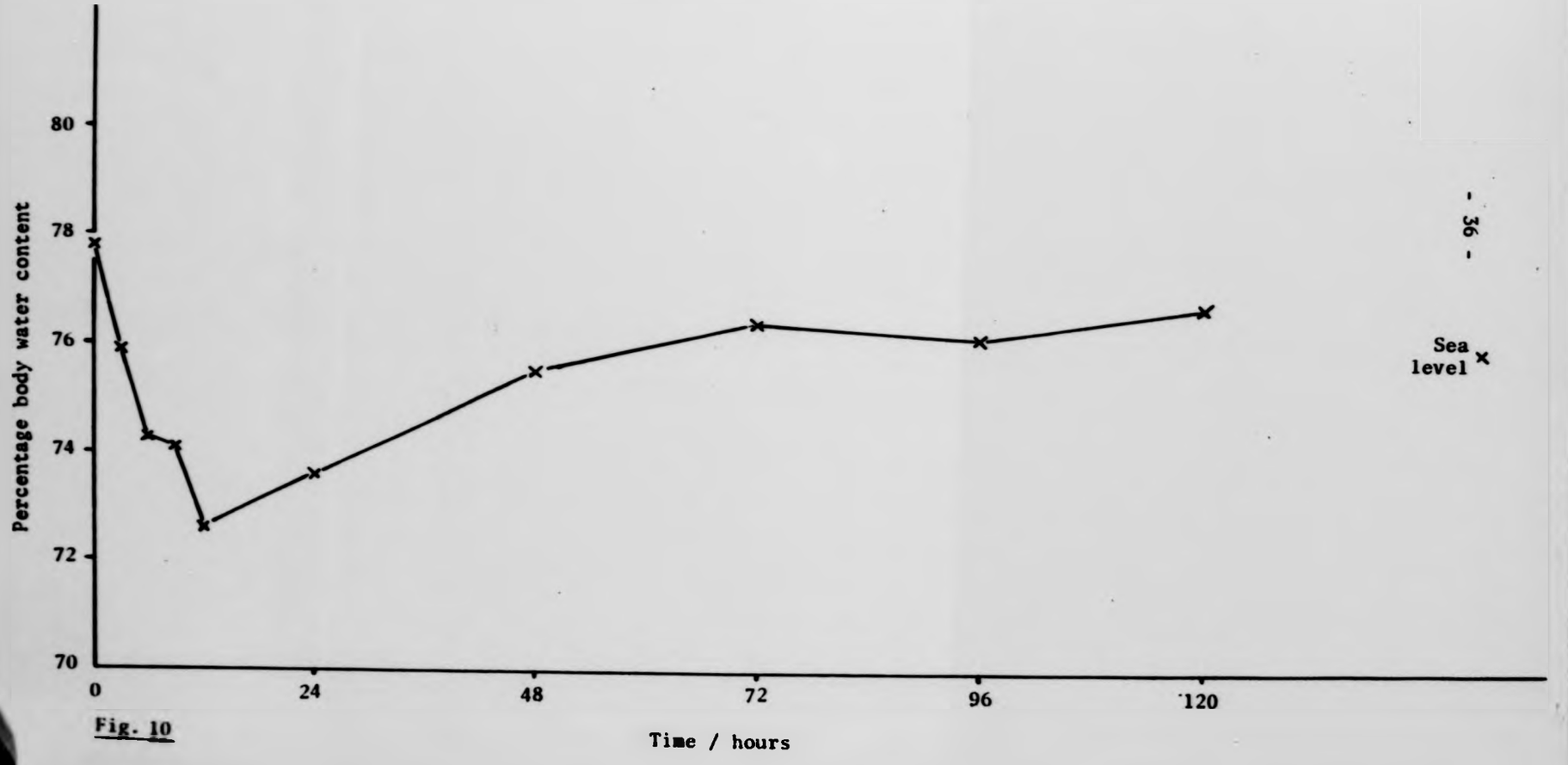

Percentage body water content
80
78
76
74
72
70
0
24
48
72
96
120
Time / hours
Sea level

Fig. 10

DISCUSSION

It was Myers (1949) who first observed the euryhaline nature of some cichlid fish. Bayoumi (1969) noted the occurrence of Tilapia zillii in the gulf of Suez (Egypt) in a salinity of almost 42.7‰.

Mortality and the increase of plasma osmotic concentration following transfer to salt water were the parameters used in the present study as indicators of the adaptability of that particular species to salt water, and the results given extend the findings of Lotan (1960) who observed that in S. niloticus the rise in the plasma osmotic concentration with increasing salinity was an indicator of the osmoregulatory mechanism of the fish. The present findings also agree with Clarke (1973) and Assem and Hanke (1978, 1979) who recorded increases in the plasma osmotic concentration of S. mossambicus which reached their maximum after 6-9 hours in salt water. These increases were adjusted after the first day in salt water, and when they transferred the fish from salt water to fresh water the plasma osmotic concentration readjusted within 6 hours. In both cases normal physiological levels were attained in 6 days.

The present findings (Table 3) more or less corroborate these findings. For example Fukusho (1969) observed no mortalities when he transferred S. mossambicus to a salinity of 24.4‰ whereas in S. niloticus of 2.9 and 5.7 g. mean weight Maruyama (1974) recorded 70-90% mortality in salt water of 21.6‰. The reduced

tolerance of S. niloticus has been observed by Fukusho (1969) in his comparative study of the specific salinity tolerance in S. mossambicus, S. niloticus and Tilapia sparrmani at different concentrations of sea water and in sea water of 32.7‰.

It has been found in salmonids that the minimum period required for attaining balance between the influx and efflux of water and ions across to osmoregulatory organs was 40 hours from transfer at temperature ranging between 5 and 11°C (Houston, 1959; Conte and Wagner, 1965; Jackson, 1981). Thus in the gradual acclimation experiments (Tables 1 and 2) of the present study, a minimum of 2 days sojourn in each salinity was provided for the fish. This was thought to be adequate to alleviate the osmotic stress imposed on the fish by each transfer, and appeared a reasonable time to allow acclimation to high levels of salinity concentration.

Since transferring fish from one salinity to a higher one was a very successful regime of acclimation, it is probable that gradually increasing the salinity over a known period through continuous introduction of sea water into the tanks might have been even more satisfactory as it would have reduced the stress caused to the fish by handling trauma during physical transfer. However besides the fact that this is a complicated technique, especially if it is to be applied on a commercial scale, no significant differences between the two methods could be obtained from the findings of previous workers. Thus for instance Teng et al. (1979) working with S. aureus used a variety of methods to constantly increase salinity whereas Maruyama (1974) working with S. niloticus used a method similar to the one used in the

present study, and in each case there was no difference in fish survivals attributable to any of these different methods.

The results in Table 1 agree in broad terms with the field results of Osborne (1979) who managed to transfer juveniles of S. spilurus from 2‰ to 40‰ in 4 days with total mortality of 65.3%. Differences between Osborne's total mortality and that presented here for the same fish species may be related to differences in the salinity concentration between the two studies, or to superior fish quality or handling, but the results are broadly similar.

The physiological osmotic concentration values (Figs. 6-9) were virtually identical to the figures given by Krogh (1939) and Brown (1957) (270-344 mOsm/kg and 360-419 mOsm/kg) for fresh water and sea water teleosts respectively. They also agree with Farmer and Beamish (1969) who recorded 332 and 335 mOsm/kg in fresh and sea water S. mossambicus. The present study values for S. niloticus were relatively high compared with the others, though they did agree with Mahdi (1972) who recorded a high plasma chloride in S. niloticus.

The haematocrit values given in the present study for S. aureus/S. niloticus hybrids agreed with those given by Mahdi (1972) for S. niloticus (34%). Hattingh (1971) obtained a lower value for S. mossambicus (24%). However all of these values were lower than those reported for salmonids. Jackson (1981) working with rainbow trout recorded a haematocrit value of 43% in fresh water, while Zeitoun et al. (1974) working with the same fish recorded 39% at 20‰ salinity.

The mean body water contents given in the present study for S. aureus/S. niloticus was a typical value for teleost fish (Holmes and Donaldson, 1969). It was similar to the values given for the "Malacca hybrid" tilapia by Tan (1971) who recorded 77.82% body water content for small fish (between 80 and 120 g.) and 74.3% for bigger ones (360-400 g.). It was also in agreement with Pandian and Roghurman (1972) who recorded values of between 74.8% and 80% body water contents in S. mossambicus.

The dual effect of the transfer on the plasma osmotic concentration and on the body water content have been demonstrated in steelhead trout, the "seagoing" rainbow trout (Salmo gairdneri) by Houston (1959) and in fresh water rainbow trout by Jackson (1981). The decline in the body water content of the hybrids of S. aureus/ S. niloticus (Fig. 10) agrees with that recorded by Maruyama (1975) who observed that there was a depression of 8.55% of the body water content in starved S. niloticus after it had been transferred to salinity of 15.8‰ and Jackson (1981) who recorded a loss of 3.3% of the body water content in rainbow trout after it had been transferred to sea water for 48 hours.

Survival of fish in full strength sea water or in fresh water requires efficient functional osmoregulatory mechanism. In simple terms fish drink small amounts of water and discharge copious urine in fresh water while they drink large volumes of water and discharge small amounts of concentrated urine in sea water (Smith 1930). With respect to the levels and distribution of water and electrolytes within the fish's body the transfer of the fish from

fresh water to sea water requires alterations in such regulatory mechanisms to meet the demands of the new medium. These alterations take place in response to the osmotic stress affecting the fish undergoing transfer. Dehydration is caused by the osmotic difference difference between the internal osmotic concentration of the fish plasma and the osmotic concentration of the external salinity which in the present study were means of 332.75 mOsm/kg and 633.4 mOsm/kg respectively.

This may well result in a net influx of ions through the gills in an attempt to concentrate the plasma. There also occurs a selective uptake of the monovalent ions and a high rate of water absorption through the gut wall (Shehadeh and Gordon, 1969). The monovalent ions remain in the plasma until the new active transport mechanisms start to operate (Jackson, 1981).

These three factors viz. dehydration, ion influx and the intestinal monovalent uptake account for the increased plasma osmotic concentration that occurs immediately after transfer. Following the transfer two distinct phases have been described by Houston (1959), in steelhead trout. An "adjustive-phase" takes place immediately after the transfer and is characterised by an increase in the plasma osmotic concentration followed by a "regulative-phase" during which stabilisation of the electrolyte concentration is maintained. The relative contribution of the various factors in either of the two phases is not known at present for Sarotherodon species.

However, Rawdon and Cornish (1973) reported a 2.5 fold increase in the fore-intestine water absorption from sea water adapted S. mossambicus

over that from fresh water fish. Potts et al. (1967) working with the same species found that the highest permeability was in the fish from fresh water and the lowest was with fish in 200% sea water. Among non-cichlid fishes, Shuttleworth and Freeman (1973) recorded that in Anguilla dieffenbachii 10% or less of the rate of addition of ions to the fish as a whole entered through the gills. Kirsch and Mayer-Gostan (1973) working with the European eel (Anguilla anguilla) and Potts et al. (1970) working with the Atlantic salmon (Salmo salar) both estimated that in sea water adapted fish about one third of the ion influx is through the gut and two thirds through the body wall.

The presence of an adjustive and a regulative phase in the tilapias is shown clearly in Figs. 6-9. Compared with the results obtained from cold water fish, it can be seen that the adjustive phase is considerably faster in the present study. According to Houston (1959) and Conte and Wagner (1965) working with steelhead trout, the anadromous form of Salmo gairdneri, and Jackson (1981) working with rainbow trout (Salmo gairdneri), the adjustive phase for this species is 80 hours for the former fish and 60 hours for the latter one. The hybrids S. aureus/S. niloticus (Fig. 9) in the present studies showed a typical picture of a warm water fast adjustive phase. Immediately after the transfer the plasma osmotic concentration went up, reaching a maximum 450 mOsm/L within 12 hours. At this point the active secretion of ions started and the withdrawal of ions from the blood began, so that between 48 and 96 hours post transfer a balance had been attained between the various inflow and excretion levels.

Jackson (1981) recorded a lethal osmotic gradient level of 5 mOsm/L/h for rainbow trout. It has been found in the gradual acclimation experiments that 24 hours are sufficient for recording the mortality level of each transfer. On the other hand it was found from the effect of salinity change on the plasma osmotic concentration experiment that the increasing order of the osmotic gradient after 24 hours ($\frac{\text{osmolality}}{24}$) was 0.92, 3.5, 4.15 and 6.8 mOsml/kg/h for S. mossambicus, S. spilurus, S. aureus/S. niloticus hybrids and S. niloticus respectively. This provides additional evidence for the differences in osmoregulatory efficiency observed between these species.

Moreover the present results might suggest that if the osmotic gradient exceeds 6 mOsm/kg/h in the first 24 hours following transfer to salt water, this could lead to observable mortalities among Sarotherodon species depending on the species and the strength of the sea water employed.

The increase in the plasma osmotic concentration was matched by a decrease in body water content (Fig. 9 and 10). This finding agrees with Shuttleworth and Freeman (1973) who postulated that in Anguilla dieffenbachii the increase in the plasma osmotic concentration was due to the removal of water, and to the addition of ions to the internal body fluids.

It can be concluded that the mortality rate is a useful means of evaluating the success of any pre-acclimation regime and determining optimum acclimation time. The plasma osmotic concentration is a very useful method in assessing the fish's ability in terms

of adaptive direct transfer to salt water. It is encouraging for the prospect of tilapia culture in the marine environment that in the present study the species were all able to be acclimated to full sea water provided they were allowed gradual acclimation.

CHAPTER 4

THE EFFECT OF HIGH SALT DIET ON THE DIRECT TRANSFER OF *S. MOSSAMBICUS*, *S. SPILURUS* AND *S. AUREUS*/*S. NILOTICUS* HYBRIDS TO SALT WATER

INTRODUCTION

It has been noted in the previous chapter that gradual acclimation through higher salinities can facilitate the final transfer of tilapias to full sea water and that the first 24 hours after the transfer represent the critical period in which the fish either show signs of successful acclimation or fail to acclimate. There is therefore a very short period during which physiological changes might be induced in order to alleviate osmotic stress.

Stimulation of the osmoregulatory system of fish has been attempted in a number of ways, such as by rearing young fish at elevated temperature, varying the normal photoperiod, hormonal treatment, and using moist diets or high salt diets (Knutsson and Grav, 1976; Gunnes, 1979; Gallis et al., 1979; Jackson, 1979).

Zaugg and McLain (1969) working with coho salmon fry (Oncorhynchus kisutch) found that by feeding a diet enriched with inorganic salt (4 and 8% NaCl) it was possible to achieve a more effective transition from fresh to sea water. Their explanation was that the ingestion of elevated quantities of salts stimulated the swallowing of sea water in the marine environment and activated the excretory process responsible for the elimination of these salts.

Basulto (1976) carried out similar experiments with Atlantic salmon (Salmo salar) and found that again the tolerance to sea water was greater in fish fed on diets enriched with salt

(12% NaCl). Recently the same findings have been demonstrated by Jackson (1979) who recorded that in rainbow trout (Salmo gairdneri) the chances of survival after transfer from fresh to sea water were increased following the feeding of a high salt diet (10% NaCl). He demonstrated that two weeks after the fish commenced feeding on the high salt diet, the experimental fish achieved a survival rate increment of 33.8% over that of the control fish.

It is well known that in salmonids that undergo smoltification, physiological changes occur while fish are still in fresh water which serve to provide a preadaptation to marine condition (see review by Hoar, 1976). However reports of enhancement of acclimation to salt water by feeding high salt diet have not to date been extended to non-salmonids. The fish species used in the present study do not undergo smoltification changes in the wild though they were shown (vide infra Chapter 3) able to withstand direct transfer to salinities of between 60% sea water i.e. 21.6‰ (S. aureus/S. niloticus hybrids and S. niloticus) and 75% sea water i.e. 27‰ (S. aureus, S. mossambicus and S. spilurus).

It would appear that the possibility of inducing preadaptation would be a useful means of enhancing transfer survival rates in tilapias transferred to the marine situation.

A series of experiments was therefore designed in order to allow an assessment of the benefits of applying a salt diet prior to salinity transfer. In Chapter 3 it was shown that commercially farmed Sarotherodon species are capable of surviving direct transfer into salinity levels which are specific for each species. Thus for

25 g. fry (initial trial has shown that the small pellet size required for smaller fish proved too soluble) which is the smallest size at which feeding of a salt diet can be attempted, S. mossambicus is totally susceptible to a salinity of 85% sea water (30.6‰), S. spilurus to a salinity of 80% sea water (28.8‰) and S. aureus/S. niloticus hybrid to 70% sea water (25.2‰).

Groups of fish were therefore exposed to a high salt diet for a period of up to four weeks, and sequential salinity vulnerability estimated in terms of reduction in mortality on direct transfer to the known lethal salinity threshold and its correlation with ability to control plasma salt concentration (osmolality) following feeding of high salt diet.

MATERIALS AND METHODS

Fish

S. mossambicus, *S. spilurus* and *S. aureus*/*S. niloticus* hybrids were used in the study. All of the fish had been reared in the tropical hatchery to an average size of 25 g. before use.

Experimental Design

The effect of high salt diet on plasma osmolality and on ability to survive transfer to high (lethal) salinity

Following preliminary studies which indicated that a high salt diet containing 10% NaCl was readily acceptable to young *Sarotherodon* fry and that it was possible to measure a correlation between uptake of such a diet and increase in plasma osmolality, feeding trials to study the effect of varying times of exposure to the high salt diet on ability to survive direct transfer into an otherwise fatally high salinity were carried out.

Feeding of high salt diet was carried out in 3 glass aquaria measuring 100 x 40 x 30 cm, in fresh water. The glass tanks were prepared as described in Chapter 2. Half of the water was renewed every week. One tank was used for each of the species i.e. *S. mossambicus*, *S. spilurus* and *S. aureus*/*S. niloticus* hybrids. One hundred and twenty fish (25 g. average weight) from each of *S. mossambicus* and *S. spilurus*, and 100 fish of the same average weight from the stock of *S. aureus*/*S. niloticus* hybrids were used

(only 100 hybrids were available due to hatchery difficulties). The water quality in the tanks was monitored every 2 weeks. The fish were allowed to settle for 2 weeks during which time they were given their standard diet. Before the high salt diet started to be fed, 20 S. mossambicus, 20 S. spilurus and 20 S. aureus/ S. niloticus hybrids directly transferred to salinities of 85% (30.6‰), 80% (28.8‰), and 70% (25.2‰) sea water respectively to serve as controls of the salinity tolerance of non-preacclimated fish.

High salt diet was supplied three times a day at a level of 5% of fish body weight per day for a period of 4 weeks. Two lots of 10 fish from each of the species were transferred weekly for testing mortality levels following transfer to their lethal salinity as described earlier, and supported by the control fish results.

Each week three S. mossambicus and three S. spilurus were bled for plasma osmotic concentration estimation as described in Chapter 2. No blood samples were taken from the hybrids of S. aureus/S. niloticus because of the lack of adequate fish numbers.

High Salt Diet

Standard food was prepared as described in Chapter 2. The high salt diet was prepared according to the same procedures except that 10% of the maize usually included in the diet was replaced by sodium chloride, so that the high salt diet contained only 3% maize as indicated in Table 4.

Ingredient	% Weight
Vitamin mixture	1
Mineral mixture	4
Oil	5
Carboxy Methyl Cellulose	2
Fish meal	58
Dried Distillers Soluble	5
α cellulose	10
Starch	2
Maize	3
Sodium Chloride	10

High salt diet analysis [Dry weight (moisture free) basis]

Nutritional Composition	% Weight
Crude Protein	36.51
Ether Extract	6.12
Ash	7.22
NFE	50.15

TABLE 4. Nutritional composition of the high salt diet

RESULTS

Effect of High Salt Diet on Fish Survival

Feeding of the salt diet prior to exposure to a previously lethal salinity did improve survival levels. These effects are shown in Fig. 11a, b and c. S. mossambicus, which in the controls were incapable of surviving a salinity equivalent to 85% sea water, showed up to 84% survival in the different tests, and the results for S. spilurus (up to 50%) and the hybrids (up to 62%) were also significant. The time for maximal physiological advantage, following feeding of the salt diet, varied between the species. S. mossambicus and S. aureus/S. niloticus hybrids both showed best survival after two weeks of salt diet feeding whereas in S. spilurus this was not achieved until 3 weeks of feeding although some improvement in survival was already extant after only one week.

Effect of High Salt Diet on Plasma Osmotic Concentration

The feeding of high salt diet had an appreciable effect on blood plasma osmotic concentration in the two species in which it was studied. Tables 5 and 6 show that the plasma osmolality (mOsm/kg) of both species were higher than those of the controls throughout the experimental periods. Maximum values for S. mossambicus (377 mOsm/kg, SD = 6) and for S. spilurus (390 mOsm/kg, SD = 18) were recorded after 3 weeks of feeding the salt diet. Mean levels that were maintained throughout the experimental period however were 351 mOsm/kg (SD = 16) for S. mossambicus and 345 mOsm/kg

Figure 11a

Effect of prior feeding of high salt diet (10% sodium chloride) on the survival of S. mossambicus following transfer to salinity of 85% sea water (30.6‰)

Two lots of 10 fish of average weight 25 g. fed on the diet were transferred weekly to salt water for 4 successive weeks. After transfer the fish were held for 72 hours in salt water during which time mortalities were counted every 12 hours.

Figure 11b

Effect of prior feeding of high salt diet (10% NaCl) on the survival of S. spilurus following transfer to salinity of 80% sea water (28.8‰)

The fish were treated as in Fig. 11a except that they were transferred to salinity of 80% sea water.

Figure 11c

Effect of prior feeding of high salt diet (10% NaCl) on the survival of S. aureus/S. niloticus hybrids following transfer to salinity of 70% sea water (25.5‰)

This fish were treated as in Fig. 11a except that they were transferred to salinity of 70% sea water.

Figure 11a

Effect of prior feeding of high salt diet (10% sodium chloride) on the survival of S. mossambicus following transfer to salinity of 85% sea water (30.6‰)

Two lots of 10 fish of average weight 25 g. fed on the diet were transferred weekly to salt water for 4 successive weeks. After transfer the fish were held for 72 hours in salt water during which time mortalities were counted every 12 hours.

Figure 11b

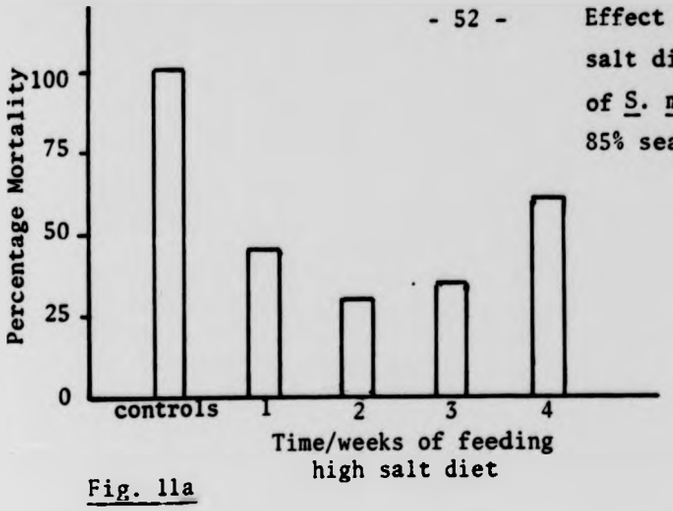
Effect of prior feeding of high salt diet (10% NaCl) on the survival of S. spilurus following transfer to salinity of 80% sea water (28.8‰)

The fish were treated as in Fig. 11a except that they were transferred to salinity of 80% sea water.

Figure 11c

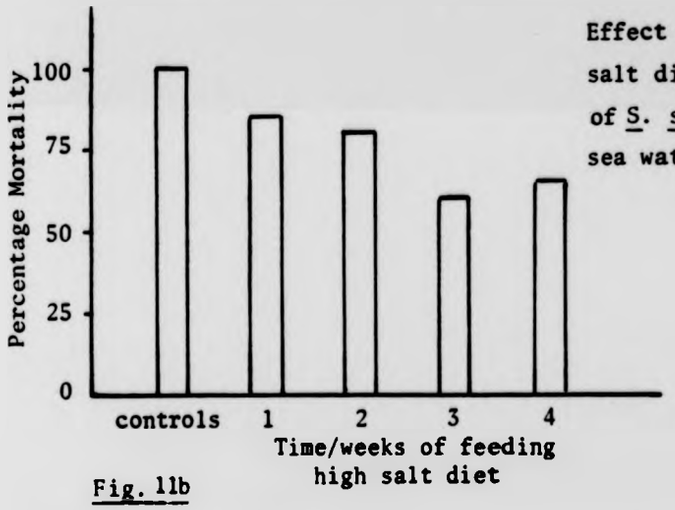
Effect of prior feeding of high salt diet (10% NaCl) on the survival of S. aureus/S. niloticus hybrids following transfer to salinity of 70% sea water (25.5‰)

This fish were treated as in Fig. 11a except that they were transferred to salinity of 70% sea water.



