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Title: Oxygen Consumption During Acclimation to Increasing Salinity in the Freshwater Euryhaline Cichlid Oreochromis niloticus (Linnaeus, 1757).

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Abstract approved: _____
Richard A. Tubb

Two sets of experiments measured changes in the oxygen consumption of the euryhaline teleost Oreochromis niloticus during acclimation to progressively increased salinity. Six acrylic respirometers were constructed for this purpose. The salinities tested were 9, 18, 27 and 36 ppt, with 36 ppt considered as full strength seawater. Fish were placed in respirometers and allowed to acclimate for 5 days at each experimental salinity. Changes in the rate of oxygen consumption of the treatment fish (SW) were compared to freshwater control fish (FW). There was a significant increase in the plasma Na^+ concentration of seawater acclimated fish over the control freshwater fish at the end of the experiment. There was no significant change in the plasma K^+ concentration of seawater-acclimated fish and there was no significant differences in the dry weights between freshwater and seawater-acclimated O. niloticus. The

constancy of water contents reflected the homeosmotic nature of this euryhaline fish, and its resistance to dehydration during acclimation. Oxygen consumption of fish was lower in 9 ppt than in other salinities including freshwater. Oxygen consumption was maximum at 27 ppt, though it represented a lower osmotic stress on the fish than full strength seawater. At a salinity of 27 ppt fish probably encountered an "osmotic adaptive threshold" where the salt excretory mechanism via chloride cells was fully activated.

OXYGEN CONSUMPTION DURING ACCLIMATION TO INCREASING SALINITY IN THE FRESHWATER EURYHALINE CICHLID *Oreochromis niloticus* (Linnaeus, 1757)

by

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Typed by researcher for Salam A. Alablani

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"He who teaches me one letter, I become his slave for ever."

- Old Arabic proverb.

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Preface

Kuwait is a small country with limited renewable resources. The natural fisheries need to be supplemented by aquaculture. Local supplies of fresh fish vary widely and a constant source of fish is needed for a growing population. Fresh water is an expensive and scarce resource and culture of seawater tolerant fish is an attractive aquaculture potential that needs to be developed. In addition to the fish species that exist naturally in the Kuwaiti waters, exotic species were imported and subjected to extensive research as candidates for aquaculture. Tilapia (family: Cichlidae), a genus tolerant of salt water, was selected as a candidate in 1981. Research at **Kuwait Institute for Scientific Research** (**KISR**) is oriented toward a better understanding of the biology and behavior of the animal, and adapting it to a commercial aquaculture in saline water.

One of the difficult problems is acclimating freshwater fish to brackish or saltwater systems. The energy cost of the acclimation process needs to be measured and analyzed to improve aquaculture practices. Tilapia have been acclimated, in Kuwait, to full strength seawater, but with no adequate knowledge of the energetic cost and/or changes in the metabolic rate of the acclimated fish.

Oxygen Consumption During Acclimation to Increasing Salinity in the Freshwater Euryhaline Cichlid *Oreochromis niloticus* (Linnaeus, 1757)

INTRODUCTION

The culture of several species of *Tilapia*, *Sarotherodon* and *Oreochromis* in the arid countries of the mideast and tropical islands has required that these species be reared in brackish or saline water. The transfer of a freshwater species into a saltwater environment is possible for only a few species in these genera, and the energetic requirements of adapting to seawater are high. Aquaculture is a very practical science and most assessments of successful transfer from freshwater species to seawater seems to have been based on survivorship. More complete understanding of the energetic costs for acclimation may improve culture practices. Acclimation of *Tilapia* and related genera to seawater usually involves gradual transition from dilute to full strength seawater, but changes in energetic demands for individual fish have not been adequately measured during the process. The costs of salinity acclimation have serious consequences for aquaculture and deserve careful investigation.

Several researchers have measured oxygen consumption of *Tilapia* after full acclimation to seawater. The purpose of this investigation was to measure oxygen consumption as a

measure of energy expenditures of Oreochromis niloticus (Linnaeus 1757) during gradual acclimation from freshwater to full strength seawater.

LITERATURE REVIEW

The species of Tilapia (family cichlidae) were reviewed and revised by Lowe-McConnell (1959). The substrate spawners were placed in the genus Tilapia and the mouthbrooders under a new genus Sarotherodon. Recently, Trewavas (1982) revised the genus Sarotherodon and on the basis of parental care divided the genus into Oreochromis, Sarotherodon and Danakilia. The species selected for experimentation was Oreochromis (Oreochromis) niloticus. O. niloticus is probably the most widely cultured freshwater fish in the world, and has been transferred from Africa to almost every tropical country in the world (Bardach et al, 1972; Riedel, 1965). Several species of Tilapia, Sarotherodon and Oreochromis are major sources of proteins in developing countries, but there is a growing consensus that O. niloticus can become the world's most important warmwater cultured fish in the near future (FAO, 1980). Consequently, a large body of information on O. niloticus has been developed and research is continuing on genetics, nutrition, reproduction, physiology and aquaculture techniques for more efficient production.

The species can utilize many organic waste materials and as a hardy omnivore adapts well to harsh conditions (Balarin and Hatton, 1979). There is some evidence

indicating that O. niloticus can feed on the cyanobacteria (Moriarty, 1973; Moriarty and Moriarty, 1973a and 1973b).

Research on the acclimation of O. niloticus and other cichlids to brackish water has received less attention than freshwater culture systems. The acclimation of a euryhaline fish such as O. niloticus to seawater involves a high degree of osmoregulatory stress (Parvatheswararao, 1965). The general principle of osmoregulation was proposed by Krogh (1937). Osmoregulation in freshwater involves the production of large volumes of dilute urine to compensate for the net branchial water influx, and simultaneous active branchial salt uptake to compensate for the net renal and extra-renal salt loss. In seawater the opposite situation exists. Fish must drink water to compensate for water lost by diffusion through permeable and semi-permeable membranes, and actively excrete salts through the opercular and branchial epithelium via the chloride cells. When O. niloticus is transferred from freshwater to seawater the reversal of osmoregulatory processes almost certainly requires large energy expenditures.

Oxygen consumption is a reasonably good method of measuring energy costs, and increases when a freshwater fish is transferred to a saline medium (Parvatheswararao, 1965; Madanmohan Rao, 1965). Dissolved oxygen concentrations and oxygen consumption rates are crucial factors in the design and management of intensive and semi-intensive culture

systems. It is necessary to be aware of the dissolved oxygen requirements of the cultured fish since low dissolved oxygen levels limit "scope for activity" (Jobling, 1981). Body size influences osmoregulatory ability of fishes and the ability to adapt to osmotic stress (Parry, 1958; Hickman, 1959; Houston, 1961). Several researchers have worked on the respiratory performance of Tilapia in general, using closed respirometers (Ahmed and Magid, 1969; Job, 1969a, 1969b; Kutty, 1972), and with flow-through respirometers (Farmer and Beamish, 1969; Beamish, 1970; Bashamohideen and Parvatheswararao, 1976a, 1976b). There are, however, many contradictory reports regarding oxygen consumption of Tilapia at different salinities. According to Job (1969a, 1969b), oxygen consumption for O. mossambicus was highest in an isosmotic medium at a salinity of 12.