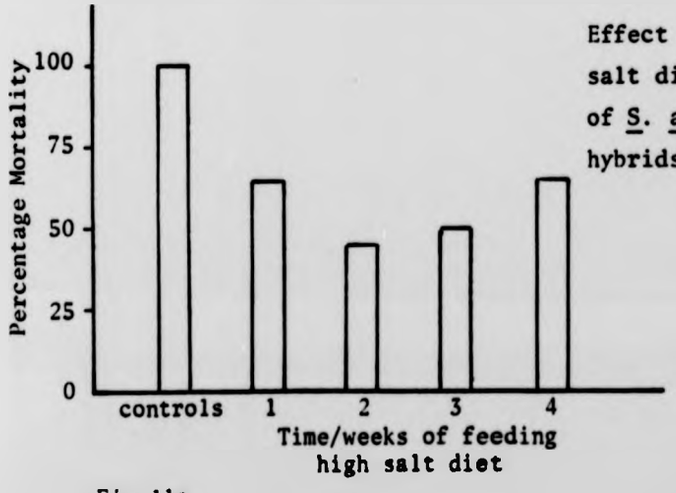
Effect of feeding high salt diet on survivals of S. mossambicus in 85% sea water

Fig. 11a



Effect of feeding high salt diet on survivals of S. spilurus in 80% sea water

Fig. 11b



Effect of feeding high salt diet on survivals of S. aureus/S. niloticus hybrids in 70% sea water

Fig. 11c

Treatment regime	Fish Weight (g)	Osmolality of blood plasma in mOsm/kg	Mean ± (S.D.) of plasma osmolality
Control (continuously held in fresh water)	25.6 22.4 23.1 20.3	330 326 340 335	333 ± 5
One week after first feeding of salt diet	21.6 24.2 27.5	354 322 364	346 ± 22
Two weeks after first feeding of salt diet	26.2 20.4 29.1	328 336 366	343 ± 20
Three weeks after first feeding of salt diet	30.4 25.0 25.6	384 371 376	377 ± 6
Four weeks after first feeding of salt diet	22.6 27.5 31.2	360 366 349	358 ± 8

TABLE 5. Effect of feeding high salt diet (10% sodium chloride) on the plasma osmotic concentration of S. mossambicus

Treatment regime	Fish Weight (g)	Osmolality of blood plasma in mOsm/kg	Mean ± (S.D.) of plasma osmolality
Control (continuously held in fresh water)	27.4 25.5 30.1 23.3	336 340 320 329	331 ± 8
One week after first feeding of salt diet	25.0 22.2 27.1	377 301 393	357 ± 39
Two weeks after first feeding of salt diet	24.4 32.1 30.5	366 328 355	349 ± 19
Three weeks after first feeding of salt diet	36.7 26.2 31.2	387 374 411	390 ± 18
Four weeks after first feeding of salt diet	25.5 24.1 33.3	344 339 349	345 ± 5

TABLE 6. Effect of feeding high salt diet (10% sodium chloride) on the plasma osmotic concentration of *S. spilurus*

(SD = 22) for S. spilurus, were not significantly different from those of the controls which did not receive the high salt diet. A correlation test between the numbers of weeks of feeding the salt diet and the increase in plasma osmolality indicate no correlation in either S. mossambicus and S. spilurus, which emphasised the considerable ability of both species to exert osmoregulatory control of excess salt in the diet.

DISCUSSION

There was no evidence that the incorporation of high dietary sodium chloride (10% NaCl) in the diet resulted in any signs of clinical disease or behaviour derangement. This agrees with the observations on other fish genera by previous workers, such as Shaw *et al*. (1975) and Basulto (1976) who used levels of up to 12% sodium chloride with Atlantic salmon (*Salmo salar*) (which are smoltifying fish) and Jackson (1979) who used 10% sodium chloride diet with rainbow trout (*Salmo gairdneri*) (which do not smoltify and were thus more akin to the tilapias in this respect).

The feeding of high salt diet to the *Sarotherodon* species had a marked effect in lowering mortalities following direct transfer to what would otherwise be lethal salt water levels. No previous work of this nature has been carried out with *Sarotherodon* species, but these results do agree with those of Zaugg and McLain (1969) who reported an increase in salinity tolerance of various Pacific salmon and with those of Basulto (1976) who reported a higher percentage survival in Atlantic salmon (*S. salar*) in sea water, after feeding an elevated level of dietary sodium chloride. The slight increase in mortalities observed in those fish fed for the longest period on high salt diet with all fish of the present study agrees with Jackson (1979) who reported best survival of rainbow trout (*S. gairdneri*) after 2 weeks of feeding on high salt diet as opposed to feeding for shorter or longer periods.

Feeding of salt and its absorption into the blood resulted

in a biphasic rise in the osmotic concentration of the plasma similar to that recorded by Jackson (1979) in the rainbow trout (S. gairdneri). Continuous uptake of salt affected the plasma by raising its osmotic concentration to a level slightly higher than that of those which were fed on the standard diet, with a mean increase of 6.91% for S. mossambicus and 8.84% for S. spilurus. These findings show that feeding of high salt diet did stimulate the osmoregulatory organs and that both fish species were able to regulate the osmotic concentration of the plasma throughout the experimental period. The treatment also significantly enhanced their subsequent transferability to salt water. The lower increase of plasma osmotic concentration in S. mossambicus emphasised the extreme euryhalinity of this species.

Basalto (1976) pointed out that because of the possible interference by feeding of high inorganic salts with food utilisation, a shorter period of salt treatment might be better and Jackson (1979) reported that although he fed high salt diet for 12 weeks to rainbow trout (S. gairdneri), the peak of effect of the diet on enhancing survival in sea water took place after only two weeks. It was on this basis that feeding of the salt diet was restricted to a four-week period and this was justified by the findings.

CHAPTER 5

THE EFFECT OF THERMAL SHOCK ON THE ABILITY OF *S. MOSSAMBICUS* AND *S. AUREUS*/*S. NILOTICUS* HYBRIDS TO SUCCESSFULLY TRANSFER TO SALT WATER

INTRODUCTION

In the previous chapters it was indicated that the salinity tolerance among the tilapias is apparently species dependent, and it was possible to alleviate to certain limits the osmotic stress associated with transfer to salt water by prior feeding of high dietary sodium chloride. Stress caused by water temperature fluctuation is a regular concomitant of fish transportation in tropical situations and when the fish are transferred to salt water under such circumstances then there is the possibility of a dual stress resulting from change in both salinity and temperature.

Spaas (1959) reported that in the Cichlidae acclimation to increasing temperature took place more quickly than that to decreasing temperature. The effect of decreasing temperature on tropical fish species was investigated histologically by Allanson (1966) who reported that when temperatures were lowered to the minimal temperature capable of tolerance by any species, food movement in the gut ceased and undigested food remained in the gut. Additionally, there were extensive cellular disruption and degenerative changes in parenchymatous organs. These findings were confirmed for S. mossambicus subjected to low temperatures by Allanson et al. (1971), who reported however that S. mossambicus was able to maintain a normal physiological level of plasma osmolality despite low water temperature (11°C) provided the salinity was at least 5‰. There was however a considerable reduction in the plasma osmolality when the fish were maintained in fresh water.

According to Whitfield and Blaber (1976) the maximum salinity tolerance of the cichlid Tilapia rendalli occurs at temperatures of between 20-28°C and the osmotic concentration of the plasma rises with increasing salinity. Farghaly et al. (1973) working with Tilapia zillii reported an increase in the concentration of most of the blood parameters with increasing salinity and temperature (from 16.5°C in fresh water up to 30°C in salt water of 39.5‰ salinity).

The experiments described in the present chapter measure the effect of sudden thermal shock and combined thermal and salinity shocks on the survival and the plasma osmotic concentration in S. mossambicus and S. aureus/S. niloticus hybrids.

MATERIALS AND METHODS

Fish

S. mossambicus and *S. aureus*/*S. niloticus* hybrids fry were obtained from the tropical hatchery of the Institute of Aquaculture at the University of Stirling. The fry were reared in the normal 50 L. tanks (Chapter 2) to a mean fish weight of 10 g. prior to use for the experiments. All the fish were held at 25°C ± 1 prior to the start of the experiments.

Experimental Design

Sixteen glass tanks of 50 L. capacity were set up as described in Chapter 2. Eight tanks were filled with fresh water and 8 with water of 60% sea water salinity (21.6‰). Two tanks from each set were maintained at 15°C ± 1, two at 20°C ± 1, two at 30°C ± 1 and the remaining two at 35°C by means of a 200 W. combined heater-thermostat. These were monitored daily and for security two heater units were installed in each tank.

Duplicate sets of 15 fish from each species were removed from the holding tanks and used in each viability test. The "single stress-temperature shock" tests were carried out by directly transferring the fish from the holding temperature of 25°C to either the higher temperature of 30 or 35°C or the lower temperature of 20 or 15°C. Tests for the combined shock of temperature and salinity were carried out in the same way but employing the salt water tanks at the transfer.

The plasma osmotic concentrations were determined only with the extreme temperatures of 15 and 35°C, at which the effect of temperature on the plasma osmolality was measured either within the fresh water or as a combined effect with salinity. Thus 30 fish from each of the species were transferred to those temperatures either from fresh water to fresh water or from fresh water to salt water. Following transfer the fish were treated for sampling as described in Chapter 3, and procedures for osmolality determination are already described in Chapter 2.

RESULTS

A. Effects of Thermal Shock on Fish Survival

The results show that thermal shock either to high temperatures (30 or 35°C), or to low temperatures (20 or 15°C) did not, within the ranges used, affect the survival of *S. mossambicus* during the experimental time course. In fresh water with a cold shock to 15°C the fish suffered from an apparent chill coma with no sign of obvious cold adaptation by the end of the experiment. *S. aureus/S. niloticus* hybrids showed no mortality under thermal shock either to higher or to lower temperatures. But in cold medium a further feature was the manifestation in both the *S. mossambicus* and the *S. aureus/S. niloticus* hybrids of *Saprolegnia* infection of the skin which occurred within four days of transfer.

B. Combined Effects of Thermal and Salinity Shock on Fish Survival

The combined effects of thermal (either to high or to low temperatures) and salinity (21.6‰) shock did not affect the survival of *S. mossambicus*. In contrast *S. aureus/S. niloticus* hybrids did not tolerate the combined shock of low temperature at 15°C and the same salinity concentration with almost 100% mortality recorded at the end of this trial. Moreover exposure of this fish to a combined shock of high temperature and salinity resulted in average mortalities of 30 and 43.3% on transfer to 30 or 35°C respectively (Table 7).

Treatment / Hours after transfer	Percentage mortality in 21.6‰ salinity following indicated temperature transfer			
	25 - 25°C	25 - 30°C	25 - 35°C	25 - 15°C
0	0	0	0	0
24	0	16.6	16.6	0
48	0	20.0	43.3	53.3
72	0	30.0	43.3	90.0
96	0	30.0	43.3	93.3
120	0	30.0	43.3	93.3
Total percentage mortality after 1 week	0	30.0	43.3	93.3

TABLE 7. Combined effect of thermal shock (sudden transfer from 25°C to either higher temperatures of 30 and 35°C or to lower temperature of 15°C) and salinity of 60% sea water (21.6‰) on the survival of *S. aureus*/*S. niloticus* hybrids

The average fish weight was 9.3 g., and duplicates of 15 fish were used in each transfer. Mortalities were recorded daily after the transfer.

General observations

The clinical signs of cold stress were immediate, very rapid, motion across the water as the fish appeared to try to escape. This was associated with extreme darkening in colour. Some of the fish then settled horizontally on the bottom of the tank, while others lay on their sides. Eventually a noticeable disorientation of movements appeared.

C. Effects of Thermal Shock on Plasma Osmotic Concentration

The results of the examination of blood samples following the direct transfer from the holding temperature of 25°C to higher temperature of 35°C are shown in Figs. 12 and 13, while those which demonstrate the result of transfer to colder water of 15°C are shown in Figs. 14 and 15 for the S. mossambicus and the hybrids of S. aureus/S. niloticus respectively. In fresh water exposure to high temperature affected both species to some degree with a slight increase in their plasma osmotic concentration being noted. The highest increases were recorded from the initial physiological level of 322 up to 384 mOsm/kg for S. mossambicus and from 334 up to 372 mOsm/kg for S. aureus/S. niloticus hybrids. For S. mossambicus the rise from the initial level reached its maximum 24 hours after the transfer and as in the direct transfer studies described in Chapter 3, the fish adapted readily to this thermal change and their plasma osmotic concentration decreased again by the end of the trial. The hybrids of S. aureus/S. niloticus displayed considerable variability but no general

Figures 12 and 13

Effect of high temperature shock on the plasma osmotic concentration of *S. mossambicus* and *S. aureus*/*S. niloticus* hybrids.

The fish were directly transferred from the holding temperature of 25°C to 35°C. Each point represents an average value from three fish; the standard deviation is also shown. The figures show that the high temperature shock affects the plasma of both species by increasing their osmotic concentrations. Although in the first day the increase was more significant with *S. mossambicus*, this species was more physiologically stable by the end of the trial.

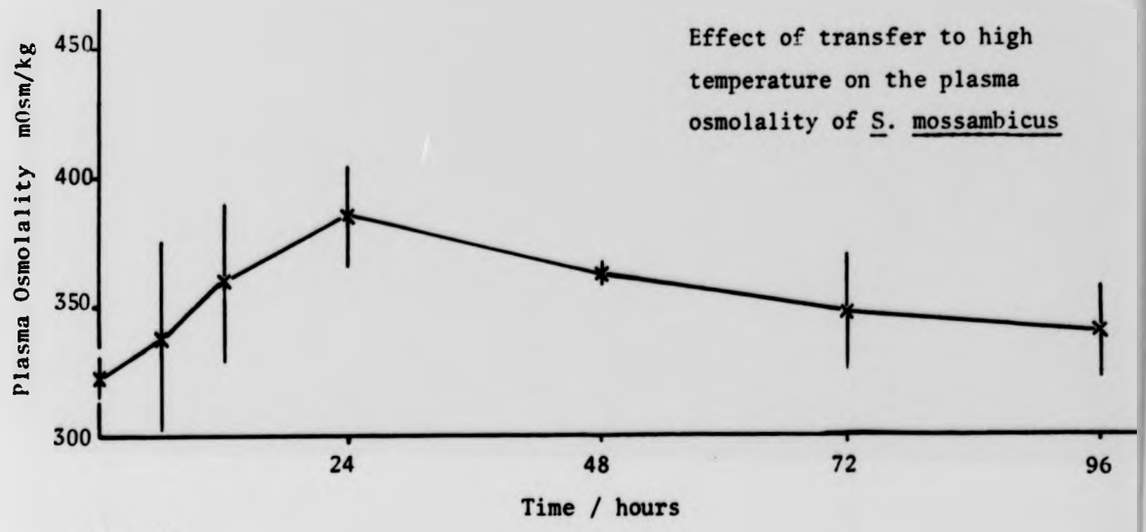

Effect of transfer to high temperature on the plasma osmolality of S. mossambicus
Plasma Osmolality mOsm/kg
450
400
350
300
24
48
72
96
Time / hours

Fig. 12

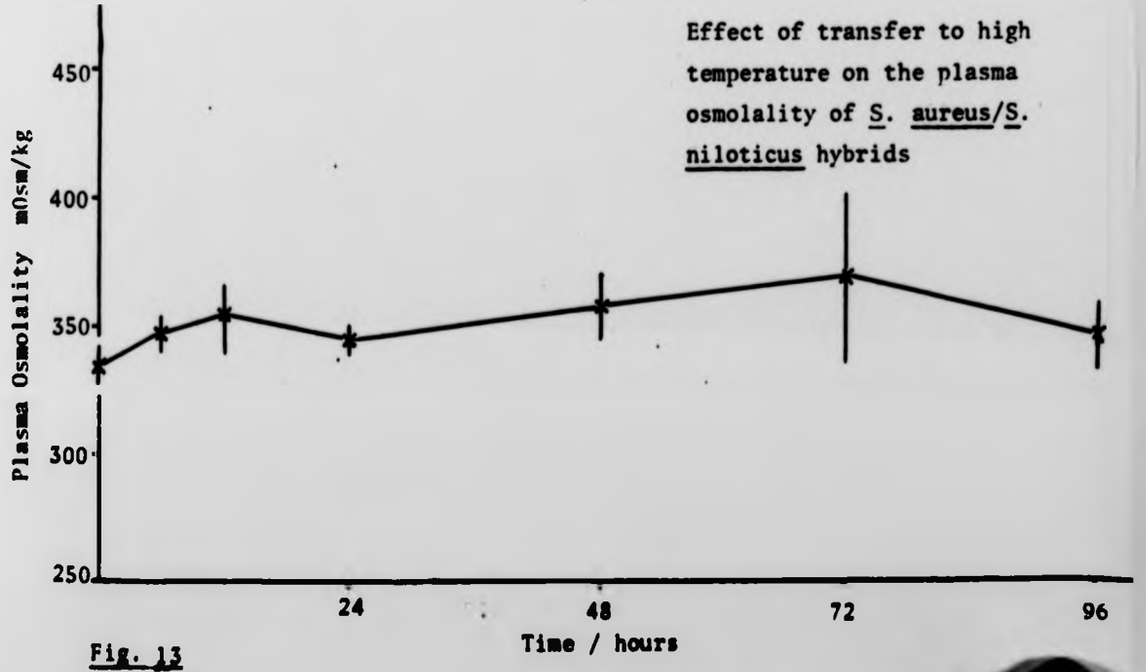

Effect of transfer to high temperature on the plasma osmolality of S. aureus/S. niloticus hybrids
Plasma Osmolality mOsm/kg
450
400
350
300
250
24
48
72
96
Time / hours

Fig. 13

trend post transfer. Although the second peak was significantly higher than the initial level ($p = 0.05$) the osmotic concentration of the plasma decreased again towards the end of the trial, which indicated that this fish was also able to tolerate the thermal shock to high temperature, but to a lesses extent than S. mossambicus.

Figs. 14 and 15 demonstrate the effects of direct transfer to cold fresh water for S. mossambicus and S. aureus/S. niloticus hybrids respectively. The graphs of the osmolality were different again for the two species. In the case of S. mossambicus the osmotic concentration of the plasma rose for the first 24 hours (up to 365 mOsm/kg) and decreased thereafter. It rose again at 72 hours, albeit to a level only slightly higher than before. While the hybridss of S. aureus/S. niloticus were not affected by cold thermal shock, and only a slight decrease in the plasma osmolality took place immediately after the transfer, the plasma osmolality stabilised thereafter on a level slightly higher than the initial physiological level of 340 mOsm/kg. Both S. mossambicus and S. aureus/S. niloticus appeared to be very tolerant to both high and low temperature shock as death at low temperature in the S. aureus/S. niloticus hybrid appeared to be primarily due to Saprolegnia infection.

D. Combined Effect of Thermal and Salinity Shock on the Plasma Osmotic Concentration

Following the direct transfer from the holding temperature of 25°C to higher temperature of 35°C and salinity of 50% sea

Figures 14 and 15

Effect of low temperature shock on the plasma osmotic concentration of S. mossambicus and S. aureus/S. niloticus hybrids.

The fish were directly transferred from the holding temperature of 25°C to the trial's low temperature of 15°C. Each point represents an average value of three fish; the standard deviation is also shown. The figures demonstrate that the hybrids were not affected by the cold shock, whereas the second increase in osmolality of S. mossambicus displays the lesser ability of this fish to regulate the osmotic concentration of the plasma at low temperature in fresh water.

Effect of transfer to low temperature on the plasma osmolality of S. mossambicus

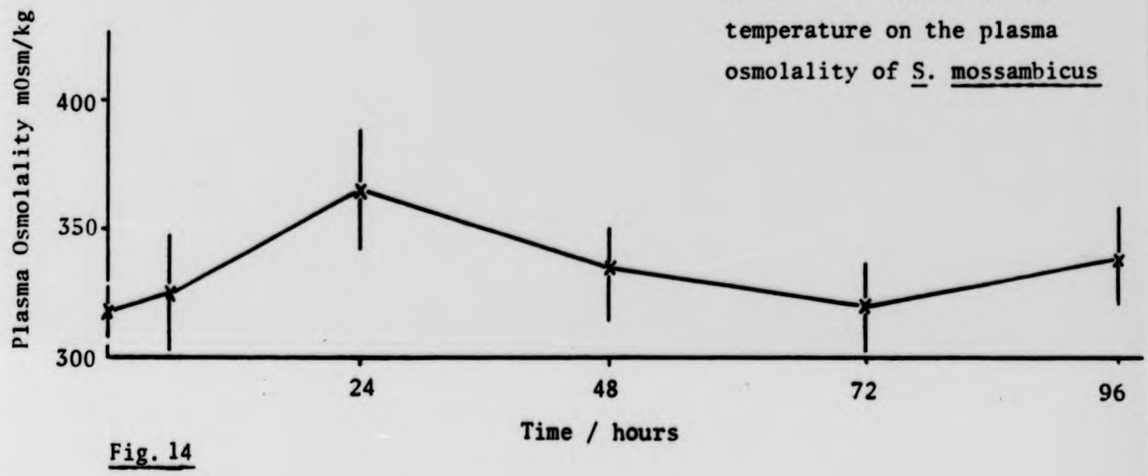


Fig. 14

Effect of transfer to low temperature on the plasma osmolality of S. aureus/S. niloticus hybrids

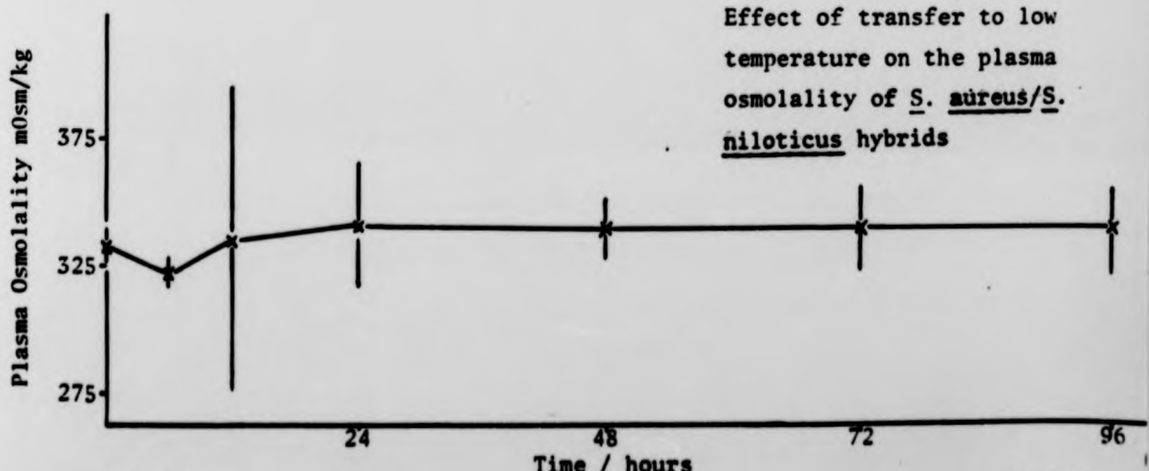


Fig. 15

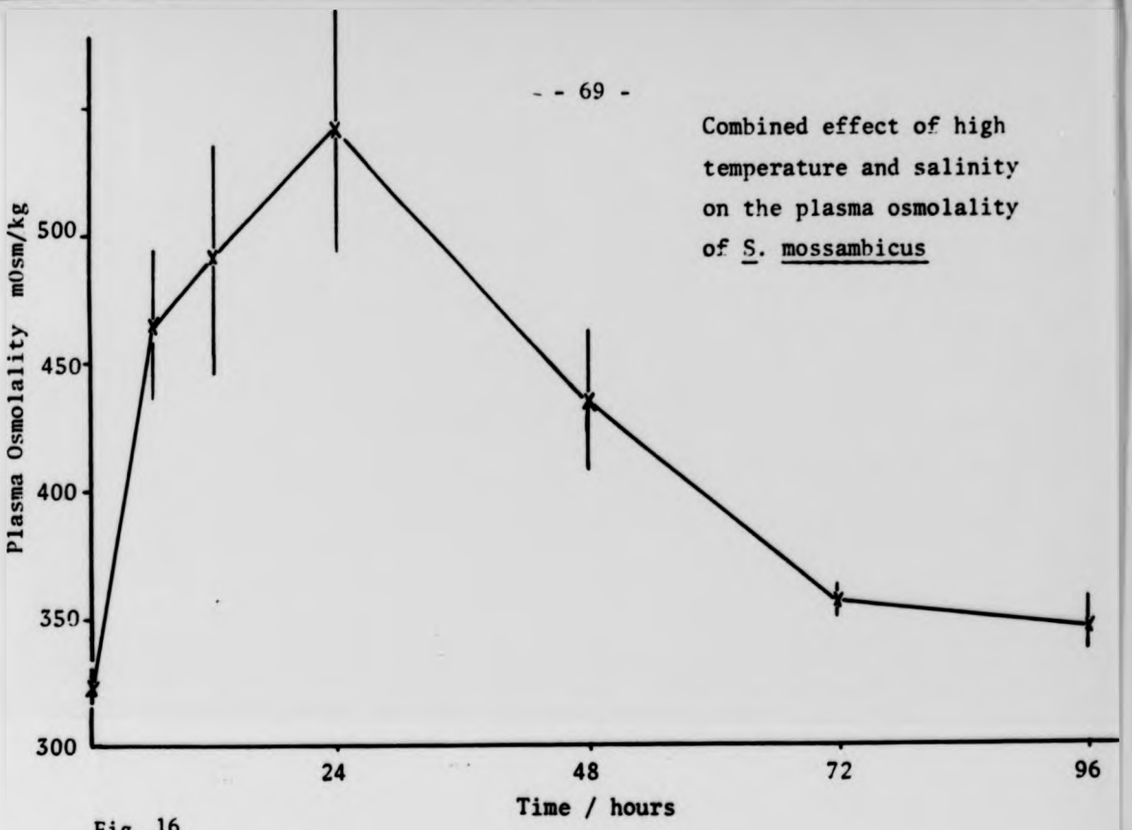
water (21.6‰), the combined osmoregulatory stress affected both species by increasing their plasma osmotic concentrations to 541 mOsm/kg for S. mossambicus and to 503 mOsm/kg for S. aureus/S. niloticus hybrids. This represents a very high increase of 219 and 169 mOsm/kg from the initial physiological levels for each species in fresh water. It has been found in Section B above that the S. mossambicus passed this trial with no mortality. This demonstrates the marked ability of this fish to withstand a very high plasma osmotic concentration during the first 24 hours, regulate it and survive after transfer to high temperature and salinity. Despite the number of mortalities recorded with the hybrids of S. aureus/S. niloticus during this trial (Section B above) those fish which did survive were also able to tolerate high plasma osmotic concentration and to regulate their plasma osmolality towards a new level by the end of the trial (Figs. 16 and 17).

The combined effect of low temperature and salinity is shown in Figs. 18 and 19. From Fig. 18 it can be seen that S. mossambicus shows an irregular concentration curve indicating osmoregulatory difficulties and an unstable physiological response from the fish. The plasma osmotic concentration increased from the initial physiological level up to 435 mOsm/kg in the first 12 hours and then decreased between 12 and 72 hours, till it increased again and reached 401 mOsm/kg by the end of the trial. Despite these unusual responses there was no mortality recorded in this trial (Section B above). In contrast to their good tolerance to low temperature by itself (Section C above) the hybrids of S. aureus/S. niloticus were not able to tolerate the

Figures 16 and 17

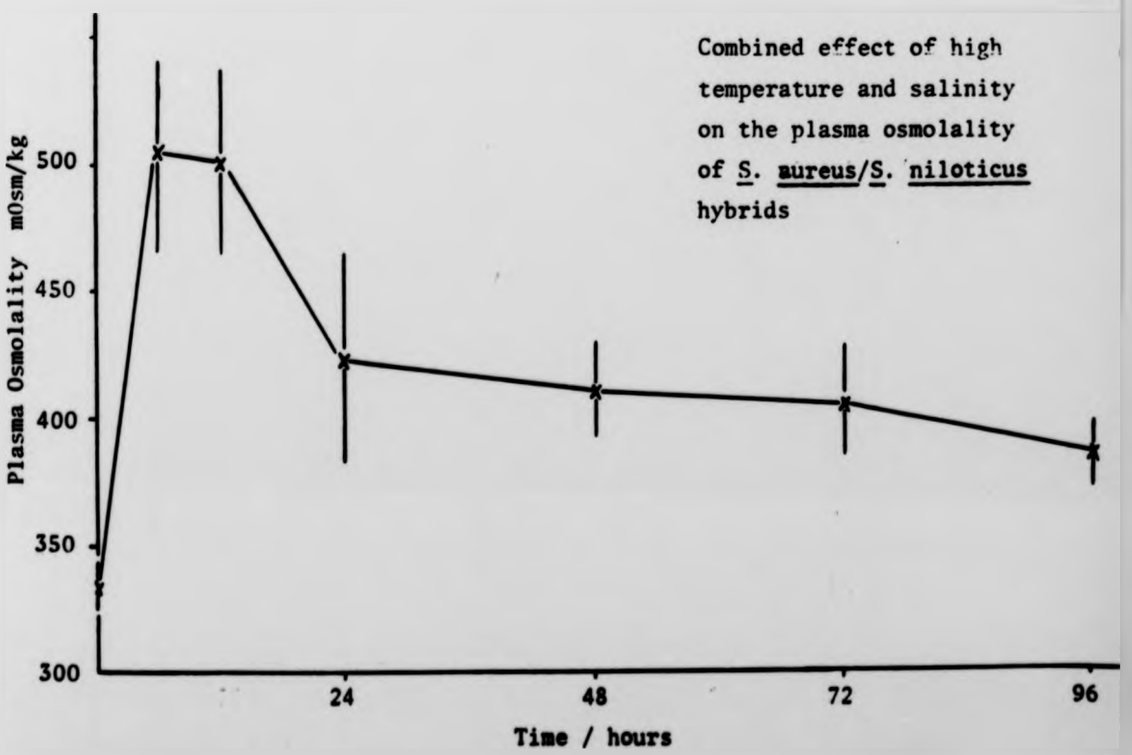
Combined effect of high temperature and salinity shock on the plasma osmotic concentration of S. mossambicus and S. aureus/ S. niloticus hybrids.

The fish were directly transferred from fresh water at holding temperature of 25°C, to a salinity equal to 60% sea water (21.6‰) at temperature of 35°C. Each point represents an average value of three fish; the standard deviation is also shown. The graphs demonstrate that both species were highly tolerant of these shocks; thus after the initial increase of their plasma osmolality, this fish was readily able to return to the relatively low level of 346 mOsm/kg by the end of the trial.



Combined effect of high temperature and salinity on the plasma osmolality of S. mossambicus

Fig. 16



Combined effect of high temperature and salinity on the plasma osmolality of S. aureus/S. niloticus hybrids

Fig. 17

Figures 18 and 19

Combined effect of low temperature and salinity shock on the plasma osmotic concentration of S. mossambicus and S. aureus/ S. niloticus hybrids.

The fish were directly transferred from fresh water at 25°C holding temperature to a salinity equal to 60% sea water (21.6‰) at temperature of 15°C. Each point represents an average value of three fish. The standard deviations are also shown. The graphs demonstrate that the hybrids were totally susceptible to these shocks, whereas the S. mossambicus were hardly able to regulate the plasma osmolality and underwent osmoregulatory problems.

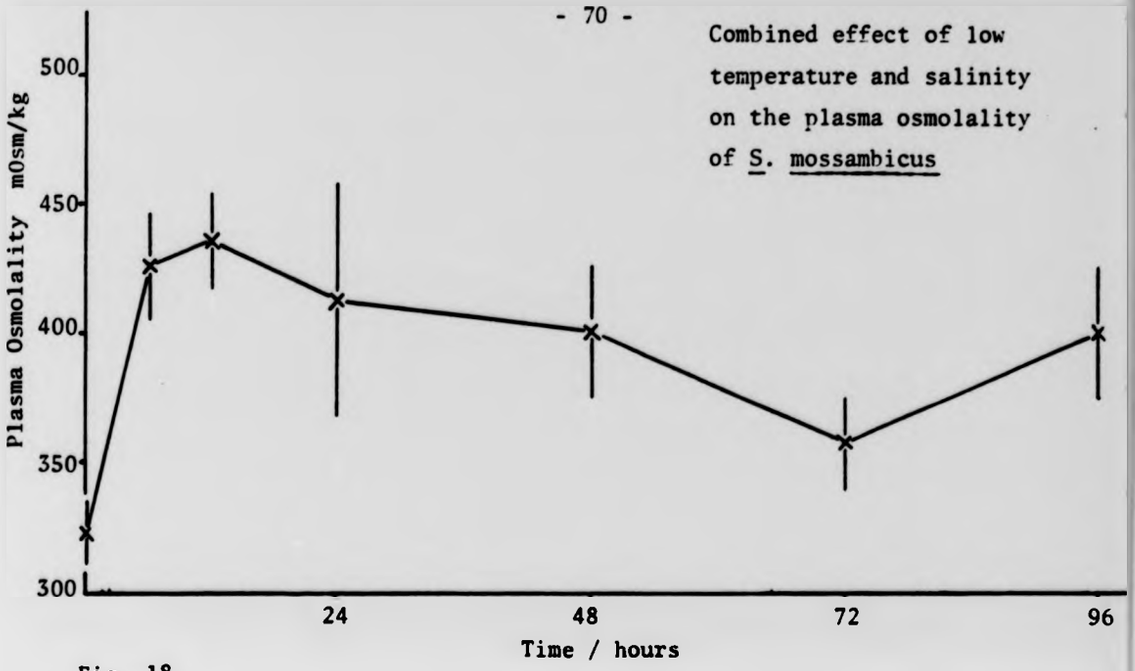

Combined effect of low temperature and salinity on the plasma osmolality of S. mossambicus
Plasma Osmolality mOsm/kg
500
450
400
350
300
24
48
72
96
Time / hours

Fig. 18

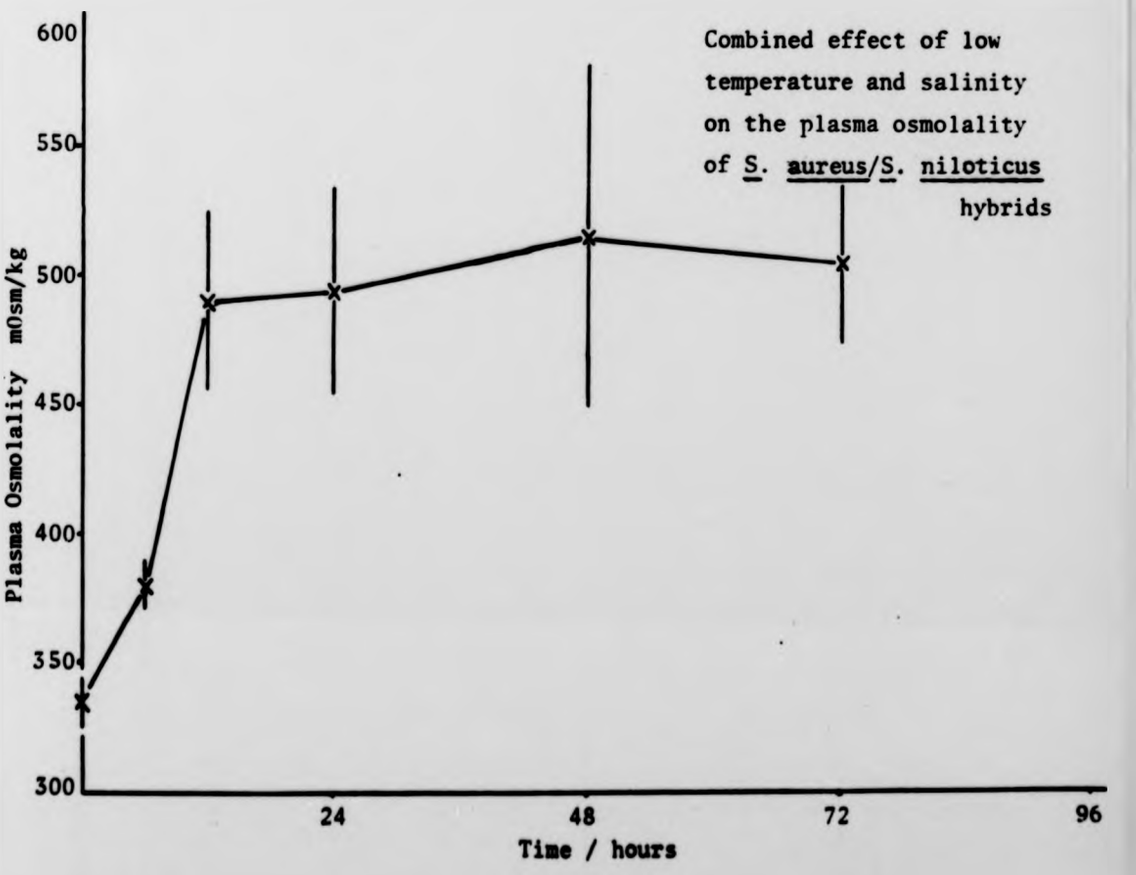

Combined effect of low temperature and salinity on the plasma osmolality of S. aureus/S. niloticus hybrids
Plasma Osmolality mOsm/kg
600
550
500
450
400
350
300
24
48
72
96
Time / hours

Fig. 19

combined effect of low temperature and salinity. Fig. 19 shows that initially the plasma osmolality increased as for S. mossambicus, but the rise, instead of levelling after a certain period, remained high and the mean value never returned to a new level as described in Chapter 3. All of the hybrids were dead after 72 hours when the mean value of their plasma osmotic concentration was 505 mOsm/kg.

DISCUSSION

It has been observed by previous workers that the cichlids of the genera Tilapia and Sarotherodon are both able to survive over a wide range of water temperatures, in some cases from 8 to 40°C, but the temperature to which they can completely adapt upon direct transfer depends on the temperature to which they have been acclimated (Spaas, 1959; Maar et al., 1966; Maruyama, 1975).

The results of the present study show that in fresh water at a maintenance temperature of 25°C S. mossambicus is better able to withstand direct transfer to a higher temperature than to a lower one. In contrast S. aureus/S. niloticus hybrids showed no significant differences in their response to higher or lower temperature. The ability of S. mossambicus to adapt to a higher temperature was noted earlier by Allanson and Noble (1964) who reported that S. mossambicus were fully acclimated when directly transferred from 25°C to 30°C whereas those which transferred from 25°C to 15°C were not fully acclimated even after 20 days. With carp (Cyprinus carpio) reared at warm water temperature Albrecht (1974) found that 30 days were required for full acclimation of this fish from 30°C to 15°C.

Regarding the effect of high temperature Bishai (1965) observed that S. niloticus is not affected by a sudden change to high temperatures, but he did not provide an adequate pre-acclimation time at the temperatures to which he was subjecting

his fish. Denzer (1967) determined an upper lethal temperature limit of 42°C for S. niloticus. Generally the previous results described above for S. mossambicus and S. niloticus indicated that these fish were able to withstand high temperature changes provided they received gradual acclimation. The results of the present study are in agreement with this in the light of the ability of the two species used to withstand high temperatures shock of at least 10°C when transferred within fresh water. Maar et al. (1966) state that Tilapia and Sarotherodon spp. were only able to withstand a temperature difference of between ± 5.6 and ± 8.4°C from the initial acclimated temperature, but these results are in disagreement with both the findings of previous authors and the present study, and raise the possibility that there were underlying pathological problems associated with these fish.

In the present study, the hybrid fish showed particular abilities which show affinities with the findings of Sarig (1969) who demonstrated the possibility that temperature tolerance may be passed from one of the parents of a hybrid to the offspring. Pruginin (1975) reported S. aureus to be the species most resistant to low temperature among the Sarotherodon. These findings support the present observations which indicated that S. aureus/S. niloticus hybrids were more tolerant to direct transfer to low temperature (without significant alteration in their plasma osmolality levels) than S. mossambicus within fresh water. It is unfortunate that an S. mossambicus hybrid was not able to be included in the study, since Potts et al. (1967) have shown that in fresh water S. mossambicus has a low permeability to sodium and high permeability to water

and the reverse was true in sea water. Allanson et al. (1971) have suggested that both renal failure and high water permeability at a low temperature of 11°C could be involved in the secondary chill coma which they described, leading to the death of fish. In the present study S. mossambicus was observed to have slightly increased plasma osmotic concentration which is surprising as renal failure and high water permeability as suggested by Allanson et al. (1971) should cause a decrease in osmotic concentration. However the rapid decrease in plasma osmotic concentration reported by Allanson et al. (1971) resulted after transfer to 11°C whereas a transfer to 13°C caused no decrease. Certainly the general observations of "chill coma" experienced after transfer to cold water and the subsequent Saprolegnia infection observed in the present study agree well with the clinical observations of Allanson et al. (1971).

No previous work relating to the effects of high temperature shock on plasma osmotic concentration in Sarotherodon species have been traced although the effects of direct transfer to high temperature on some other blood parameters of Tilapia spp. and some other fish species have been reported. For example Forghaly et al. (1973) observed an increase in almost all of the cellular and organic components of the blood of Tilapia zillii associated with transfer to high temperature. Thus the small increase in plasma osmotic concentration associated with high temperature shock in fresh water which was experienced by the fish of the present study may have been associated with haemoconcentration and indicates that these fish are readily able to tolerate a high temperature shock in fresh

water of 10°C or more, with no signficant physiological problems, and is in complete disagreement with the general view of Maar et al. (1966).

The marked ability of both S. mossambicus and S. aureus/S. niloticus hybrids to withstand very high plasma osmotic concentrations (Figs. 16 and 17) agrees with the results of Lotan (1960) who recorded a value of 586 mOsm/kg during the gradual acclimation of S. niloticus (suspected aureus) to high salinities. However both fish of the present study either subjected to high temperature within fresh water itself or to high temperature and salt water again displayed the characteristic pattern of the bi-phasic curves of plasma osmolality already described in Chapter 3. However when the cold stress or the combined stress of low temperature and salinity was applied the two species behaved in different ways. The most important difference was observed with the combined effect of low temperature and salinity which might be attributed to the difference in the salinity tolerance between the two species. Thus as has been shown in Chapter 3 salinity of 60% sea water (21.6‰) was just below the lethal limit for the hybrids of S. aureus/S. niloticus, and since the same salinity concentration was used in the present study, it is possible that the low temperature shock affected this species by lowering its transferability to such ""relatively" high salinity, causing the severe mortality which was indicated by the sustained very high levels of plasma osmotic concentration. It seems that similar observations might be found with S. mossambicus if a higher salinity was to be applied although with such euryhaline

fish the salinity stress would probably have had to be greater. These findings are supported by those of Whitfield and Blaber (1976) who observed that the maximum salinity tolerance for T. rendalli was achieved at temperatures of between 20 and 28°C. However the hybrids of S. aureus/S. niloticus maintained stable levels of plasma osmolality during transfer to lower water temperature in fresh water (Fig. 15). Similar findings have been reported for cyprinid fish by Grigo (1975) who found that the plasma osmotic concentration of the carp (Cyprinus carpio) was not affected by cold shock when he transferred this fish from 30 to 15°C.

CHAPTER 6

THE EFFECT OF REDUCED WATER QUALITY AND FOOD DEPRIVATION ON THE ABILITY OF *S. MOSSAMBICUS* AND *S. NILOTICUS* TO SUCCESSFULLY TRANSFER TO SALT WATER

INTRODUCTION

The difficulties of maintaining young fish in a fresh water environment in desert areas or in holding or transporting fish for lengthy periods of time in less than ideal conditions prior to introduction to sea water areas are likely to present additional stress in the farm if poor water quality or poor, intermittent feeding take place. However, in a developing country these undesirable husbandry practices can occur. It was therefore considered important to consider the influence of these additional stresses on the acclimation of fish to sea water.

A number of water quality parameters are likely to be significant, including ammonia levels, nitrite levels, oxygen concentration and pH. While some data are available on responses of Sarotherodon species to individual factors there is only circumstantial information on the combined effects of all of the various factors contributing to poor water quality in hatchery conditions.

Thus there is a great deal of experimental data on the tolerance and the lethal limits of oxygen, carbon dioxide and pH for tilapias. For example the lowest oxygen tolerance level recorded was 0.1 mg/L for S. mossambicus (Maruyama, 1958) and for S. niloticus (Magid and Babiker, 1975). Dusart (1963) reported that S. macrochir were able to live in swamps and to tolerate carbon dioxide levels as high as 72.6 mg/L. Most of the experimental work carried out to determine the pH limits was really

concerned with the general enhancement of pond productivity rather than specific effects of pH on fish in particular. George (1975) reported that S. niloticus were able to tolerate high pH levels (between 8-11) in ponds in the Sudan. Reite et al. (1973) working with S. grahami in Lake Magadi, Kenya, established a tolerance range of pH between 5 and 11 over 24 hours. According to Fryer and Iles (1972) S. grahami is one of the most resistant of the Sarotherodon species, but it is likely that most of the other Sarotherodon species are able to tolerate similar levels, as these would have corresponded with conditions, in the first case, in an actively photosynthesizing and secondly, in a dying out aquatic plant environment.

The separate effects of high salt diet and of thermal shock on salt water acclimation have been discussed in the two previous chapters. It is well known that in nature tilapias are able to survive in extremely adverse conditions and are frequently found in habitats where no other fish could exist (Dusart, 1963; Coe, 1965; Fryer and Iles, 1972; Caulton, 1976). Most such environments are likely to include occasional exposure to thermal shock, poor environmental water quality and shortage of food so this probably explains the very considerable hardiness of the genus.

The present experiments were designed to investigate separately the effects of exposure to poor water quality (oxygen concentration, pH, ammonia and nitrite nitrogen) and reduced food supply on fish survival (in fresh water and following transfer to salt water) and on plasma osmotic concentrations. S. mossambicus and S. niloticus were used in the present series of experiment.

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MATERIALS AND METHODS

Fish

Sarotherodon mossambicus and S. niloticus were obtained from the tropical hatchery of the Institute of Aquaculture at the University of Stirling. A total of 350 fish of S. mossambicus and 350 of S. niloticus (3 g. average weight) were used for the water quality experiments (Table 8) and 150 fish (4 g. average weight) of each species were used in the starvation experiment.

Experimental Design

A. Water quality experiments

Two types of experiments were conducted as shown in Table 8. The first was to examine the effect of water quality on fish survival in fresh water. In this experiment the water was not filtered or renewed (Class A). The fish were held there under the accumulation of environmental wastes (i.e. ammonia, CO_2, solids, nitrite and pH change) until mortalities took place without transfer of fish to salt water. In the second experiment, the water was partially changed (Class B), but unfiltered, and subgroups of fish were transferred weekly to salt water from one week onwards for five weeks. Thus the first trial could indicate the critical levels involved to be related into the observed effects of salinity transfer in the second trial. The experimental procedures were as follows:

Fish species	Type of experiment	No. of fish	Fish Weight (g)	Type of water	Duration in fresh water	Salinity to which the fish transferred (viz. tolerance level) (‰)
S. niloticus	Effect of water quality on fish survival	60	3.0	Class A	Until mortalities take place	-
S. mossambicus	Effect of water quality on fish survival	60	3.0	Class A	Until mortalities take place	-
S. niloticus	Effect of water quality on P.O.C. and on fish survival in salt water	180	2.78	Class B	5 weeks	21.6
S. mossambicus	Effect of water quality on P.O.C. and on fish survival in salt water	180	3.3	Class B	5 weeks	27.0

TABLE 8. Summary of water quality trials

Class A = The water was not renewed or filtered
Class B = The water was partially changed
P.O.C. = Plasma osmotic concentration

1. Effects of water quality on fish survival in fresh water (Class A water)

Four tanks of the normal 50 L. capacity were prepared as described in Chapter 2, but the filters were removed from these tanks and the water was not changed throughout the trial (Class A). Two of the tanks were stocked with 30 S. mossambicus (3 g. average weight) each, and the other two stocked with 30 S. niloticus (3 g. average weight) each. The fish were maintained while there developed continuous accumulation of environmental wastes. The fish mortality was recorded every day.

Solids per se were not measured directly, therefore a scale of water quality, including turbidity was devised. The criterion was based on the distance through which a fish of 4 cm standard length could no longer be seen in very turbid water as follows:

0 - Water very pure and clear, no settled waste

1 - Water slightly turbid as some faeces spread on the bottom and all fish were easily seen throughout the tank

2 - Water of medium turbidity as faeces and debris covered some of the bottom and also moved through the water column. Sight distance 15 cm +.

3 - A lot of faeces and debris on the bottom and stuck on the internal sides with the water colour almost light brown. Sight distance between 10 cm and 15 cm.

4 - Dull, heavy levels of debris and suspended matter as the sight distance is between 5 cm and 10 cm.

5 - Very heavy levels of suspended matter and debris, water very turbid, with sight distance between 2 cm and 5 cm.

Additional observations were made of behaviour and feeding. Feeding activity was described by another scale (slightly modified after Jackson, 1979) as follows:

N - None, no feeding activity

VL - Very light, a few pellets taken as they sank and from the bottom of the tanks

L - Light, pellets taken as they sank, from the bottom of the tanks, a few from the surface

A - Active, normal level of feeding with most fish taking food from surface

VA - Very active, all the fish at the surface thrashing to obtain food.

Oxygen saturation levels were measured daily with a Kent 1520 portable oxygen meter, the hydrogen ion concentration was also measured daily by a pH meter (Corning EEL model 12). Ammonia and nitrite concentration were measured every six days or when fish mortality was observed. The methods of measuring ammonia and nitrite have been described earlier (Chapter 2).

A limited number of histological samples were taken of gill tissues, since gills are the organs likely to be affected first by poor environmental conditions.

2. Effect of poor water quality on fish survival and plasma osmotic concentration in fresh water (Class B water) and on ability to directly transfer to salt water

Two of the normal 100 L. capacity tanks were used (Chapter 2). The tanks were prepared without filters but with heaters and aeration as described above. One of the tanks was stocked with 170 S. mossambicus (3.3 g. average weight) and the other with 170 S. niloticus of 2.78 g. average weight. The fish were left in their tanks for 5 weeks. During the first 3 weeks half of the water was changed with fresh water every week. No chemical sources of ammonia or nitrite were added and thus the only source of pollution was the fish excreta and the uneaten food. One week from the start of exposure and weekly thereafter for five weeks triplicates of 10 fish from each of the species groups were directly transferred to the salinities which was earlier shown to be their maximum tolerance levels (Chapter 3), in order to provide a reliable evaluation of the effect of water quality on the transfer.

Simultaneously with each weekly transfer to salt water, 3 fish samples from each of the species were netted out and bled for the measurement of the plasma osmotic concentration. The blood sampling method was as described in Chapter 2.

B. Salt water tanks

Six tanks of 50 L. capacity measuring 50 x 40 x 40 cm each were prepared as described in Chapter 2, and contained water at

salinity concentration of 21.6‰ ± 0.5 for S. niloticus or 27‰ ± 0.5 for S. mossambicus. In all fresh and salt water tanks the temperature was kept constant at 27°C ± 0.5. After transfer to salt water the fish were held for 72 hours and the mortalities were recorded every 12 hours.

C. Effect of food deprivation on capability for direct transfer to salt water and on the osmotic concentration of the plasma

This experiment was designed to investigate the effect of food deprivation on the plasma osmotic concentration in fresh water and on the survival of the starved fish following the direct transfer to salt water. Two tanks of 100 x 40 x 30 cm were prepared as described in Chapter 2. One of the tanks was stocked with 110 S. niloticus (4.2 g. average weight) and the other was stocked with 110 S. mossambicus (3.5 g. average weight). The control fish of both species were kept in a bigger tank measuring 115 x 50 x 40 cm where they were separated by a net. The fish were held for one week to settle during which they were fed standard diet, at rate of 5% of their body weight daily, spread over three feeds. The fish were fasted for one week, after which they were then fed at the much lower level of 5% body weight per week for a period of 8 weeks. The fish were weighed at the start of the experiment and check weighed thereafter every two weeks to allow approximate food weight revision.

The estimation of the effect of salt water exposure on survival of food deprived fish was carried out by transferring

samples from the fresh water experimental tanks to salt water tanks. Triplicate samples of 10 fish of each species were netted out and directly transferred to salt water. (Each fish species was transferred to its threshold salinity concentration as described above). At the time of each transfer 3 fish were netted out and bled to determine the effects of reduced feeding on the plasma osmotic concentration.

In all fresh and salt water tanks the temperature was fixed at constant level of 27°C ± 0.5 throughout the experiment and the water quality was monitored as described in Chapter 2.

RESULTS

Effect of Water Quality on Fish Survival (Class A Water)

Water quality and mortalities

Tables 9 and 10 summarise the effect of the water quality on fish survival in fresh water. Neither S. niloticus nor S. mossambicus tolerated the chronic accumulation of the various metabolic excretory products and the subsequent deterioration in the quality of the environment. The highest average total ammonia content recorded was 19 mg/L (equal to 1.128 mg/L of unionized ammonia at pH 7.6) in the S. niloticus tanks, and 9.4 mg/L (equal to 0.613 mg/L of unionized ammonia at pH 7.65) in the S. mossambicus tanks. These levels were lethal for the respective species and thus S. niloticus appeared to be more tolerant to low quality environment than S. mossambicus. The former species was able to resist the continuous decline in water quality for 12 days before the start of mortality, but subsequently the effect was acute and lethal and 90% of the fish died within 48 hours. On the other hand S. mossambicus survived only for 8 days before the mortalities commenced, but although the environmental conditions continued to deteriorate thereafter mortalities were lower. Thus despite S. mossambicus having been affected at lower ammonia concentration they resisted longer and nine fish were still living four days after the first mortality took place.

Although the water quality conditions were deteriorating as faecal solids and various soluble metabolic excretory products were

Time in days	Total no. of fish	Mortality (%) ± S.D.	Water quality condition	Average oxygen saturation (mg/L)	Average pH	Average ammonia-N concentration (mg/L)	Average nitrite-N concentration (mg/L)	Feeding activity
0	60	0	0	7.97	6.85	0.005	0.01	VA
2	60	0	1	6.69	7.2	-	-	VA
4	60	0	1	6.38	7.35	-	-	A
6	60	0	2	6.22	7.5	1.45	0.98	L
8	60	0	2	6.22	7.5	-	-	VL
10	60	0	3	6.22	7.5	-	-	N
12	43	28 ± 7.1	4	5.90	7.6	19.0	1.48	N
14	0	100	4	6.22	7.6	-	-	N

TABLE 9. Effect of poor water quality (Class A water) on the survival of S. niloticus

The average initial weight of the fish was 3.0 g. The fish were held with the continuous accumulation of various metabolic wastes until the mortalities took place. Two 50 L. tanks of 30 fish each were used.

Time in Days	Total no. of fish	Mortality (%) ± S.D.	Water quality condition	Average oxygen saturation (mg/L)	Average pH	Average ammonia-N concentration (mg/L)	Average nitrite-N concentration (mg/L)	Feeding activity
0	60	0	0	7.97	6.9	0.005	0.01	V
2	60	0	1	6.22	7.4	-	-	A
4	60	0	2	5.90	7.5	-	-	L
6	60	0	3	5.90	7.6	2.1	0.76	N
8	54	10 ± 4.7	3	5.70	7.6	-	-	N
10	37	38.3 ± 16.5	4	5.70	7.65	7.6	0.33	N
12	9	85 ± 7.1	5	5.70	7.65	9.4	0.48	N
14	1	98.3 ± 2.4	4	5.70	-	-	-	-

TABLE 10. Effect of poor water quality (Class A) on the survival of S. mossambicus

The initial average fish weight was 3.0 g. Two 50 L. glass tanks of 30 fish each were used. The fish were held under conditions of continuous accumulation of various metabolic wastes until mortalities took place.

accumulating, the oxygen content and the pH of the water did not significantly alter. The total nitrite-nitrogen changed within relatively narrow limits and the maximum value recorded was 1.45 mg/L with S. niloticus.

Feeding and behaviour

The feeding activity was observed to serve as an indicator of the degree of stress induced in the fish by the environment, and the differing resistance between the two fish species. Thus with respect to the different resistance time between the two fish species, both of them actively fed during the first two days, but about four days before the first mortalities took place most of the fish ceased to feed. The stressed fish were lethargic and gathered in groups near the bottom. During the last two days they showed very slow swimming movements and did not respond to the presence of fresh food or to external stimuli.

Gill samples showed lamellar hyperplasia and in most cases the tips of the secondary lamellae were swollen, indicative of a polluted environment.

Effect of Water Quality (Class B Water) on Fish Survival, Direct Transfer to Salt Water and Plasma Osmotic Concentration

The effect of poor quality water (Class B) on the survival of S. niloticus prior to salt water transfer is shown in Table 11. It can be seen that in partially changed water the total ammonia concentration reached a maximum level of 6.4 mg/L (equal to 0.359 mg/L

Time in Weeks	Total no. of fish	Mortality (%)	Water quality condition	Average oxygen saturation (mg/L)	Average pH	Average ammonia-N concentration (mg/L)	Average nitrite-N concentration (mg/L)	Feeding activity
0	180	0	0	7.97	6.85	0.004	0.01	VA
1	147	0	1	6.22	7.3	1.4	0.98	A
2	114	0	2	6.22	7.4	2.8	0.68	L
3	81	0	3	5.90	7.4	6.4	0.48	A
4	48	0	3	6.22	7.3	3.7	0.25	A
5	15	0	3	6.69	7.3	0.35	0.82	A

TABLE 11. Effect of poor quality water (Class B water) on the survival of S. niloticus

The partially changed water did not affect the survival of S. niloticus. The decrease in the fish number was as a result of fish samples being transferred to salt water from this trial, the fish survival following transfer to salt water is shown in Fig. 20.

of unionized ammonia at pH 7.4), three weeks from the start of the experiment. However total ammonia concentration decreased thereafter and went down as low as 0.35 mg/L (equal to 0.018 mg/L of unionized ammonia at pH 7.3) by the end of the experiment, presumably due to the accumulation of bacteria capable of metabolising ammonia on the surface of the tanks. Although ammonia concentration decreased, other faecal and bacterial solids continued to increase thereafter and these did not appear to cause any additional mortality. Comparing the values in Table 11 with those in Table 9, it can be seen that the highest level of ammonia concentration recorded was far below the lethal level of 19 mg/L total ammonia determined in the first trial.

The effect of prior exposure to the poor environment (Class B water) on the survival of S. niloticus after direct transfer to salinity of 60% sea water (21.6‰) is shown in Fig. 20. The highest mortalities were recorded with the batch transferred in the first week. These mortalities and also those in the following three weeks were significantly higher than the controls. At the fifth week the mortalities decreased to 23.3% at which they did not significantly vary from the controls ($p = 0.05$).

The poor environment (Class B water) affected the plasma osmotic concentration by increasing it. The results are given in Tables 12 and 13 which show that during the first week of exposure to deteriorating water qaulity, the osmotic concentration of the plasma of both S. niloticus and S. mossambicus increased significantly ($p = 0.01$) from 340 mOsm/kg (S.D. = ± 8) to 371 mOsm/kg

Treatment regime	Fish Weight (g)	Osmolality of blood plasma (mOsm/kg)	Mean ± S.D. of plasma osmolality (mOsm/kg)
Control	2.7	337	340 ± 8
	2.1	331	
	2.4	348	
	3.3	344	
One week in partially changed water	3.7	377	371 ± 5
	2.4	371	
	2.5	366	
Two weeks in partially changed water	3.3	339	337 ± 8
	3.2	328	
	3.8	344	
Three weeks in partially changed water	3.4	348	347 ± 9
	3.7	344	
	4.3	339	
Four weeks in partially changed water	4.2	348	347 ± 10
	3.8	344	
	4.5	339	
Five weeks in partially changed water	4.7	339	349 ± 10
	4.5	349	
	5.1	360	

TABLE 12. Effect of poor quality water (Class B) on the plasma osmotic concentration of S. niloticus

Treatment regime	Fish Weight (g)	Osmolality of blood plasma (mOsm/kg)	Mean ± S.D. of plasma osmolality (mOsm/kg)
Control	4.8	335	324 ± 11
	3.4	312	
	3.2	324	
One week in partially changed water	6.0	414	374 ± 35
	3.7	350	
	4.5	358	
Two weeks in partially changed water	4.1	366	376 ± 18
	4.8	398	
	5.3	366	
Three weeks in partially changed water	7.2	312	335 ± 20
	5.4	344	
	4.0	349	
Four weeks in partially changed water	6.3	301	313 ± 13
	5.0	312	
	4.7	328	

TABLE 13. Effect of poor quality water (Class B) on the plasma osmotic concentration of *S. mossambicus*

Figure 20

Effect of prior exposure to poor water quality (Class B water) on the survival of S. niloticus after direct transfer to salinity of 60% sea water (21.6‰).

Triplicates of 10 fish were used in each transfer. The initial average weight of fish was 2.78 g. The fish were held in salt water for 72 hours after each transfer, during which the fish mortalities were counted every 12 hours.

controls

experimental fish

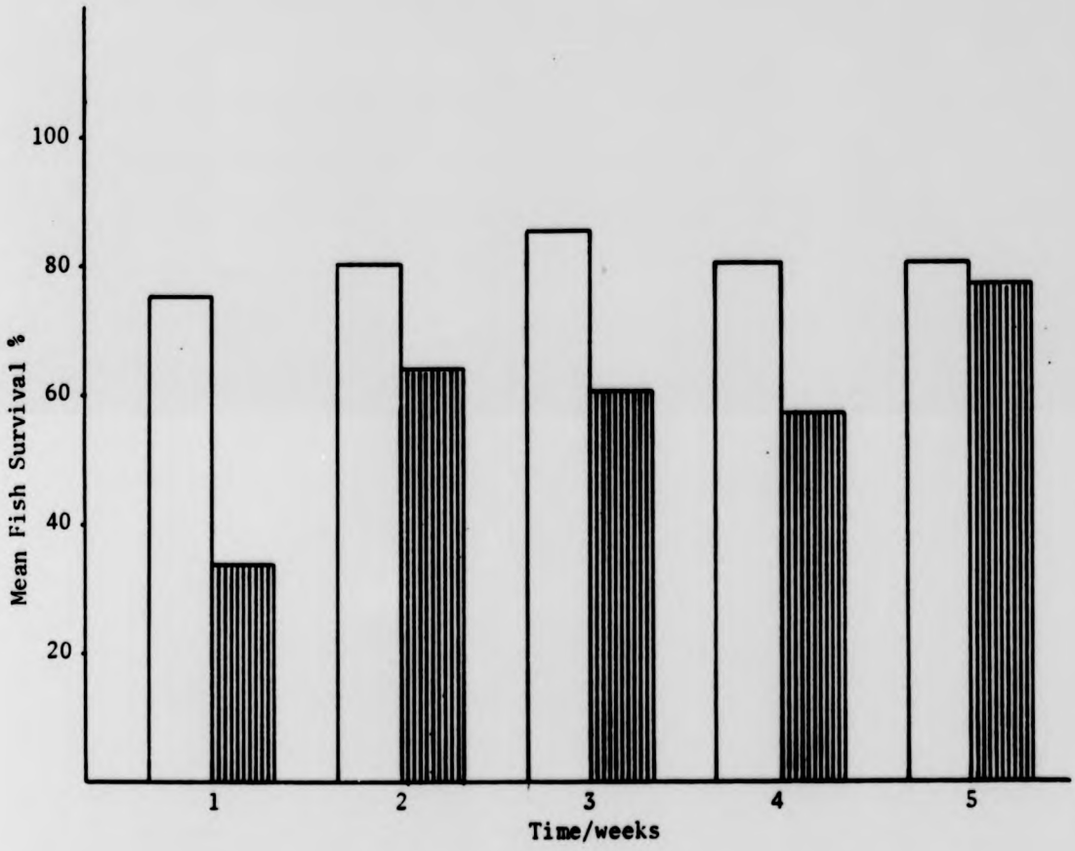

Fig. 20

(S.D. = ± 45) for S. niloticus, and from 324 mOsm/kg (S.D. = ± 11) to 374 mOsm/kg (S.D. = ± 35) for S. mossambicus. After the first week the osmotic concentration of the plasma for S. niloticus returned to a new level which almost equalled the initial physiological level and remained so throughout the rest of the experimental period. The mean value during this period was 341.5 mOsm/kg (S.D.= ± 7.7). On the other hand the osmotic concentration levels for S. mossambicus remained high till the end of the second week when the level was still as high as 376 mOsm/kg (S.D. ± 18). Moreover S. mossambicus did not tolerate the poor environment (Class B water) for more than one week. Thus high mortality took place by the second week. It was not possible to carry out the transfer to salt water by the third week as 74.7% of the fish had died.

Effect of Food Deprivation on the Direct Transfer to Salt Water and on the Plasma Osmotic Concentration

The effect of reduced feeding on fish survival after direct transfer to salt water is shown in Figs. 21 and 22 for S. mossambicus and S. niloticus respectively. Reduced food intake resulted in fewer fish surviving the transfer to salt water both with S. niloticus and S. mossambicus. The number of fish surviving decreased with the passing of each week except for the first sample from S. mossambicus (two weeks after commencement of reduced feeding) where the experimental fish showed slightly better survival than the controls.

Tables 14 and 15 show the effect of reduced feeding on the

Figures 21 and 22

Effect of prior reduced feeding on fish survival following direct transfer to salt water in S. mossambicus and S. niloticus respectively.

S. mossambicus were directly transferred to salinity of 75% sea water (27‰) while S. niloticus directly transferred to 60% sea water (21.6‰). Triplicate samples of 10 fish from each of the species were used in each trial. Post salt water transfer the fish were held for 72 hours and mortalities were counted every 12 hours.

control

experimental fish

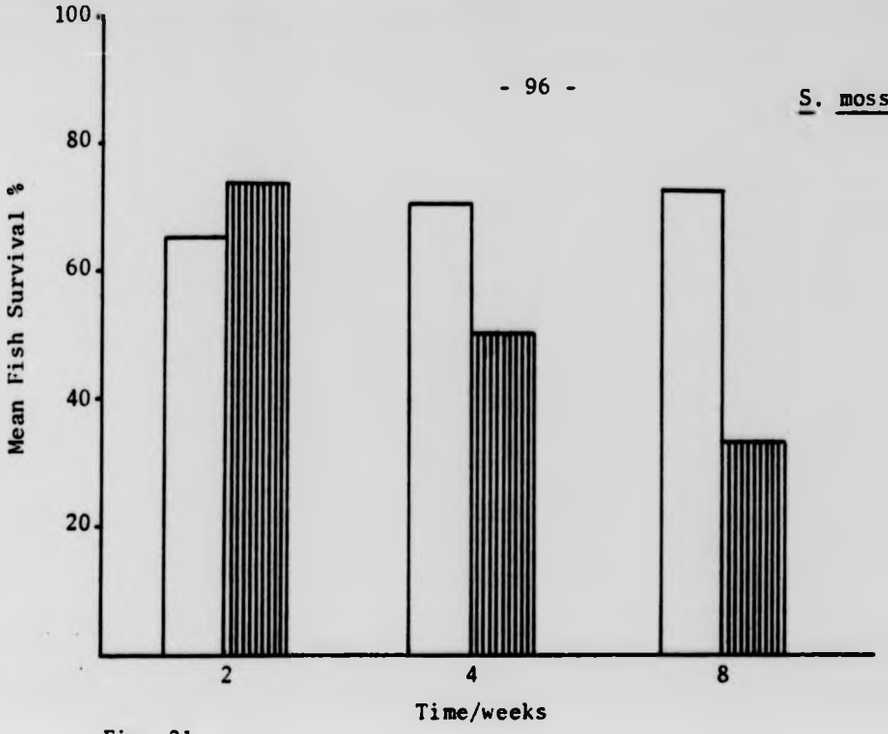
S. mossambicus
Mean Fish Survival %
100
80
60
40
20
2
4
8
Time/weeks

Fig. 21

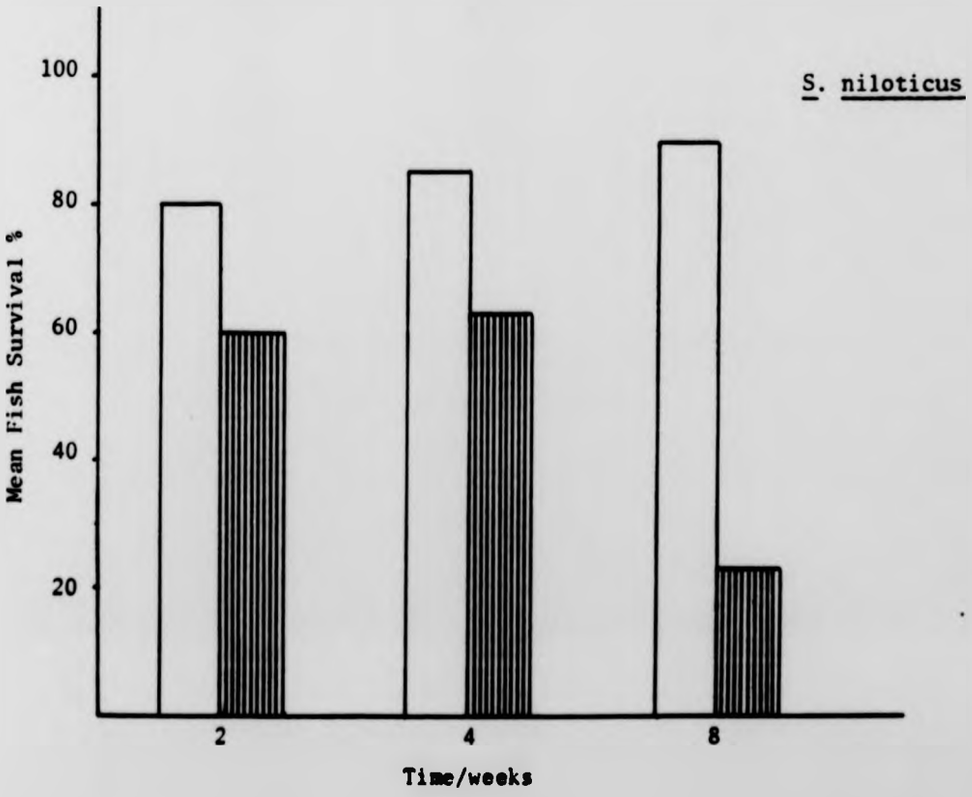
S. niloticus
Mean Fish Survival %
100
80
60
40
20
2
4
8
Time/weeks

Fig. 22

Treatment regime	Fish Weight (g)	Osmolality of blood plasma (mOsm/kg)	Mean ± S.D. of plasma osmolality (mOsm/kg)
Control	5.1	336	
	4.2	343	339 ± 8
	4.5	348	
	3.8	330	
Two weeks on reduced feeding	5.0	328	
	3.5	320	327 ± 7
	4.8	334	
Four weeks on reduced feeding	4.8	355	
	4.5	360	356 ± 3
	3.2	355	
Eight weeks on reduced feeding	5.5	339	
	4.2	350	342 ± 6
	4.3	339	

TABLE 14. Effect of reduced feeding on the plasma osmotic concentration of S. niloticus

Treatment regime	Fish Weight (g)	Osmolality of blood plasma (mOsm/kg)	Mean ± S.D. of plasma osmolality (mOsm/kg)
Control	3.5	312	
	3.1	330	317 ± 14
	4.8	300	
	3.3	327	
Two weeks on	4.4	299	
reduced feeding	3.7	339	323 ± 21
	3.8	333	
Four weeks on	5.1	322	
reduced feeding	4.5	312	317 ± 5
	3.9	317	
Eight weeks on	4.9	344	
reduced feeding	4.2	398	365 ± 29
	4.7	353	

TABLE 15. Effect of reduced feeding on the plasma osmotic concentration of S. mossambicus

osmotic concentration of the plasma. In the case of S. niloticus the reduced feeding did not significantly alter the osmotic concentration levels. Thus after two weeks the plasma osmolality decreased by 20 mOsm/kg compared with the control fish levels. Thereafter they increased to reach levels slightly higher than the initial ones. On the other hand in S. mossambicus, the osmotic concentration of the plasma remained more or less stable during the first four weeks on reduced feeding and it was not until eight weeks that the osmotic concentration of the plasma recorded a significant ($p = 0.01$) increase of 48 mOsm/kg over the initial control levels.

DISCUSSION

Effect of Poor Quality Water

In the present study the effect of poor environmental conditions on the host was evaluated in terms of fish survival, plasma osmotic concentration in fresh water, and effect of prior holding in water of poor quality on subsequent transfer to salt water. In such an environment ammonia was found to be the main metabolic product that affects fish survival and gill epithelium. This agrees with Burrows (1964) who reported that of a range of accumulated excretory products tested on hatchery-reared salmonids, ammonia was the principal excretory product that affected both gills and physical stamina.

In relation to observed lethal levels (Tables 9 and 10), there is little data on Sarotherodon species per se reported elsewhere. Barry and Stickney (1979) reported a 48 hours median lethal concentration (LC 50) of unionized ammonia of 2.4 mg/L for S. aureus. This result is relatively higher than those obtained for both species of the present study S. niloticus and S. mossambicus. However it has been reported by a number of previous authors that the effect of mixtures of ammonia and other compounds (as in the accumulated excretory wastes of the present study) are of greater toxicity than either substance alone, i.e. combination of ammonia and phenol (Herbert, 1961), zinc and ammonia (Herbert and Shurben, 1964). For further details see review by Alabaster and Lloyd (1980).

A number of physiological effects are reported to be likely

to occur in a wide variety of fish suffering from ammonia toxicity. These may have been responsible for lowering the ability of the fish to tolerate salinity. Ammonia related gill epithelium damage has been demonstrated by several authors (Kuhn and Koecke, 1956; Burrows, 1964; Reichenbache-Klinke, 1967; Flis, 1968a; Smart, 1976). The lethargy and loss of appetite of the fish of the present study agrees with the observations of Forster and Smart (1979) who found that exposure of rainbow trout to sublethal levels of unionized ammonia caused lethargy, loss of appetite and poor growth during the first two weeks of their growth rate experiment. Increases in urine excretion have been reported by Lloyd and Orr (1969) who suggested that in rainbow trout exposed to sublethal ammonia, this diuresis was caused by the ammonia inducing an increased water permeability which may indicate a stress imposed by the water intake on the kidneys and a water balance in general. However results presented here do not show the expected decrease in osmotic concentration normally associated with high water intake; on the contrary a small increase was observed. The reason for this is not understood but could be the result of osmotically active metabolites in the blood being unusually high due to the poor water quality.

The regulation of sodium and chloride ions in teleosts have been extensively studied (Holmes and Donaldson, 1969; Bentley, 1971; Lutz, 1972; Maetz, 1974; Whiting, 1979). But the plasma sodium and chloride relationships in fish subjected to stressful conditions are less well known although Tomasso et al. (1980) reported that 24 hours (LC 50) of ammonia did not affect the plasma sodium and chloride values in the channel catfish (Ictalurus punctatus). The external ammonia concentration did not significantly

affect the chloride values in the plasma of rainbow trout (Swift, 1981). These limited findings are however in agreement with those in Table 12 of the present study.

The ammonia and nitrite values in Tables 9 and 10 suggest inherent differences in resistance between the two Sarotherodon species used. The values of nitrite which could be resisted by S. niloticus (Table 11) are higher than the figures of 0.19-0.39 mg/L nitrite-nitrogen recorded by Russo et al. (1974), for 4 days medial lethal concentration in rainbow trout, and the figures of 0.55 mg/L nitrite-nitrogen recorded by Smith and Williams (1974) for mortality tests with the same species, but in view of the very different natural habitats of the two species this is not very surprising.

Effect of Reduced Feeding

The effects of starvation on fish are somewhat poorly documented. Swallow and Fleming (1969) observed partial depletion of the liver glycogen and resorption of the liver tissue in their 20 days experiment on the starvation of S. mossambicus held at 28 ± 1°C. Shimma et al. (1976) observed a sharp drop in the glycogen content in rainbow trout held at 17°C during early fasting and at the fortieth day of starvation there was a tardy fall of plasma glucose level. Thus although the euryhaline fish of the present study (S. mossambicus, S. niloticus) could normally tolerate the salinity transfer used they failed to survive in these known tolerable salinities after they have been deprived

of food, suggesting that the high energy requirement for salt excretion and also the protein requirement for salt cell production could no longer be met. Other studies on starvation and osmotic stress in tilapias are not available and hence, comparisons of the figures obtained in the present study (Tables 14 and 15) have to be made with studies such as those of Soivio and Oikari (1976) who reported that after one month starvation and handling of the Northern pike (Esox lucius) the plasma sodium was not significantly altered, and with those of Tripelett and Calaprise (1974) who observed that in the Pacific salmon the starvation caused by the spawning migration did not affect the plasma sodium levels. Such comparisons, however unreliable, do agree with the present findings that starvation alone did not affect the plasma osmotic concentration.

CHAPTER 7

EFFECT OF TRANSFER TO SALT WATER ON THE GROWTH RATE OF TILAPIAS

INTRODUCTION

During the last 20 years there has been a limited number of experiments performed relating to the transfer of the commercially cultured tilapia species to salt water in those areas lacking plentiful fresh water (Zaneveld, 1959; Lotan, 1960). They have, however, been limited in scope, and occasionally the results have been conflicting. None have been carried out using known genetically typed species or defined hybrids.

Although the primary aim of such studies has been to allow culture in arid areas, by use of saline water, a second advantage claimed has been a reduction or abolition in reproduction under such conditions. Chervinski and Yashouv, in 1971, claimed that in sea water ponds S. aureus grew as well as in fresh water ponds but with the advantage that they did not reproduce in such conditions. In the present study (Chapter 3 vide infra) S. aureus has been observed to be one of the most resistant of the Sarotherodon species to sea water. This inability to reproduce in sea water especially if it applied to the other euryhaline commercially cultured tilapia as well could provide a good natural method of reducing over-population which is one of the major problems besetting pond culture of tilapias in fresh water.

Most of the workers in this field have demonstrated an almost equal rate of growth in fresh water, at different concentrations of salinity and in full strength sea water (Zaneveld, 1959; Chervinski, 1961 , 1966; Chervinski and Yashov, 1971; Chervinski and Zorn, 1974).

Canagaratnam (1966) working with S. mossambicus even claimed that the percentage growth rate of this species in sea water was double that of fish maintained in fresh water. However, this does not take into full account the problems of adaptation to salt water indicated earlier.

In the previous chapters of this thesis it has been shown that adaptability to salt water is species dependent, thus S. aureus, S. mossambicus and S. spilurus were found to be more capable of survival in salt water than the hybrids of S. aureus/ S. niloticus and S. niloticus in that order. Moreover the ability to tolerate the sudden exposure to salt water without mortality was also varied. The former three species were able to tolerate with zero mortality the direct transfer to salinity concentration of 65% sea water (23.4‰) while the latter two species tolerated 50% sea water (18‰) (See Chapter 3).

The present experiments were therefore designed to investigate, under similar circumstances to the acclimation studies, the effects of different concentrations of sea water on the growth rate of these species.

MATERIALS AND METHODS

Fish

All the fish of the present experiments, viz. S. aureus, S. mossambicus, S. spilurus, S. aureus/S. niloticus hybrids and S. niloticus, were obtained from the tropical hatchery of the Institute of Aquaculture of the University of Stirling. All were of proven genotypically defined species (McAndrew, 1981). The fish were first fully acclimated to their new media, i.e. 50% sea water (18‰), 75% sea water (27‰) or full sea water (36‰), then after feeding and full activities had resumed, held for another week at their respective salinities before the trial, during which period they were fed a commercial trout diet. The weight and length of each fish were determined at the start of each trial and every two weeks during it. The food level fed was adjusted every two weeks accordingly.

Experimental Design

A. Preliminary experiment

Two pilot trials were carried out, one with the highly euryhaline S. mossambicus (0.835 g. mean weight) and the other with the less euryhaline hybrids of S. aureus/S. niloticus (3.15 g. mean weight). For each of the trials four of the normal 100 L. capacity glass tanks (Chapter 2) were used, i.e. for fresh water, salinities of 50% sea water (18‰), 75% sea water (27‰) and full strength sea water (36‰). The results of these trials

showed that both species were able to withstand the prolonged exposure for 12 weeks to all salinities including full sea water of 36‰. The growth responses were different however. Thus S. mossambicus grew equally in fresh water and at all salinities while S. aureus/S. niloticus hybrids maintained lower growth rate in full strength sea water with a total loss of 26% of the initial number of fish at this salinity.

B. Main experiments

In view of the above findings seven main experiments were designed covering shorter periods. Three of these experiments were of six weeks and were conducted with S. aureus (3.32 g. mean weight), S. spilurus (2.67 g. mean weight) and S. niloticus (2.19 g. mean weight). For each trial, triplicates of 15 fish were used in fresh water as control, and at each experimental salinity i.e. 50%, 75% and 100% sea water of 36‰ salinity.

In order to investigate the size effect upon the growth responses at different salinities, another four experiments were carried out with S. mossambicus and S. spilurus. For two of them, triplicates of 15 fish (6 g. average weight) from each of the species were used in fresh water and at 50%, 75% and full sea water of 36‰ salinity for a period of six weeks. Larger sizes of 18.5 g. average weight from the same species were used in the last two experiments, but because of this average size a smaller number had to be used in each experimental tank. Thus triplicates of 6 fish from S. mossambicus were used in fresh water and at each

experimental salinity. Moreover a different experimental regime was applied with this size of S. spilurus since it has been the experience of other workers with this species (Balarin, personal communication) and also in the present study (vide supra) that S. spilurus, especially when over 10 g. size, is very aggressive when transferred from tank to tank, and in some cases the aggressive behaviour appears very suddenly. This phenomenon frequently led to continuous fighting and one big male fish usually dominated the tank. Thus in order to avoid such behaviour with the big fish of the present experiment three fish were used only, two of them in each set were separated by a small net device, and the third one was left free in each experimental tank. Table 16 summarises the different experimental regimes of the present study.

C. Feeding regime

For all of the experiments the standard diet described earlier was used (see Chapter 2). Since the optimum feeding rate and daily feeding frequencies for tilapias are not clearly defined yet (Jauncey, personal communication), two different feeding regimes were applied. The fish under 4 g. average weight were fed till satiation, while those of over 4 g. were fed at a rate of 4% of their body weight daily (dry diet weight/wet fish weight). In both cases the fish were hand fed and the daily rations were spread over three meals.

Fish species	Serial number	No. of fish in each tank	Mean fish weight (g)	Feeding regime
S. aureus	1	15	3.32	satiation
S. spilurus	2	15	2.67	satiation
S. niloticus	3	15	2.19	satiation
S. mossambicus	4	15	6.48	4%
S. spilurus	5	15	5.82	4%
S. mossambicus	6	6	18.18	4%
S. spilurus	7	3	19.83	4%

TABLE 16. Summary of the experimental regimes used in the present study. Each experiment was carried out in triplicates in fresh water and at 50%, 75% and 100% sea water of 36‰ salinity. Each of the experiments lasted 6 weeks.

Fish species	Serial number	No. of fish in each tank	Mean fish weight (g)	Feeding regime
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S. spilurus	5	15	5.82	4%
S. mossambicus	6	6	18.18	4%
S. spilurus	7	3	19.83	4%

TABLE 16. Summary of the experimental regimes used in the present study. Each experiment was carried out in triplicates in fresh water and at 50%, 75% and 100% sea water of 36‰ salinity. Each of the experiments lasted 6 weeks.

D. Holding facilities

Four recycling unit systems were constructed, for fresh water, 50% sea water (18‰), 75% sea water (27‰) and full strength sea water of 36‰ salinity. Each unit consisted of six self-cleaning circular tanks of 40 litre capacity each, a 68 litre header tank and a 40 litre settling tank. The header tank contained a smaller 30 litre tank containing gravel and cotton wool to act as a biological filter. The settling tank contained a screen to filter the water before it was pumped through the biological filter in the header tank. The water from the header tank then fed the circular tanks by gravity at a rate of 22 L/min. Water temperature was maintained at 29°C ± 0.5 throughout the experimental period by means of seven combined heater-thermostat units per system. The water was artificially aerated by means of air-stones in each circular tank and in the header tanks to provide a continuously available dissolved oxygen level above 90% saturation (Fig. 23).

Figure 23

Layout of one of the four recycling units.

1 : Settling tank
2 : Water pump
3 : Biological filter (topped with cottonwool and gravel underneath)
4 : Header tank
5-10 : Experimental tanks

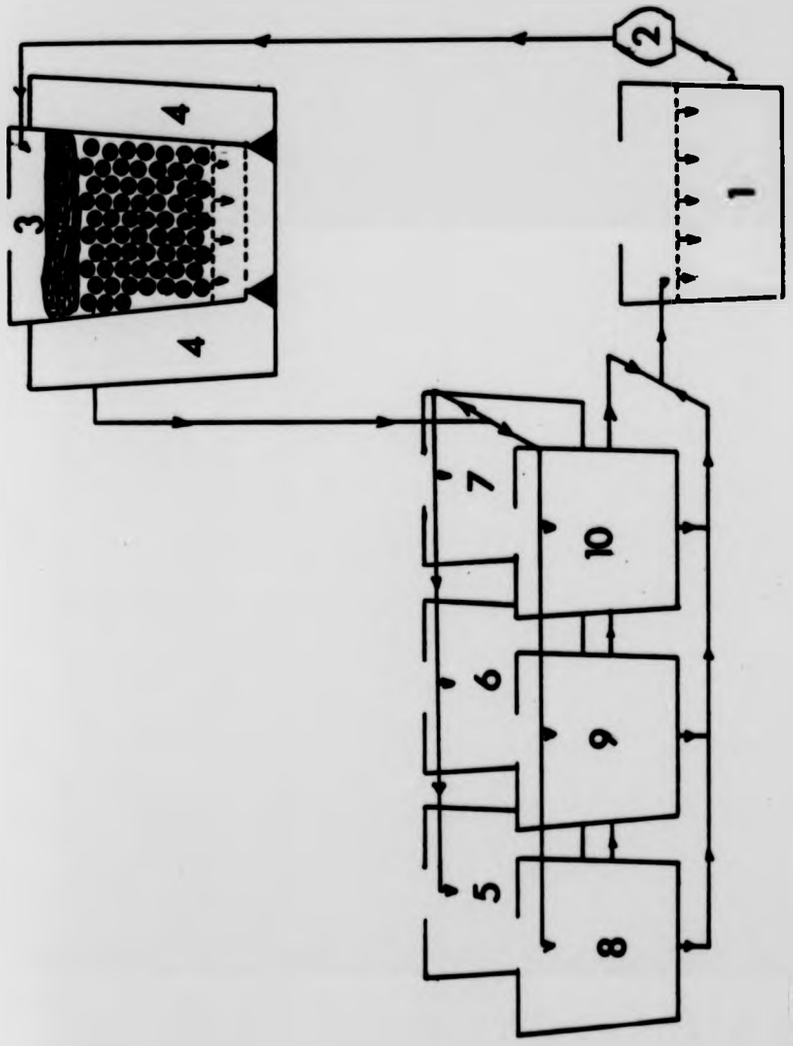

Fig. 23

RESULTS

A. Growth Responses in Sarotherodon aureus, S. spilurus and S. niloticus (2.72 g. ± 0.56 mean weight) in Fresh Water at 50%, 75% and 100% Sea Water

Sarotherodon aureus and S. spilurus seemed to be very well acclimated to all salinities including full strength sea water of 36‰ salinity. Throughout the experimental period each of these species maintained an equal growth rate value with no significant difference in fresh water and at all experimental salinities. It has been demonstrated in Chapter 3 that S. niloticus showed less capability for direct transfer to salt water than all of the other species of the present study. This disadvantage has been emphasised in these results since despite the apparent initial adaptability of this species to full sea water of 36‰ salinity, mortalities took place as in previous experiments at the beginning of the third week. The total mortality amounted to 33.7% by the end of the trial. However survivors from this species in sea water appeared to be fast growing and maintained the highest specific growth rate values among both the control and other experimental salinities but the high level of mortality makes this result questionable. While comparisons between the specific growth rate values of S. niloticus in fresh water and those in the salinities of 50% and 75% sea water for the same species show that there was no significant difference between the values in fresh water and those in 50% sea water. The values recorded in 75% sea water were significantly lower than the control ones ($P = 0.05$).

Salinity treatment ‰	Mean initial weight (g)	Grand mean	Mean final weight (g)	Grand mean	Mean increment (g)	Mean Specific growth rate	Increase in growth expressed as percentage of control
0.05 Fresh water control	3.46		19.08				
	3.18	3.28	19.65	18.89	15.61	4.17	100
	3.21		17.92				
18	3.03		21.31				
	3.64	3.35	23.19	22.35	19.00	4.52	119.17
	3.41		22.54				
27	3.76		19.05				
	3.18	3.22	17.29	17.23	14.01	4.0	91.42
	2.73		16.15				
36	3.94		21.18				
	3.23	3.43	19.84	20.55	17.12	4.26	104.88
	3.11		20.62				

TABLE 17. Growth rates and salinities data for S. aureus (3.32 g. mean initial weight)

Salinity treatment ‰	Mean initial weight (g)	Grand mean	Mean final weight (g)	Grand mean	Mean increment (g)	Mean Specific growth rate	Increase in growth expressed as percentage of control
0.05 Fresh water control	2.43		10.17				
	2.62	2.59	10.45	10.44	7.85	3.32	100
	2.73		10.68				
18	2.57		10.42				
	2.67	2.56	10.36	10.32	7.76	3.31	100
	2.43		10.17				
27	3.12		9.96				
	2.67	2.85	9.31	9.61	6.76	2.88	78.25
	2.75		9.55				
36	2.68		9.93				
	2.89	2.69	10.32	10.26	7.57	3.19	92.84
	2.50		10.51				

TABLE 18. Growth rates and salinities data for S. spilurus (2.67 g. mean initial weight)

Salinity treatment ‰	Mean initial weight (g)	Grand mean	Mean final weight (g)	Grand mean	Mean increment (g)	Mean Specific growth rate	Increase in growth expressed as percentage of control
0.05 Fresh water control	1.90		9.30				
	2.08	1.96	10.53	9.73	7.77	3.80	100
	1.92		9.36				
18	2.36		10.98				
	2.18	2.28	9.97	10.59	8.31	3.67	91.94
	2.30		10.80				
27	2.30		9.19				
	2.60	2.43	9.40	9.21	6.78	3.17	70.38
	2.38		9.01				
36	2.57		10.35 *				
	1.94	2.10	12.45	11.05 *	8.95 *	4.0 *	107.51 *
	1.78		10.35				

TABLE 19. Growth rates and salinities data for S. niloticus (2.19 g. mean initial weight)

* 33% were lost at this salinity

Figure 24

Growth rate response of S. aureus (3.32 g. mean weight) in fresh water and at the different salinities.

- ○ Fresh water control
- ● Salinity of 50% sea water (18‰)
- △ Salinity of 75% sea water (27‰)
- ▲ Full strength sea water of 36‰ salinity

The mean increments and specific growth rates are to be found in Table 17. With respect to the different responses at different salinities, the growth rate graphs observed with the species of the present study were virtually similar.

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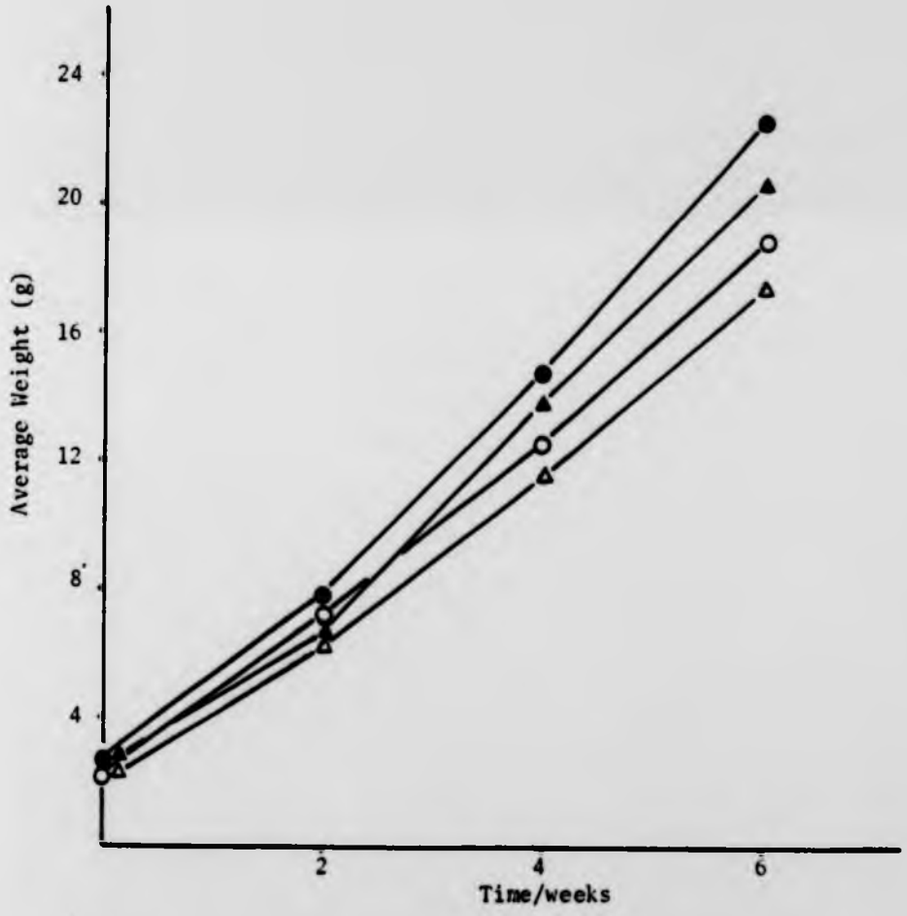
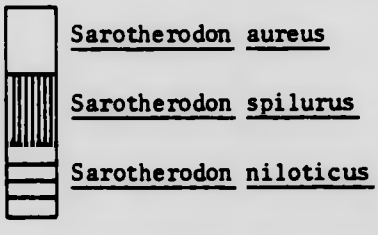


Fig. 24

Figure 25

Specific growth rate values of S. aureus, S. spilurus and S. niloticus (2.73 g. mean weight) in fresh water and at the different salinities.

These data were obtained from Tables 17-19, to give a common comparison between the three species.



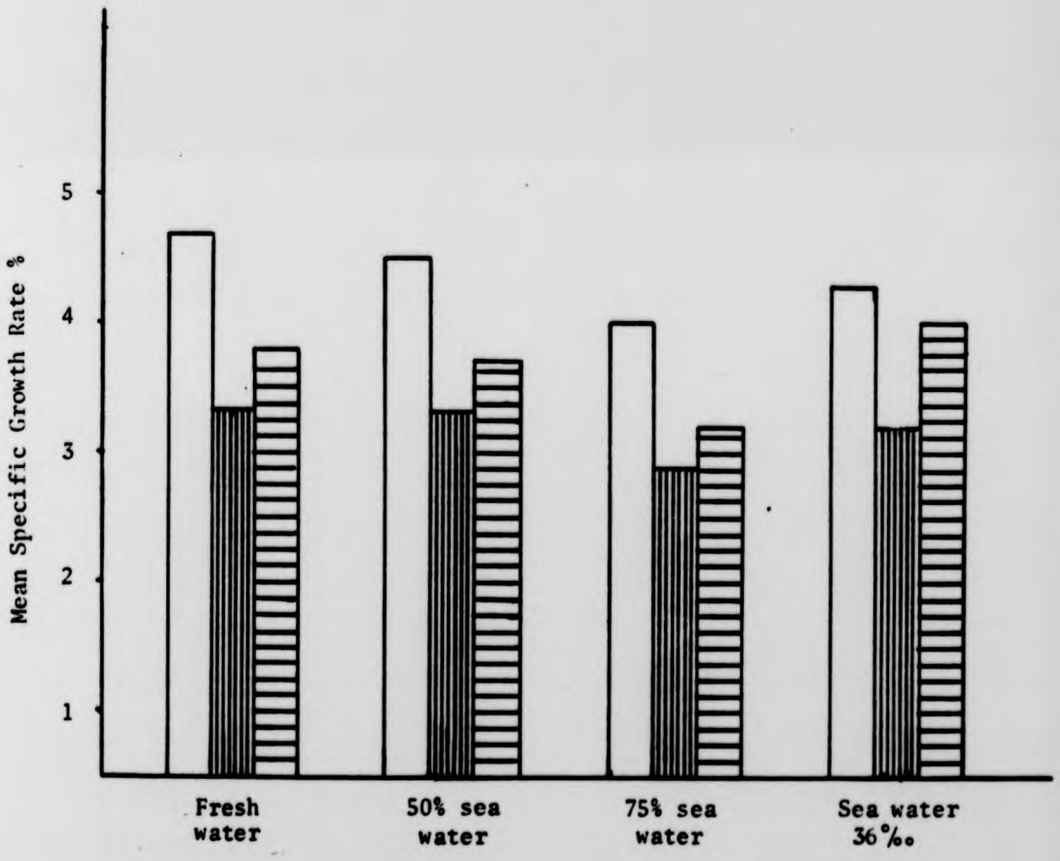

Mean Specific Growth Rate %
5
4
3
2
1
Fresh water
50% sea water
75% sea water
Sea water 36‰

Fig. 25

Tables 17, 18 and 19 summarise the experimental data obtained with S. aureus, S. spilurus and S. niloticus respectively. It can be seen from the tables that S. aureus maintained the highest specific growth rate values among the other candidates (Fig. 25).

B. Size Effect on Growth Responses in S. mossambicus and S. spilurus

Tables 20-23 show the growth rate and salinity data for the different size batches of S. mossambicus and S. spilurus used in this study. Two different sizes (6.48 g., 18.18 g., and 5.82 g., 19.83 g. mean weight) were used from each of the respective species. The results demonstrated that, as before, both S. mossambicus and S. spilurus were highly adaptable to prolonged exposure to the different experimental salinities (viz. 50%, 75% and 100% sea water of 36‰ salinity) without any mortality in all cases. In S. mossambicus the size difference did not have any effect on the subsequent growth rates at the different experimental salinities. Moreover, within the same fish size there was no significant difference ($P = 0.05$) between the specific growth rate values of the control group and all the other groups, at the different experimental salinities. Generally S. spilurus maintained higher growth rate figures than S. mossambicus. This species significantly maintained equal specific growth rate values with the groups of 5.82 g. initial weight in all cases, while in the bigger size groups of initial weight 19.83 g. the group adapted to full strength sea water did not grow as well as the control group (difference significant at $P = 0.01$) (see Table 23 for specific growth rate values). This

Salinity treatment ‰	Mean initial weight (g)	Grand mean	Mean final weight (g)	Grand mean	Mean increment (g)	Mean specific growth rate	Increase in growth expressed as percentage of control	Food conversion ratio
0.05 Fresh water control	6.74		17.55					
	6.77	6.74	17.55	17.66	10.92	2.29	100	2.27
	6.72		17.88					
18	5.93		14.34					
	6.40	6.20	16.37	16.10	9.9	2.28	98.57	2.37
	6.28		17.49					
27	6.08		17.60					
	6.72	6.48	18.24	18.22	11.74	2.45	111.8	2.11
	6.64		18.85					
36	6.40		20.55					
	6.71	6.52	15.52	17.19	10.67	2.31	101	2.29
	6.46		15.50					

TABLE 20. Growth rates and salinities data for S. mossambicus (6.48 g. mean initial weight)

Salinity treatment ‰	Mean initial weight (g)	Grand mean	Mean final weight (g)	Grand mean	Mean increment (g)	Mean specific growth rate	Increase in growth expressed as percentage of control	Food conversion ratio
0.05 Fresh water control	18.50		50.88					
	18.28	18.50	48.24	48.83	30.33	2.07	100	2.03
	18.72		47.53					
18	18.93		46.29					
	23.32	21.52	59.49	53.64	32.12	2.17	94.47	1.93
	22.32		55.17					
27	13.06		38.82					
	14.98	14.45	38.55	38.10	23.65	2.31	99.9	1.82
	15.30		36.87					
36	19.74		40.00					
	17.58	18.25	43.17	44.64	26.39	2.14	92.69	2.13
	17.42		44.76					

TABLE 21. Growth rates and salinities data for S. mossambicus (18.18 g. mean initial weight)

Salinity treatment ‰	Mean initial weight (g)	Grand mean	Mean final weight (g)	Grand mean	Mean increment (g)	Mean specific growth rate	Increase in growth expressed as percentage of control	Food conversion ratio
0.05 Fresh water control	5.32		16.00					
	5.60	5.53	17.12	18.75	13.22	2.9	100	1.45
	5.67		21.63					
18	5.77		18.00					
	6.13	5.95	19.82	19.16	13.21	2.88	92.47	1.50
	5.96		19.65					
27	5.76		21.98					
	6.29	5.96	18.15	19.71	13.75	2.82	96.52	1.58
	5.84		18.99					
36	6.14		19.29					
	5.60	5.86	19.65	19.98	14.12	2.9	100.82	1.52
	5.85		21.00					

TABLE 22. Growth rates and salinities data for S. spilurus (5.83 g. mean initial weight)

Salinity treatment ‰	Mean initial weight (g)	Grand mean	Mean final weight (g)	Grand mean	Mean increment (g)	Mean specific growth rate	Increase in growth expressed as percentage of control	Food conversion ratio
0.05 Fresh water control	18.40		68.55					
	13.60	16.40	58.95	62.72	46.32	3.18	100	1.21
	17.30		60.64					
18	20.53		80.55					
	21.37	20.50	67.75	70.14	49.64	2.93	85.74	1.30
	19.60		62.10					
27	19.27		74.55					
	19.53	20.38	56.40	68.68	48.3	2.90	93.47	1.35
	22.13		75.11					
36	24.00		61.20					
	22.30	22.70	56.85	58.23	35.53	2.24	55.42	1.76
	21.87		56.66					

TABLE 23. Growth rates and salinities data for S. spilurus (19.83 g. mean initial weight)

retarded growth rate may however have been due to the fact that pump failure (the same pump was serving all three replicate tanks) occurred repeatedly throughout the experiment and this could have led to water quality deterioration, stress and consequently impaired growth. Tables 20-23 also show that the food conversion values were in general better in S. spilurus than those in S. mossambicus. In the former species the food conversion values were better in the control group than those in any of the salt water acclimated groups.

It is worthy of note here that newly hatched fry were frequently collected from all the tanks of S. mossambicus, showing that this species can readily reproduce in sea water under suitable conditions. In the case of the closely related S. spilurus, however, although groups of fry were found in all intermediate salinity tanks, in those of full sea water this species was not able to reproduce.

DISCUSSION

The growth rate findings of the present study agree with those obtained by most previous workers. For example Zaneveld (1959) who reported no difference in the growth rate of S. mossambicus in fresh water and at sea water salinity of 36.2‰, Chervinski and Yashouv (1971) for S. aureus in fresh water and at sea water of salinity range between 36 and 41‰, Chervinski and Zorn (1974) for S. aureus and Tilapia zilli in fresh water and sea water ponds. These previous findings and the present totally disagree with those of Canagaratnam (1966) who claimed to have observed S. mossambicus to grow very much better in full sea water than in fresh water.

Correlations between the rate of fish oxygen consumption and the rate of metabolism have been extensively examined, thus many earlier workers have demonstrated that in fully acclimated euryhaline teleosts there was no significant long term change in oxygen consumption at different salinities. The growth rates achieved in the present study are not in disagreement with earlier findings that the metabolic requirements are not correlated with salinity concentration in euryhaline teleosts. Different findings have been reported however, when the fish were subjected to an enforced high level of activity. Farmer and Beamish (1969) measured the oxygen consumption rate in S. niloticus for a range of swimming speeds, 30, 40 and 50 cm/sec in fish of 60 and 120 g. at salinities of 0.0, 7.5, 11.6, 22.5 and 30‰. They found that the respiratory rates at 7.5 and 22.5 were approximately equal, while the minimum respiratory rate occurred at 11.6‰ and the maximum at 30.0‰. Madan Mohan (1968, 1969,

1971) carried out similar works with rainbow trout (Salmo gairdneri) and stated that the minimum oxygen consumption occurred at 7.5‰ salinity. Farmer and Beamish (1969) and Madan Mohan (1971) carried out their experiments with actively forced fish. MacLeod (1977) extrapolated their oxygen consumption figures from enforced maximal activity to standard activity and he stated that at standard activity the difference in the oxygen consumption between fresh water and different salinities is less marked. He added that the standard rate of activity is more applicable to both field and fish farm conditions than the high levels of activity on which the conclusions of Farmer and Beamish (1969), and Madan Mohan (1971) were based.

MacLeod's (1977) conclusions agree with the present results where the different salinities generally did not affect the food conversion values. This may imply that in the fully acclimated fish and at routine activity levels, the proportion of energy dissipated in the osmoregulatory process is small in comparison to the total energy budget (Tables 20-23).

Among the fish of the present study S. aureus maintained the best specific growth rate values. This agrees with Chervinski and Yashouv (1971) who reported that, in a salinity concentration comparative study, S. aureus grew faster than S. mossambicus in sea water ponds. This characteristic and the extremely high adaptability of this species to salt water (Chapter 3), demonstrates the high ability of S. aureus for sea water transfer with proven faster growth rate.

The results obtained for S. niloticus were inconclusive. The fish of this species suffered high mortality in full sea water salinity of 36‰, but those which survived attained higher specific growth rate than those of the same species in fresh water or at 18‰ and 27‰ salinities. This may however, at least in part, have been due to the reduced density associated with the losses.

Conte and Wagner (1965) showed that, in steelhead trout (migratory form of rainbow trout), size at transfer was an important factor, the bigger fish being more able to tolerate transfer to salt water. Jackson (1981) carried out similar experiments with rainbow trout, and he demonstrated that the small size fish of 10 g. suffered high mortality with osmoregulatory problems following transfer to sea water compared with the larger size fish of 30 g. On the other hand Bashamohideen and Paravatheswararao (1976a, b) demonstrated that in adaptation to osmotic stress, small individuals (10 g.) of S. mossambicus relative to large (50 g. size) individuals, were better able to adapt to the imposed osmotic stress. The two fish sizes of S. mossambicus and S. spilurus used in the present study (Tables 20-23) were subjected to the same gradual acclimation regime and both sizes showed similar behaviour in the different salinity concentrations with no signs of osmoregulatory problems during the acclimation time. Both species maintained virtually no difference in the growth rates at all salinities. This confirms again the high salinity tolerance of these two species. Moreover this ability to tolerate high salinity transfer at small sizes without fail in progressive growth rate is a most significant economic advantage in terms of running a sea water fish farm with such euryhaline teleosts.

CHAPTER 8

HISTOLOGY AND ULTRASTRUCTURE OF THE CHLORIDE CELL IN FRESH WATER AND IN FULLY ACCLIMATED SEA WATER SPECIMENS OF *S. MOSSAMBICUS* AND *S. SPILURUS*

INTRODUCTION

The adaptability of Sarotherodon mossambicus and S. spilurus to a salt water mode of existence, and the influence of environmental factors during fresh water rearing on the success of subsequent transfer to salt water have been discussed in previous chapters. It has been shown that such transfer lead to a raising of the plasma osmotic concentration to a transient peak which, if the fish survive the transfer, is followed by a subsequent fall to a new level, maintained at a higher level than that which obtained when the fish were originally held in fresh water. The importance of the gills in osmoregulation has long been appreciated (Smith, 1930; Krogh, 1939). More recent work on the mechanisms of ion exchange across the gills has been reviewed by Maetz (1971, 1976).

Depending on the salinity of the external medium the gill epithelium has to pump salt from the body against a varying osmotic gradient. This represents an adaptation challenge that requires structural changes at the cellular level. Successful transfer of fish to salt water is characterised by an increase in the number of the specialised, enzyme and mitochondrion rich epithelial cells known as chloride cells and in the development within these cells of a greatly extended tubular reticulum (Shirai and Utida, 1970; Karnaky et al., 1976a). These modifications probably account for the increase in Na-K activated ATP-ase activity of the gill tissue (Epstein et al., 1967; Kamiya and Utida, 1968; Karnaky et al., 1976b; Sargent et al., 1975).

The chloride cells have been described as large, mitochondrion rich cells occurring singly in the primary and secondary epithelium of fresh water adapted fish but in larger groups in the interlamellar and afferent arterial areas of the primary lamellae of salt water adapted fish (Datta-Munshi, 1964; Shirai and Utida, 1970; Morgan and Tovell, 1973; Coleman et al., 1977; Sargent et al., 1978). The chloride cells are further identified by their extensive system of tubular membranes. The tubular membrane is an amplification of folding of the cell basolateral membrane (Philpott, 1980), permeable to large molecules such as horseradish peroxide (45A°) (Philpott, 1966), lanthanum (20A°) (Ritch and Philpott, 1969). Fine repeating particulate patterns are also apparent in freeze fraction faces of the tubules (Sardet et al., 1979).

As part of the present study on the adaptability of Sarotherodon species to salt water, it was considered useful to carry out a light and electron microscopic examination of the basic morphological and histological characteristics of the gill epithelium in S. mossambicus and S. spilurus, to determine the presence of such organelles within their respiratory epithelium, and to assess how they adjusted following transfer to salt water.

MATERIALS AND METHODS

Fish

Eight fish (of mean weight 15 g.) from each of *S. mossambicus* and *S. spilurus* were used. For each species, four of the fish came from fresh water while the other four had been adapted to sea water and maintained there for three months.

Light Microscopy

The fish were first anaesthetized in a 1:10,000 solution of benzocaine and about 0.5 cm^3 of tissue from the gill removed for fixation.

The method chosen to demonstrate the chloride cells was the Altman aniline fuchsin method for the staining of mitochondria. A similar method was used by Copeland (1948) and Shirai and Utida (1970). The actual technique used was the modification of Altman's original method given by Drury and Wallington (1967). This stained mitochondria, nucleoli and erythrocytes red, and cytoplasm and connective tissues pale yellow. The sections were then counter-stained with methyl green to demonstrate the nuclei (Gelman, 1950).

Electron Microscopy

Tissues were quickly fixed in a 2.5% solution of glutaraldehyde in 0.2M sodium cacodylate buffer of pH 7.2 for a minimum of 2 hours at room temperature. The tissues were then rinsed in 4-6 changes

of the buffer solution, dried on blotting paper and fixed for another hour at 4°C in 1% osmium tetroxide. Following osmication the tissues were dehydrated in a graded series of alcohols, viz. first in 50% with two changes of 15 minutes, next in 70% with two changes of 15 minutes, then in 80% with two changes of 15 minutes, then in 90% with two changes of 15 minutes and finally in 100% alcohol with four changes of 15 minutes each. The tissues were then immersed in a mixture of propylene oxide and resin (see Appendix 11) 75%-25% for one hour, then in a 50%-50% mixture for another hour and finally in 100% resin overnight. Finally tissues were placed in plastic capsules, covered with 100% resin and cured in an oven at 60°C for 24 hours.

Sections cut at 2 µm were first cut from the resin embedded blocks of tissues on an LKB pyramitome 11800 using glass knives. These sections were stained with 1% toluidine blue and viewed under a binocular microscope. This enabled rapid location of the desired area for further ultra-thin sectioning. The blocks were trimmed as required with a razor blade and ultra-thin sections were then cut using either diamond or glass knives on an LKB ultratome III. Sections, preferably in the gold region, i.e. 90 nm (based on the continuous interference colour index), were collected from water, onto square mesh grids.

The sections were stained by floating the grids on drops of saturated uranyl acetate solution for 20 minutes. After washing in distilled water, they were floated for 20 minutes on drops of lead citrate. They were finally rinsed in 0.02M sodium hydroxide solution

and distilled water, and after drying they were ready for viewing. All electron microscopy material was viewed in a Jeol Jem-100C electron microscope.

RESULTS

General Morphology

The structure of the gills of Sarotherodon species was typical of that of teleost fish. There were four branchial arches on either side of the pharynx, each bearing two rows of flattened primary lamellae supported by cartilaginous gill rays. Each primary lamella bore two rows of terminal secondary lamellae (see review by Conte, 1969).

Histology of the Gill Lamellae

Gill primary lamellae contain a cartilaginous centrally located gill ray which lies nearer to the afferent artery and is attached to the gill arch thereby providing support for the primary lamellae. The efferent artery is on the opposite side of the gill ray (Fig. 26).

As in other teleosts the epithelium of the tilapia primary lamellae consisted of surface squamous epithelial cells with small numbers of non-differentiated cells normally lying between the surface epithelial cells and the basement membrane. A few mucous cells were also present. Epithelial cells were frequently separated by the large mitochondria-rich chloride cells (Fig. 27). The chloride cells were distributed along both sides of the primary lamellae, both surrounding the afferent arteries and concentrated at the bases of the secondary lamellae (Figs. 28-29).

Figure 26

Cross section of primary lamellae from sea water adapted S. spilurus

AA : Afferent artery
C : Cartilage
CC : Chloride cells
EA : Efferent artery
SE : Surface epithelial cells
SL : Secondary lamellae

(x 500)

Figure 27

Oblique section of the afferent artery side of primary lamellae from fresh water S. spilurus.

This section shows the different types of gill epithelial cells.

C : Cartilage
CC : Chloride cells
ND : Non-differentiated cells
SE : Surface epithelial cells

The mucous goblet cells can hardly be seen in the gills of Sarotherodon spp.

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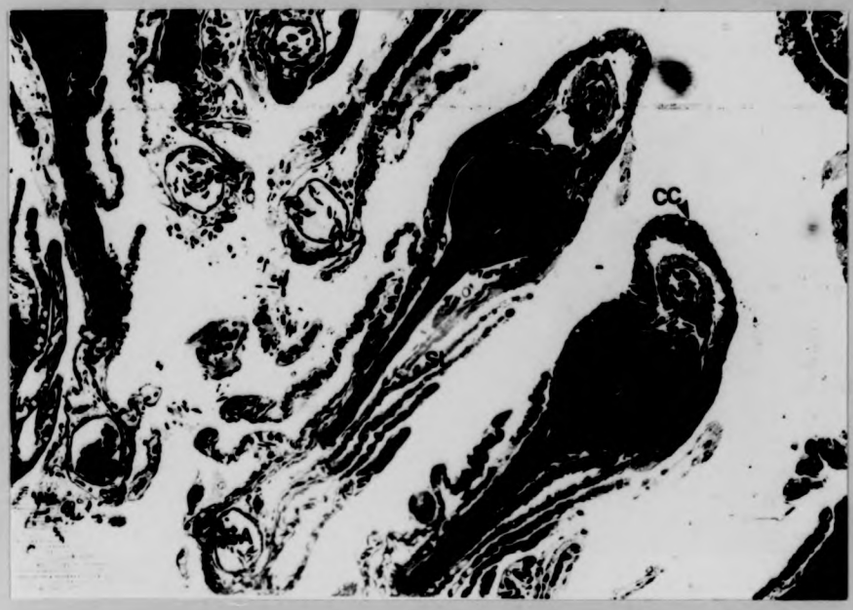

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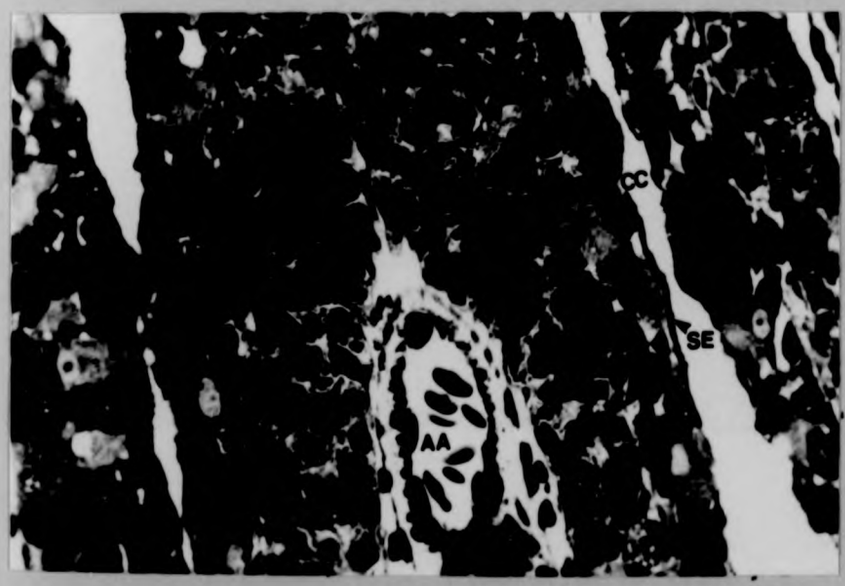

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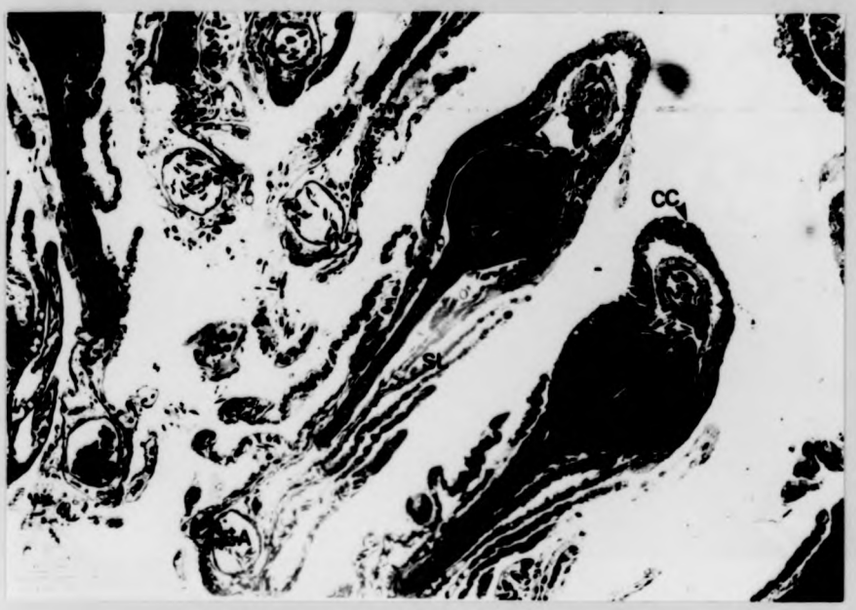

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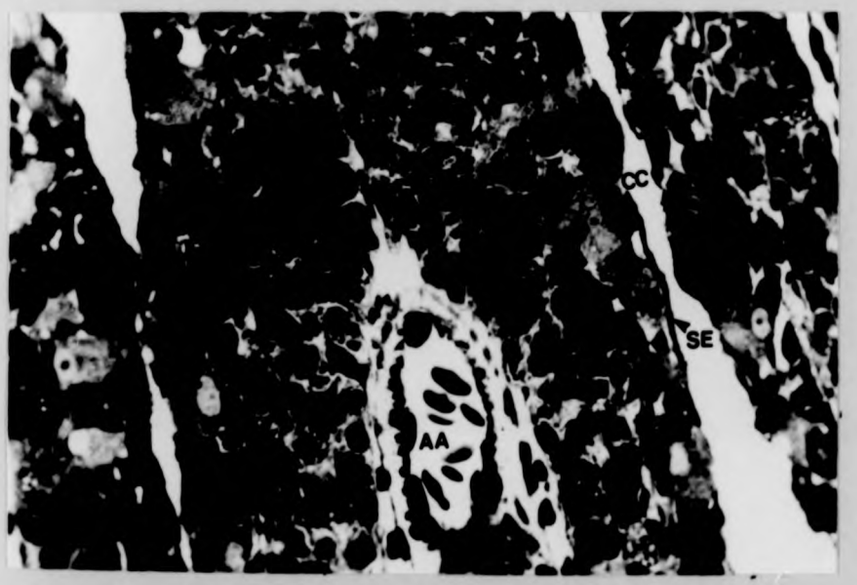

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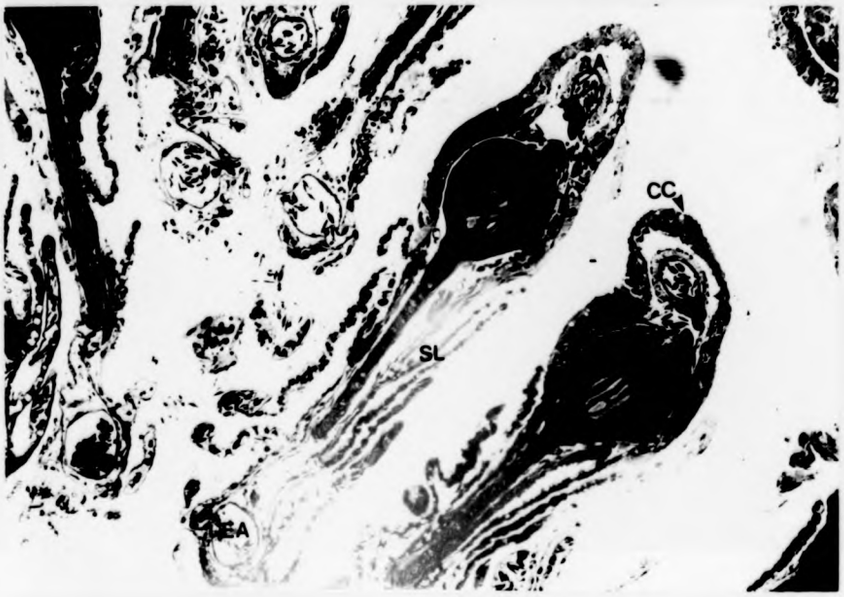

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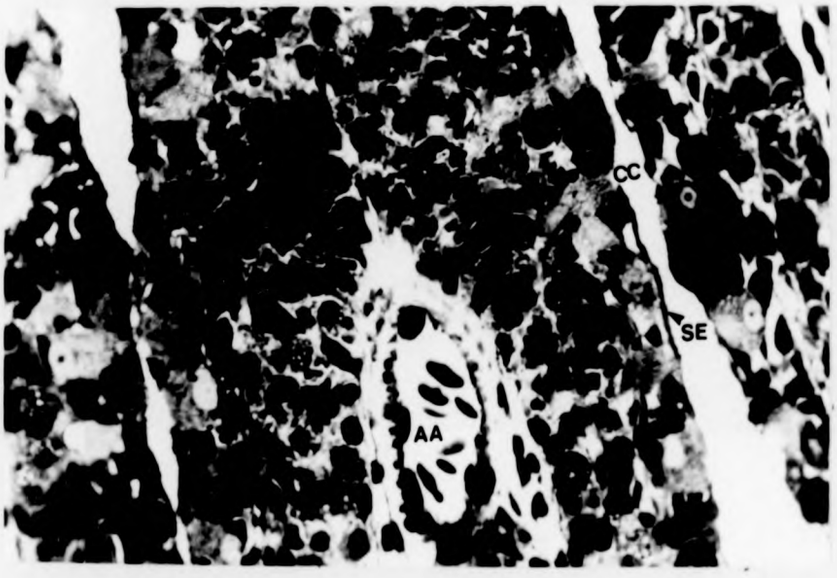

Figure 28

Oblique section at the bases of secondary lamellae from fresh water S. spilurus

The chloride cells (CC) are located at the bases between the secondary lamellae. These cells are not found on the secondary lamellae in the gills of Sarotherodon spp.

C : Cartilage
P : Pillar cells
SE : Surface epithelial cells

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Figure 29

Section of primary lamella from fresh water S. spilurus

The apical crypts are opened to the outside environment in most cases (arrows).

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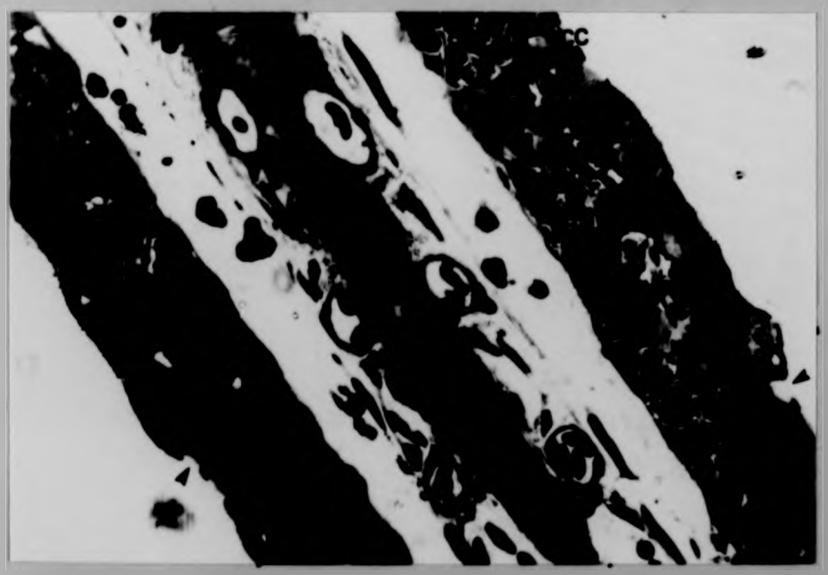

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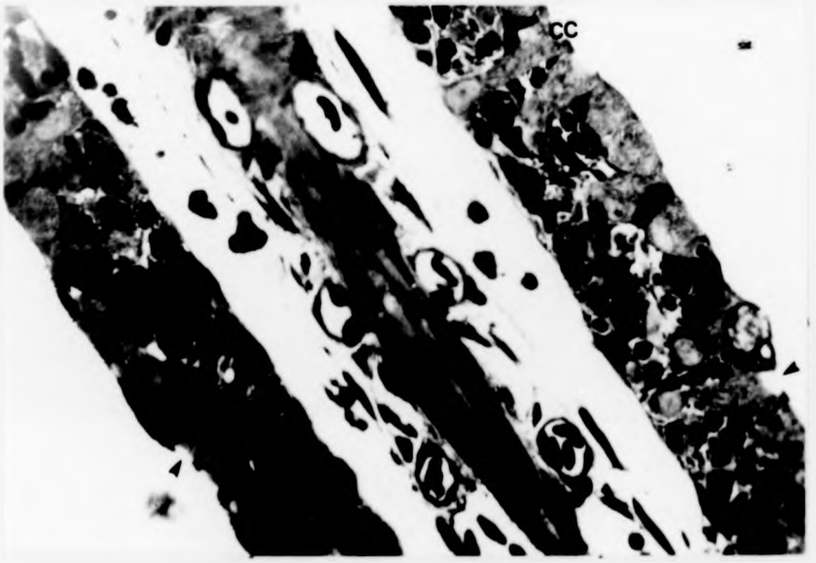

Figure 30

Oblique section of primary lamella from sea water adapted *S. spilurus*

At the same magnification as in Figs. 24 and 25, this section shows the difference in size and density of chloride cells (CC) between the two cases. The surface squamous epithelial cells (SE) extend over the chloride cells, but exposing the underlying orifices of the apical crypt (arrows).

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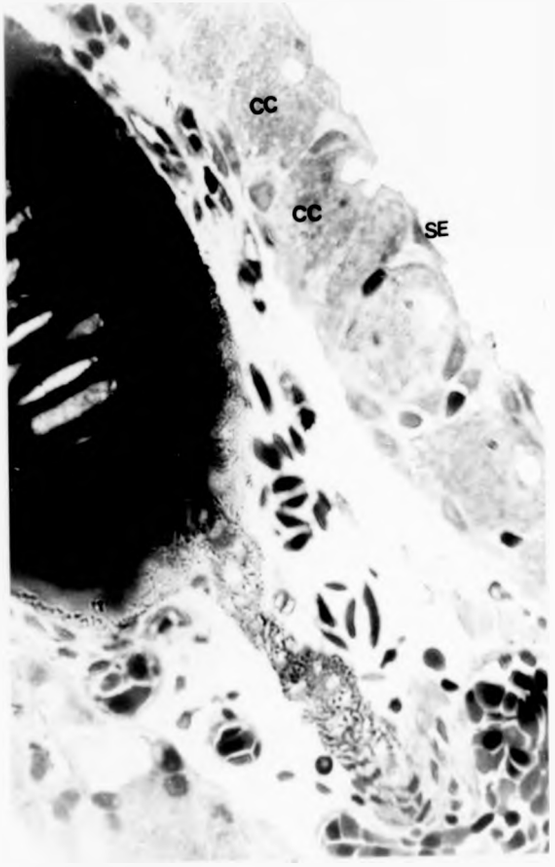

In sea water adapted S. mossambicus and S. spilurus the chloride cells were much more abundant and bigger in size than those in their fresh water equivalents. (In both S. mossambicus and S. spilurus, irrespective of whether they came from fresh or sea water, chloride cells were in most cases elongated, often extending from the basement membrane to the free surface of the primary lamella). The apical membrane was in contact with the external medium. The underlying orifices of the apical crypts of the chloride cells can be seen in Figs. 30.

The secondary lamellae projected from both sides of the primary lamellae. The epithelium of the secondary lamellae consisted of one layer of flat epithelial cells on a delicate basement membrane separating it from the endothelium of the secondary lamellar vessel, which was largely comprised of pillar cells, the supporting cells which maintain the patency of the vessel (Fig. 31). The nuclei of the epithelial cells were usually flattened and lay directly over the body of the pillar cells. Red blood cells were seen in the spaces between the pillar cells, but there were no chloride cells situated on the secondary lamellae (Figs. 28 and 31).

Ultrastructure of the Chloride Cells

The increase in size and number of chloride cells in sea water adapted S. mossambicus and S. spilurus as compared with their fresh water counterparts, observed with the light microscope, was confirmed by electron microscopy. Compared to the light microscope

Figure 31

Low power micrograph of secondary lamella from S. mossambicus

B	:	Basement membrane
BS	:	Blood space
N	:	Nucleus
P	:	Pillar cell and its cytoplasmic flanges (arrow)
R	:	Red blood cell
SE	:	Surface epithelial cell and its long nucleus (N) lie over the pillar cell body

(x 8000)

Figure 32

Chloride cell from fresh water S. spilurus

AD	:	Adjacent epithelial cell
G	:	Golgi apparatus
SE	:	Surface epithelial cell
V	:	Vesicles

The section shows the branched tubular system, the regular shape nucleus and the closed apical crypt. Note the low density of mitochondria (M) and the intercellular spaces (arrows).

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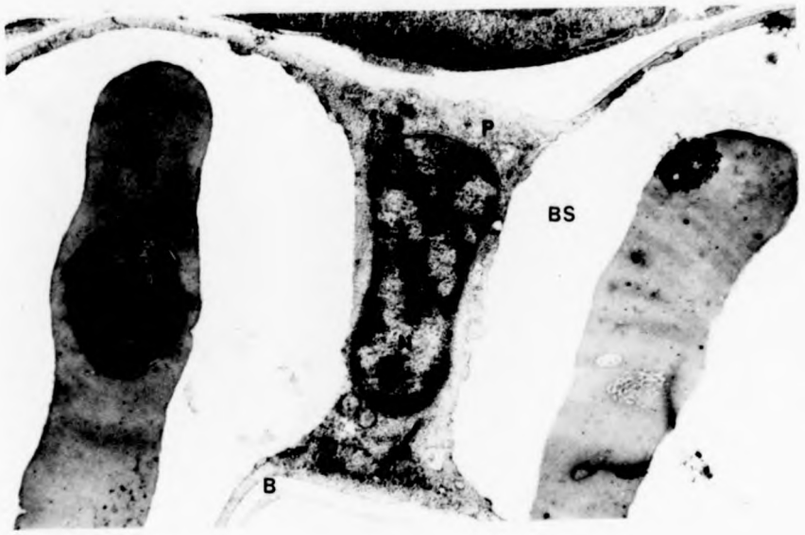

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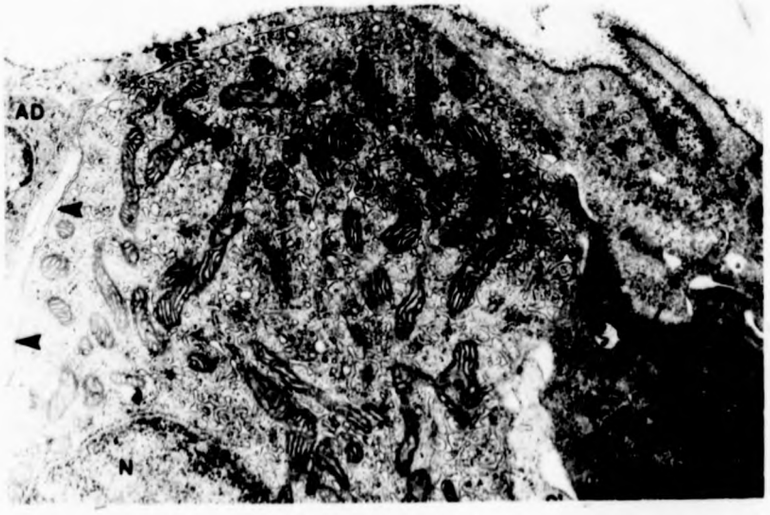

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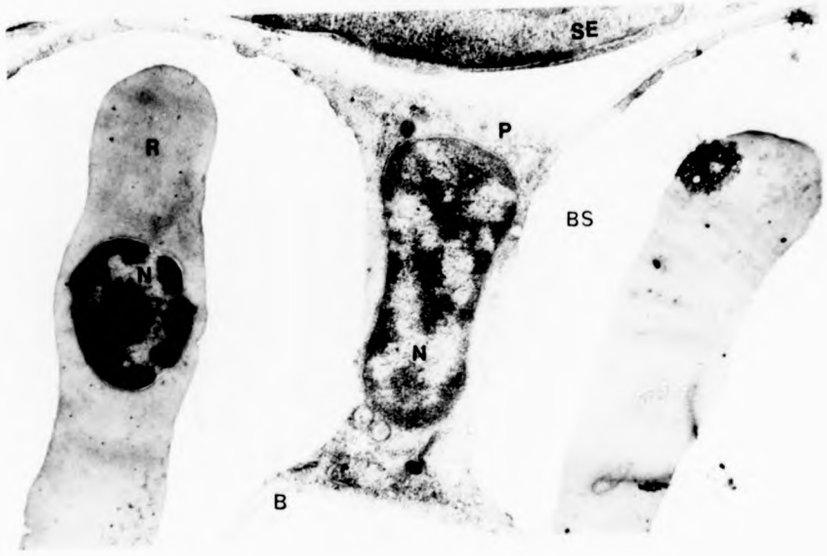

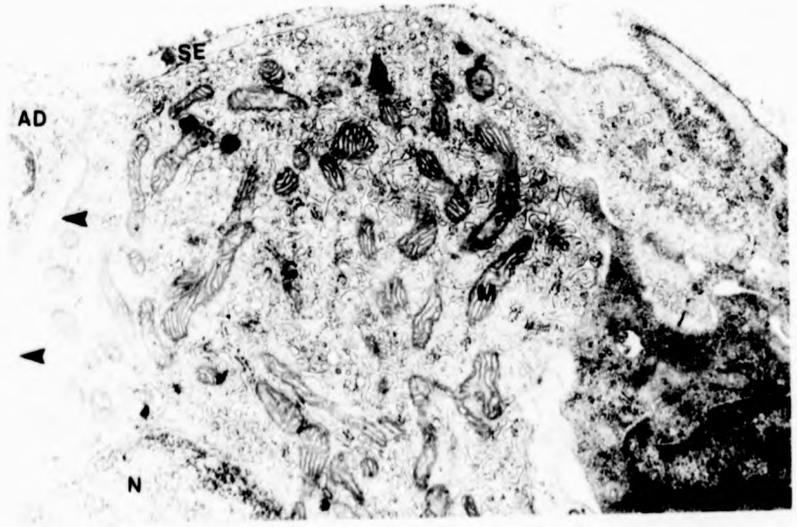

however, the electron microscope was more efficient in showing the external shape, the relative number and the overall appearance of the different gill epithelial cells, in addition to the ultrastructural contents of the chloride cells.

In both species the chloride cells from fresh water showed marked differences in overall appearance from those in sea water adapted fish. In sea water adapted fish the cells were often very electron dense with an irregularly shaped nucleus and a very dense cytoplasm, while in fresh water fish they generally demonstrated a regularly shaped nucleus and were much more electron lucent (Figs. 32-34). Intracellular spaces could be observed between adjacent chloride cells and between chloride cells and adjacent epithelial cells. In sea water adapted S. spilurus there was an interesting and consistent variation in that adjacent chloride cells were closely linked by means of extensive interdigitations of the plasmalemma in the region of the apical crypt (Fig. 35).

One conspicuous feature of many chloride cells in both fresh and sea water was the obvious apical crypt opening to the external environment. This crypt was located between the surface epithelial cells. The apical membrane of this area had obvious microvilli matching those of its closely adherent neighbouring epithelial cells. The microvilli in the apical membrane were more numerous in fresh water adapted fish than in sea water fish (Figs. 35-36). Vesicles of various sizes were concentrated in the apical region. These vesicles seemed to contain an electron dense material and they had no apparent relation with the tubules of the tubular system (Fig. 37).

Figure 33

Chloride cell from fresh water S. mossambicus.

Similar to Fig. 32, but this section also shows the direct contact of the cell with the blood vessel (BV) at the base of a secondary lamella, and the open apical crypt (AP). Intercellular spaces indicated by arrows.

(x 15,000)

Figure 34

Chloride cell from sea water adapted S. mossambicus.

Compared to Figs. 32 and 33, this section demonstrates the compact high density of mitochondria (M) and the irregular shape of the nucleus (N).

G : Golgi apparatus
NL : Nucleolus
RE : Rough endoplasmic reticulum
SE : Surface epithelial cell

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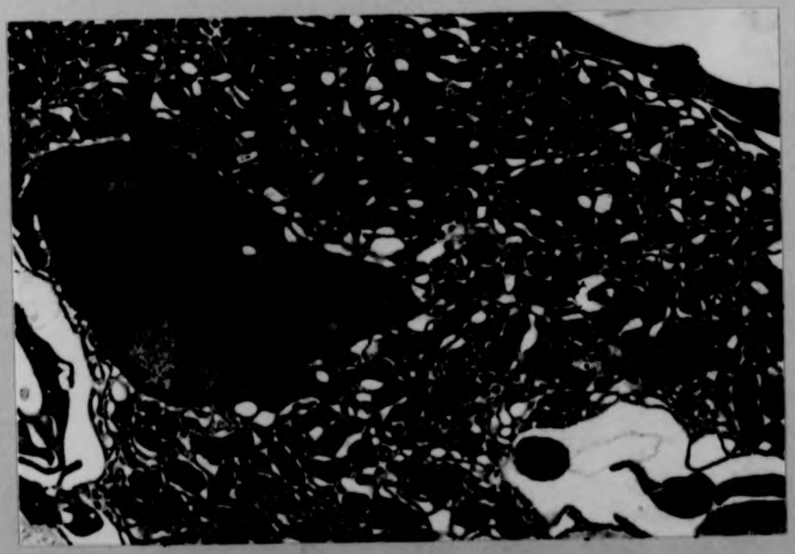

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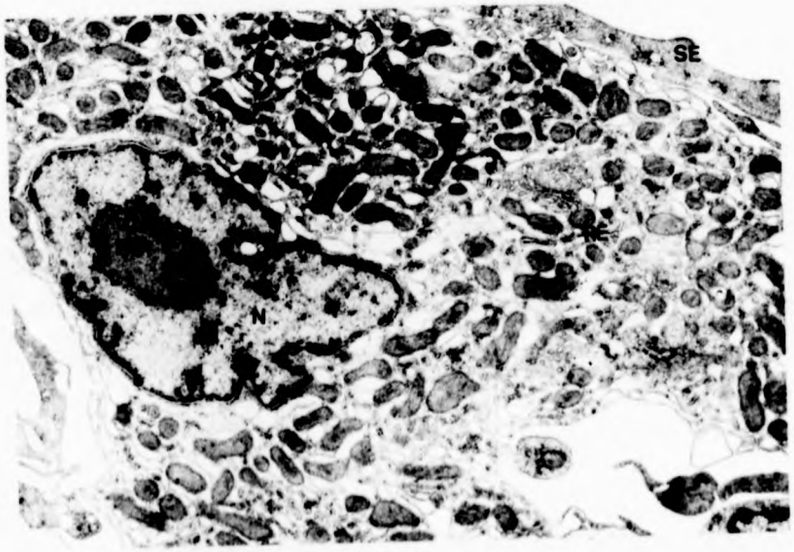

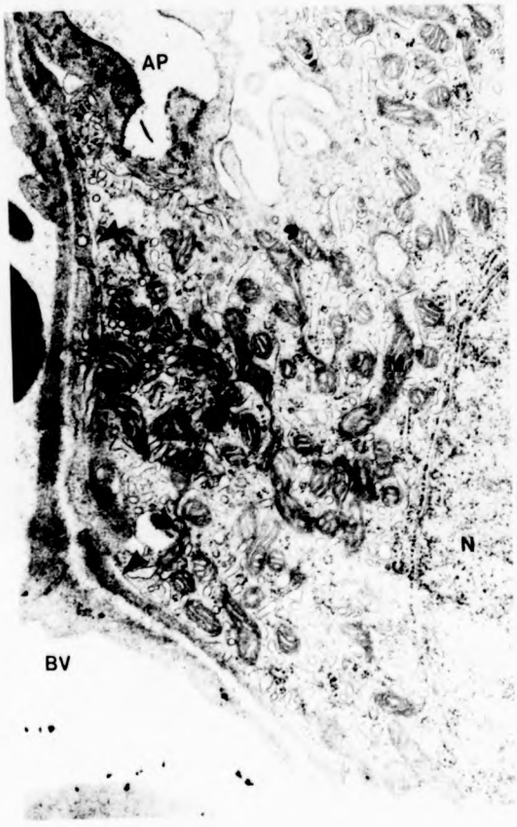
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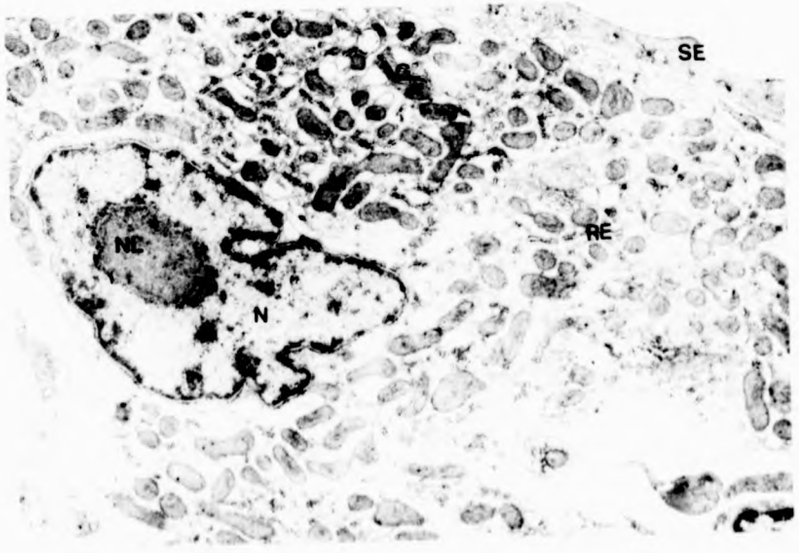
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Figure 35

Close examination of an apical crypt (AP) from sea water adapted S. spilurus

Observe the interdigitations between the adjacent chloride cells in this region (X), the absence of microvilli, the tight junctions between the surface epithelial cell (SE) and the chloride cell (one arrow), and the shallow junction between the adjacent chloride cells (two arrows).

(x 6000)

Figure 36

Close examination of an apical crypt from fresh water S. spilurus

Compared to Fig. 35, this section shows the numerous microvilli (MI) and the absence of interdigitations between the adjacent chloride cells.

M : Mitochondria
RE : Rough endoplasmic reticulum

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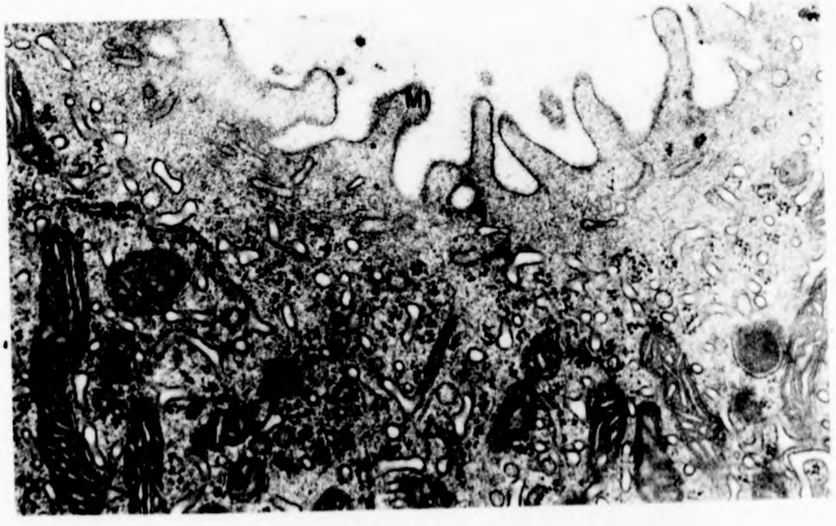

Mitochondria were numerous and evenly distributed throughout the cytoplasm. They varied in shape from round or ovoid to elongated and sinuous. Mitochondria were more numerous and slightly bigger in sea water adapted fish than in fresh water fish. The cristae of the mitochondria were in many instances irregular in shape.

An extensive tubular system consisting of branching and knob-like extensions of tubules occurred between the mitochondria. These branches of the tubular system were continuous with or opened onto the plasma membrane in the basal and lateral regions of the chloride cells. The tubular system in the seawater adapted fish appeared to be more compact than in the fresh water fish. Apart from this no other differences could be detected between fresh and sea water adapted fish (Fig. 35).

The Golgi apparatus was very highly developed and easily recognised. It was composed of three or four paired membranes roughly parallel to each other. Frequently more than one Golgi was observed in a single cell (Figs. 32-34).

Figure 37

High power micrograph of an apical crypt of chloride cell from fresh water *S. spilurus*

The vesicles (V) in this region contain an electron dense material, and they apparently do not have any evident relationship with the tubular system (TS). This apical crypt is coated with filamentous materials.

(x 30,000)

Figure 38

High power examination of the basolateral membrane of chloride cell from fresh water *S. mossambicus*

Observe the continuous tubular system with the plasma membrane (BM), and the formation of intercellular spaces (arrows).

BV : Blood vessel
RE : Rough endoplasmic reticulum

(x 50,000)

cell

ense

al

30,000)

f chloride

a
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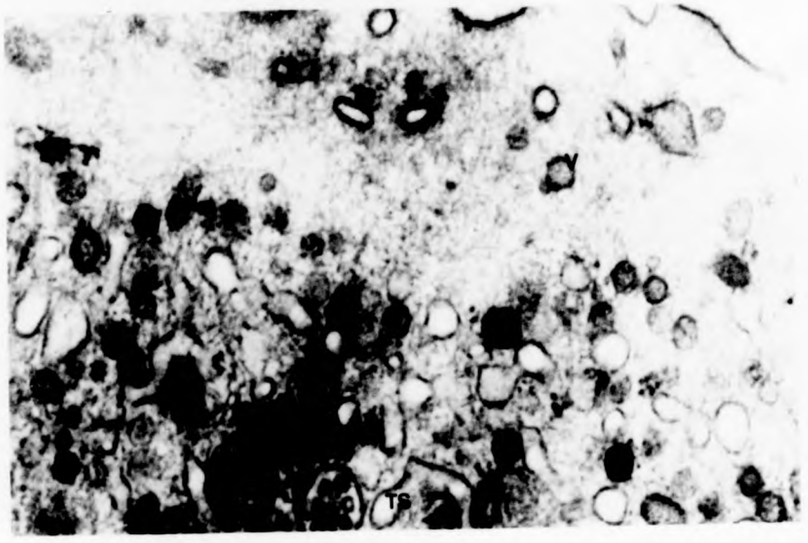
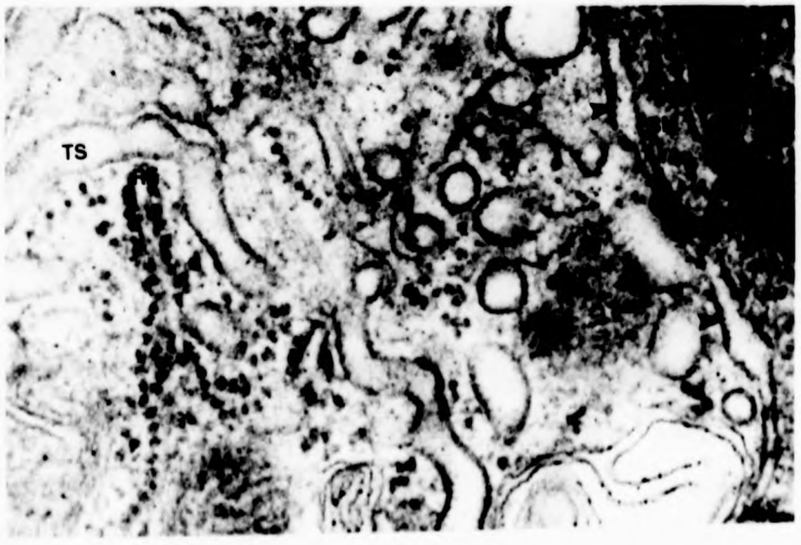
x 30,000)





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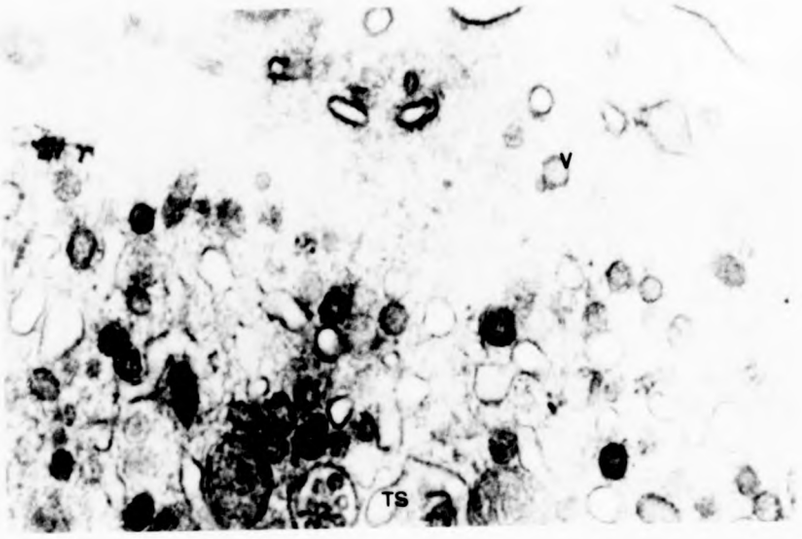

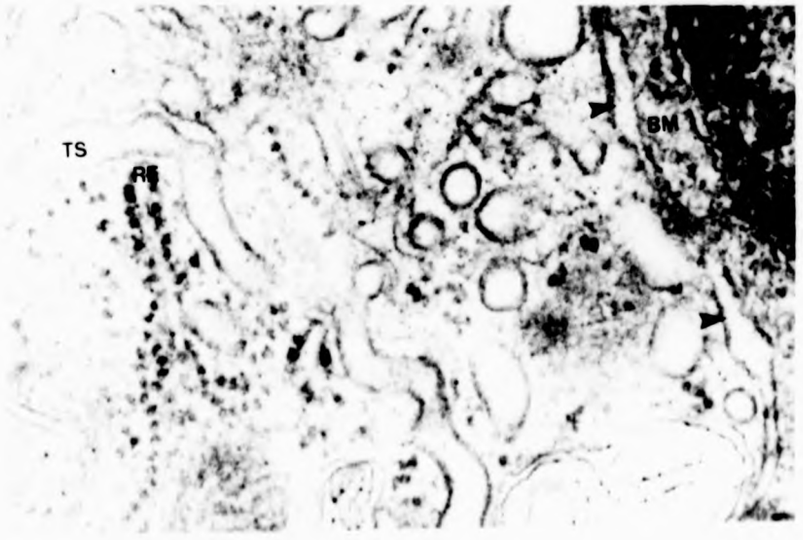

DISCUSSION

According to Farmer and Beamish (1969 and Job (1976), S. niloticus and S. mossambicus were isosmotic in a salinity of 11.6‰ and 12.9‰ respectively. This implies that in a fresh water medium, the gill epithelium with its chloride cells is experiencing an opposite, albeit lower, osmotic gradient between the inside of the fish and the external environment compared with that experienced in full strength sea water of 36‰ salinity. Thus the predominance of the relatively small cell with a lower electron density and the small number of mitochondria as observed in such fresh water fish contrasted with the big well-developed cells with highly active organelles observed in the sea water adapted fish, a fact also observed in other species.

The present work confirms for these Sarotherodon species the already established microanatomy of the gills of other fresh water and sea water teleosts (Conte, 1969; Sardet et al., 1979; Laurent and Dunel, 1980). The presence of well developed chloride cells in the gill of fresh water fish has been described by Datta-Munshi (1964) in the Indian major carp (Catla catla), by Coleman et al. (1977) in Sarotherodon aureus and by Morgan and Tovell (1973) in rainbow trout (Salmo gairdneri). However, compared to those found in the sea water adapted teleosts, the fresh water form of chloride cells have been described as the less electron dense form of these cells by Doyle and Goreki (1961) in the fresh water teleost Fundulus spp., by Straus (1963) in the guppy

(Lebistes reticulatus), and by Coleman et al. (1977) in S. aureus. The latter findings agree with the present study in respect of both S. mossambicus and S. spilurus.

Keys and Willmer (1932) first suggested the role of the chloride cells in fish osmoregulation. It has since then been reported by Copeland (1948) and by Kessel and Beams (1962) that in Fundulus heteroclitus the apical crypt which they referred to as a "secretory vesicle" was present only when the fish was in a hypertonic environment and hence secreting salt. Moreover, Getman (1950) correlated the varying number of crypts in fresh and sea water adapted Anguilla rostrata with the stage of osmotic adaptation of the animal. Shirai and Utida (1970) observed that in sea water adapted Anguilla japonica the apical crypt could be induced to disappear on transfer to fresh water and reappear when the procedure was reversed. However these findings did not seem to have been observed consistently. According to Philpott and Copeland (1963) the apical crypts were found in Fundulus spp. regardless of the environment (for full details see review by Karnaky, 1980). The findings of Philpott and Copeland (1963) agree with those of the present study in both S. mossambicus and S. spilurus. Thus the presence or absence of an apical crypt does not seem at least in the tilapias to be any sort of indicator of the salinity concentration of the environment.

The proliferation of the tubular system in response to sea water adaptation has been observed by Kessel and Beams (1962) in F. heteroclitus and a recent confirmatory study by Karnaky et al.

(1976a) indicated that, when the pup fish (Cyprinodon variegatus) was adapted to salinity of 200% sea water, the tubular system of the chloride cells underwent such extensive hypertrophy that tubules developed in virtually all of the available cytoplasmic space not already occupied by mitochondria. These findings support the present study in the light of the observations that in both S. mossambicus and S. spilurus, darker cytoplasm and more compact tubular system were consistently observed in sea water adapted fish than in their fresh water species.

The relationship between the ultrastructure of the gills and the movement of the ions across them has been studied in both fresh and sea water adapted teleosts. Sargent et al. (1978), working with Anguilla anguilla in sea water, observed the presence of intercellular spaces along with baso-lateral membrane of the cell and between the adjacent chloride cells, the continuity of these spaces with the tubular system and the short tight junctions near the apical membrane of the cell. They considered these areas to be junctions through which sodium chloride concentrated and passed outwards across the gill epithelium in to sea water. These conclusions concorded with the biochemical studies on the localization of Na^+-k^+ ATPase activity in the chloride cells reported by Ellis et al. (1977), who found that ouabain inhibition of Na^+-k^+ ATPase activity in vivo is normally effective only via the serosal route. The (^3H) labelled ouabain links specifically to the tubular system in the basolateral area of the chloride cell (Karnarky et al., 1976b). The Na^+ ion is also found in highest concentration in the basolateral area of the cell (Mizohera et al.,

1970). However, the presence of intercellular spaces along the basolateral membrane and the continuity of these spaces with the tubular system observed in sea- and fresh-water fish of the present study suggest a similar process of salt excretion to those described by Sargent et al. (1978). Furthermore it has been observed in several teleosts that adaptation to sea water did not affect the organisation of the respiratory cells, but had a marked effect on that of chloride cells which proliferated and, in the case of small or immature cells, sent interdigitations into pre-existing chloride cells. The old and the young chloride cells were linked by single strand short leaky junctions, while those in fresh water were located singly and linked with the neighbouring respiratory cells via long tight junctions (Sardet et al., 1979). Consequently these authors have suggested several routes of solutes transfer across the gill epithelium mainly along the leaky junction in sea water adapted teleosts and through the tubulo-vesicular route in fresh water teleosts. The present results agree with the above hypotheses derived for sea water adapted fish, but the presence of more than one chloride cell (up to two) adjacent to each other in fresh water, and the lack of evident relationships between the vesicles and the tubular system observed in the fish of the present study is in disagreement with them, and may suggest that in fresh water Sarotherodon spp. the fish are well able to use the shallow junctions between two adjacent chloride cells in solutes transfer. Moreover the discontinuity between the vesicles and the tubular system, and the presence of more than one chloride cell adjacent to each other in

fresh water adapted fish have been also reported by Sargent *et al.* (1978) in *A. anguilla*, and by Laurent and Dunel (1980) in certain teleosts.

GENERAL CONCLUSIONS

It is obvious from the results of this study that the Sarotherodon species used are capable to varying extents of adapting to a salt water existence. The plasma osmotic concentration as defined in the study appears to give a good indication of the capability of the fish to successfully acclimate particularly during the critical early hours following upon transfer. The fish showed the biphasic osmolality response characteristic of such transfers in other species, the response in this case taking place over a four day period post transfer. This ability to tolerate direct transfer to salt water and to regulate the subsequent increases in plasma osmolality was however only possible when the fish were held in normal environmental conditions prior to salt water transfer, showing that holding of fish in good environmental conditions prior to salt water transfer is a crucial factor for subsequent successful transfer to be achieved. It is also obvious that any form of stress other than salinity stress should be avoided during acclimation. It is desirable that both the fresh and salt water should be of high quality and fish being acclimated should all be of a similar size to eliminate the possible build up of hierarchies. Larger fish can exhibit dominance and show aggressive behaviour towards smaller ones and this may result in considerable energy expenditure which appeared to affect the smaller fish's ability to undergo transfer to sea water.

The physiological responses of the plasma osmotic concentration following thermal shock alone and combined thermal and

CHAPTER 9

GENERAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

salinity shocks demonstrate the significance of maintaining a stable temperature before and after salt water transfer. Generally the combined salinity and thermal shock result showed that the combined shock of salinity and high temperature was far more readily tolerated than the combined shock of salinity and low temperature (Figs. 15-18). These findings indicate the thermophilic nature of such fish which is highly significant but possibly foreseeable in view of the temperature fluctuations in tropical countries.

The principal aim of the experimental feeding of the high salt diet was to verify whether the salt diet had any influence upon salt water tolerance in Sarotherodon species. Although the results were positive the influence of the salt diet was rather marginal. Comparison of the feeding of the salt diets with gradual acclimation showed that the gradual acclimation regimes were more effective in stimulating the osmoregulatory organs to counteract the transfer to higher salinities up to full strength sea water; even at its maximum effect the high salt diet was never sufficient for fish to survive the direct transfer to full sea water. The advantage of the gradual acclimation method over the feeding of the salt diets was also evident in terms of time taken to achieve successful acclimation.

The differences in euryhalinity between the Sarotherodon species used in this study was first observed in the gradual acclimation experiments and later in the growth rate responses experiments. Consequently it was possible to divide the species

tested into two groups according to their euryhalinity (see Chapters 3 and 7). The first group is the very highly euryhaline species including S. aureus which appeared to achieve the highest survival and the best growth rate and food conversion performance in fresh water, at 50%, 75% sea water and full strength sea water of 36‰ salinity. This is particularly fortuitous, since S. aureus is also a large fast growing and very light coloured species, major factors in relation to ease of culture and marketing in countries of the Arabian Peninsula where such hatchery and salt water fattening systems are likely to be of value.

SUGGESTIONS FOR FUTURE WORK

In this study it was attempted to demonstrate the ability of some of the Sarotherodon species, namely S. aureus, S. mossambicus, S. spilurus, S. niloticus and the hybrid of S. aureus/S. niloticus, to tolerate transfer to salt water. All the experiments were carried out under standard laboratory conditions employing fixed temperatures and light durations. Future investigations need to consider the possible effects of different seasonal fluctuations in these environmental parameters in Arabian conditions on the fish transferability. Similar type of work with salmonid fish has been published (Conte and Wagner, 1965; Jackson, 1981) and it might be beneficial to compare such results. This could be taken a step further in some sub-tropical countries where the possibility of overwintering tilapia in sea water may offer a cheaper alternative to overwintering under cover of a greenhouse or by some other expensive method.

The response of the chloride cells as well as the physiological response of the plasma were crucial to the success of the transfer. It would be interesting to investigate the contribution of other osmoregulatory organs such as the kidneys, intestine and the skin in salt water transfer, as well as the effect of salt water on certain individual components of the plasma such as sodium, potassium, chlorideetc.

Since only one standard diet and one fixed feeding regime were used in this study, it would be of great economical importance

to clearly define the precise nutritional requirements of the commercially cultured Tilapia and Sarotherodon species in sea water since these may well vary from those of freshwater fish especially in terms of mineral salts content. Moreover, since salt water transfer affects the osmotic concentration of the plasma and the body water content, this may imply a slight modification in the body chemical composition and the nutritional quality of the flesh. A comparative study of the body chemical composition of fresh and sea water fish is likely to be of value.

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APPENDICES

APPENDIX 1

Composition of the Standard Experimental Diet

Ingredients	Percentage Composition
Fish meal	58%
Mineral mix	4%
Vitamin mix	1%
Lipid	5%
Binder (Carboxymethyl cellulose)	2%
Dried distillers solubles	5%
α-cellulose	10%
Corn starch	2%
Maize	13%
	100%

APPENDIX 2

The Composition of the Mineral Mix contained in the Standard Diet per 200 grammes

Ingredients	Weight (g)
Calcium tetrahydrogen orthophosphate	137.20
Calcium carbonate	10.90
Magnesium carbonate	18.12
Ferrous sulphate	5.98
Potassium chloride	9.96
Sodium chloride	15.94
Aluminium sulphate	0.0398
Zinc sulphate	0.796
Copper sulphate	0.1994
Manganese sulphate	0.54
Calcium iodate	0.049
Cobalt sulphate	0.1994
	199.92

The total weight was made up to 200 g. with α-cellulose

APPENDIX 3

Composition of the Vitamin Mix contained in the Standard Diet per 100 grammes

Ingredients	Weight (g)
Thiamine (B_1)	0.3
Riboflavin (B_2)	0.76
Pyridoxine (B_6)	0.18
Panthothenic acid	2.00
Inositol	7.10
Biotin	0.10
Folic acid	0.075
Para-amino benzoic acid	1.5
Choline	32.24
Niacin [Nicotine acid (B_3)]	2.66
Cyano-cobalamine (B_{12})	0.005
Vitamin A (Retinol palmitate)	10.000 IU
α-Tocopherol (E)	1.5
Ascorbic acid (C)	10.0
Menadione (K)	0.2
	58.62

The total weight was made up to 100g with α-cellulose

APPENDIX 4

Proximate Analysis of the Standard Diet on a Dry Weight (Moisture free) Basis

Components	Percentage Composition
Crude protein	37.71
Ether extract	6.49
Ash	7.42
NFE *	48.38

* NFE - Nitrogen free extractives determined by difference

(100 - 31.71 + 6.49 + 7.42)

APPENDIX 5

Chemicals used for Ammonia Determination

The following chemicals were used for ammonia determination:

1. Reagents

(a) Sodium nitroprusside solution: 0.4 g sodium nitroprusside was dissolved in 100 ml deionised water. Stored at 4°C in an amber glass bottle. This solution is stable for at least one month.

(b) Sodium hydroxide stock solution: 68 g (A.R. Grade) sodium hydroxide was dissolved in 250 ml deionised water (272 g/ litre).

(c) Phenol stock solution: 156 g A.R. Grade Phenol was dissolved in 250 ml methonal. Stored at 4°C in an amber glass bottle.

2. Analytical Reagents

(a) Citrate Buffer: 50 g of sodium citrate, 25 g of EDTA and 5 g of sodium hydroxide were dissolved in deionised water and the solution made up to 250 ml in measuring flask.

(b) Phenate reagents: 15 ml of the phenol stock solution and 10 ml of sodium nitroprusside were mixed and the solution was made up to 100 ml with deionised water. The solution was transferred to clean glass bottle and stored at 4°C.

(c) Alkaline hypochloride: 30 ml of sodium hydroxide stock solution and 5 ml of sodium hypochlorite solution were diluted to 100 ml with deionised water, transferred to an amber glass bottle and stored at 4°C.

3. Standard Solutions

To prepare a stock ammonia solution, 3.82 g of ammonium chloride was dissolved in deionised water and made up to 500 ml in a measuring flask. This gives a concentration of 40 mg ammonia-nitrogen/litre. A series of standard solutions were then prepared by diluting different volumes of this solution with deionised water in 100 ml measuring flasks so as to give ammonia concentrations ranging between 0.04 and 0.4 mg NH_4-nitrogen. The absorbencies for these standard solutions were measured at 700 nm in 1 cm cell and a calibration graph was set up accordingly.

APPENDIX 6

Calculated pKa Values for Ammonia as a Function of Temperature (0-30°C)

Temperature (°C)	pKa	Temperature (°C)	pKa
0.0	10.0826	16.0	9.5297
1.0	10.0461	17.0	9.4972
2.0	10.0099	18.0	9.4649
3.0	9.9740	19.0	9.4328
4.0	9.9384	20.0	9.4010
5.0	9.9030	21.0	9.3693
6.0	9.8678	22.0	9.3379
7.0	9.8329	23.0	9.3067
8.0	9.7983	24.0	9.2757
9.0	9.7639	25.0	9.2448
10.0	9.7297	26.0	9.2143
11.0	9.6958	27.0	9.1839
12.0	9.6621	28.0	9.1537
13.0	9.6287	29.0	9.1237
14.0	9.5955	30.0	9.0939
15.0	9.5625		

After Emerson *et al.* (1975)

APPENDIX 7

Preparations used in Nitrite Determination

Reagents

1. Sulphanilamide: 5 g A.R. Grade of sulphanilamide were dissolved in a mixture of 50 ml hydrochloric acid (S.G. 1.18) and 300 ml of distilled water. The solution was then diluted to 500 ml with distilled water.

2. N-(1-naphthyl)-ethylendiamine dihydrochloride solution: 0.5 g of N.E.D. was dissolved in 500 ml distilled water and stored in dark bottle. This solution was renewed every month.

3. Standard nitrite solution: Pure sodium nitrite was dried at 105°C in a desiccator for several hours. After cooling 0.493 g of dried material was dissolved in deionised water and the solution made up to one litre. This solution contained 10 mg NO_2-N/litre. As in ammonia estimations several concentrations of standard nitrites solutions were made up from this solution to set up a standard graph.

Sarotherodon spilurus					Sarotherodon mossambicus					Sarotherodon niloticus				
Time (hours)	No. of fish in each sample	Mean Weight (g)	Plasma osmotic concentration ± S.D.	Mean Haematocrit value	Time (hours)	No. of fish in each sample	Mean Weight (g)	Plasma osmotic concentration ± S.D.	Mean haematocrit value	Time (hours)	No. of fish in each sample	Mean weight (g)	Plasma osmotic concentration ± S.D.	Mean haematocrit value
0	6	19.7	322 ± 5	35.5	0	6	18.6	322 ± 12	36.0	0	6	15.6	342 ± 8	37.85
3	3	15.6	359 ± 15	30.5	3	3	20.3	348 ± 11	32.7	3	3	17.7	416 ± 5	32.7
6	3	18.7	382 ± 35	30.0	6	3	19.2	429 ± 20	28.3	6	3	16.5	466 ± 30	27.53
9	3	14.6	390 ± 30	33.0	9	3	17.1	419 ± 13	26.7	9	3	16.4	506 ± 42	26.24
12	3	16.4	430 ± 27	32.75	12	3	19.0	396 ± 21	25.0	12	3	13.2	535 ± 48	26.11
24	3	20.9	416 ± 40	32.5	24	3	17.5	344 ± 29	32.3	24	3	17.1	507 ± 55	30.27
48	3	19.5	368 ± 33	32.0	48	3	13.0	348 ± 16	29.0	48	3	18.3	432 ± 26	30.5
72	3	19.9	372 ± 15	30.0	72	3	15.2	349 ± 17	30.5	72	3	15.7	393 ± 37	36.6
96	3	16.5	351 ± 20	30.0	96	3	15.3	346 ± 15	30.0	96	3	14.4	367 ± 28	34.0
120	3	15.3	355 ± 15	29.0	120	3	14.7	344 ± 9	31.0	120	3	18.6	364 ± 10	31.76
Sea water value	6	18.8	354 ± 13	28.75	Sea water value	6	11.2	341 ± 14	29.8	Sea water value	6	12.6	354 ± 13	29.41

APPENDIX 8. Effect of direct transfer from fresh water to salinity of 60% sea water (21.6‰) on the plasma osmotic concentration and the haematocrit values in S. spilurus, S. mossambicus and S. niloticus

	The change in plasma osmotic concentration				The change in percentage body water content		
Time (hours)	No. of fish in each sample	Mean weight (g)	Mean plasma osmotic concentration ± S.D.	Mean Haematocrit value	No. of fish in each sample	Mean weight (g)	Percentage body water content ± S.D.
0	6	22.8	334 ± 8	32.5	5	8.9	77.866 ± 2.17
3	3	16.7	360 ± 19	26.6	3	10.6	75.8 ± 0.47
6	3	20.1	412 ± 27	25.59	3	11.4	74.21 ± 0.64
9	3	25.7	439 ± 36	26.95	3	11.5	74.18 ± 0.56
12	3	29.0	450 ± 16	24.77	3	12.55	72.65 ± 0.37
24	3	26.1	433 ± 10	23.93	3	12.4	73.62 ± 0.66
48	3	19.3	373 ± 12	34.38	3	10.2	75.45 ± 0.35
72	3	26.4	364 ± 12	38.32	3	12.3	76.3 ± 0.6
96	3	23.5	352 ± 12	36.53	3	14.4	76.13 ± 0.23
120	3	26.6	351 ± 11	35.5	3	10.0	76.61 ± 0.25
Sea water value	6	28.8	354 ± 13	28.2	4	12.4	75.92 ± 0.99

APPENDIX 9. Effect of direct transfer from fresh water to salinity of 60% sea water (21.6‰) on the plasma osmotic concentration and the percentage body water content in S. aureus/S. niloticus hybrids.

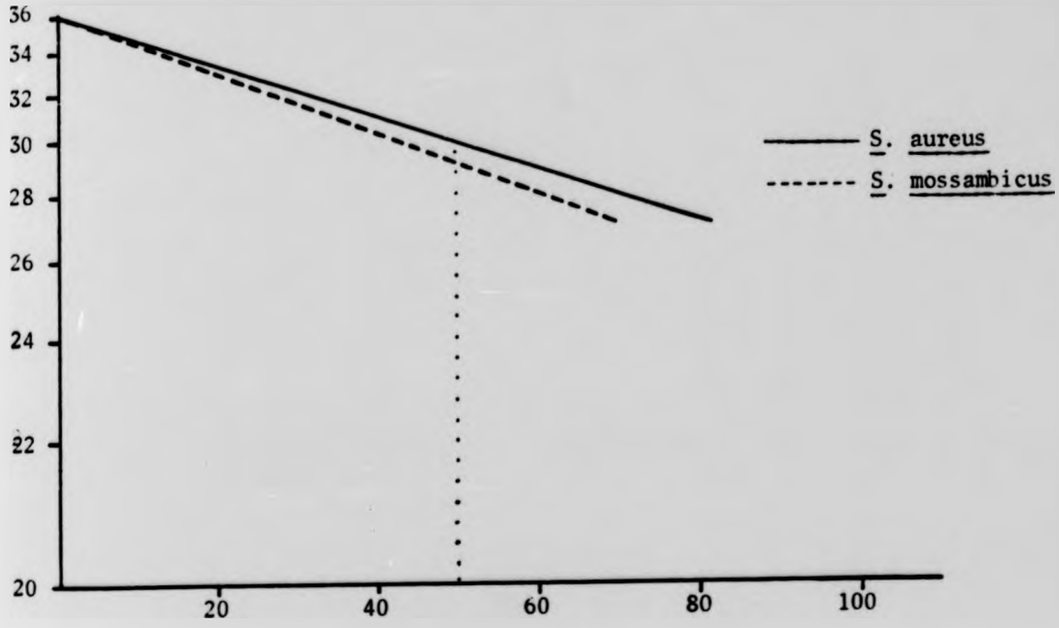
Salinity ‰	No. of test fish	Number of fish surviving									
		After 24 hours					After 48 hours				
		S. niloticus	S. niloticus/ S. aureus hybrids	S. mossambicus	S. spilurus	S. aureus	S. niloticus	S. niloticus/ S. aureus hybrids	S. mossambicus	S. spilurus	S. aureus
36	60	0	0	0	0	0	0	0	0	0	0
27	60	0	0	42	42	49	0	0	39	31	48
23.4	60	36	26	60	60	60	11	26	60	60	60
21.6	60	42	60	60	60	60	42	60	60	60	60

APPENDIX 10. Practical application

This table has been extracted from Tables 1, 2 and 3. From the semi logarithmic graph we could detect the predicted TL50. The calculation based on an interpolated value of the percentage of the fish surving at two concentrations of sea water at which less than half and more than half survived. (See Figure overleaf).

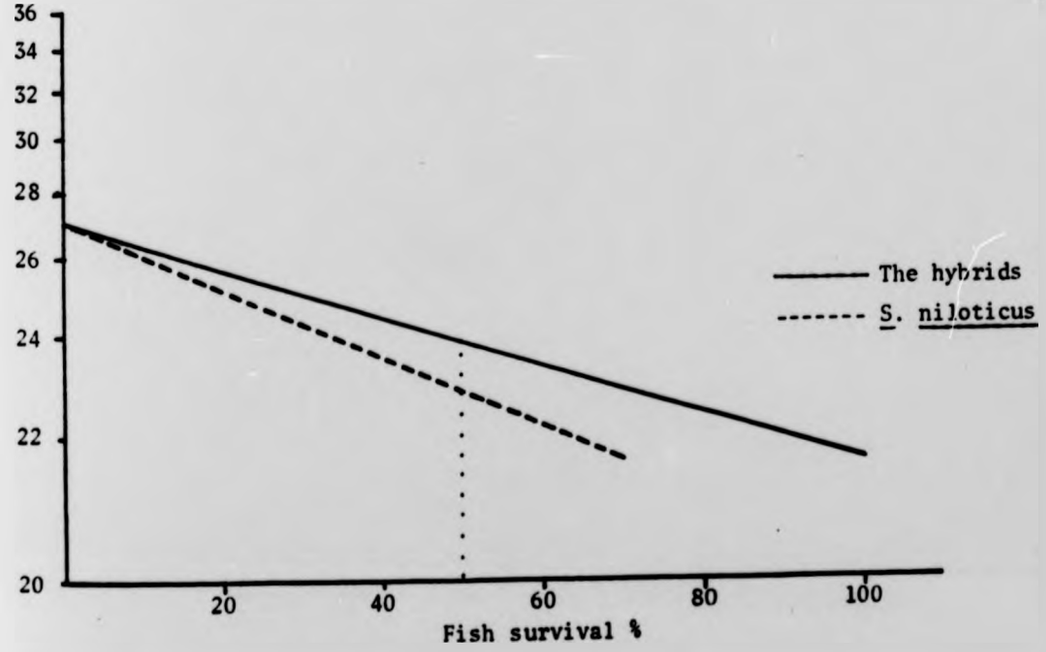
The sea water concentration on the logarithmic scale.

The percentage survival on the arithmetic scale.



24 h. TL.50 for S. mossambicus is 29‰

24 h. TL.50 for S. aureus is 29.8‰



24 h. TL.50 for S. niloticus is 22.8‰

24 h. TL.50 for the hybrids of S. niloticus/S. aureus is 23.8‰

APPENDIX 11

Mixture A and B were first prepared as follows:

Mixture A :	Epon 812	26 gm
	DDSA	71 gm
Mixture B:	Epon 812	18 gm
	MNA	15 gm

A and B were then mixed in a 7 : 3 ratio for blocks of medium hardness.

Immediately before use, the reation accelerator DMP-30 was added to a final concentration of 1.5-2%. All mixtures were well-stirred at all stages. Mixtures A and B can be stored in a refrigerator for about two months.

II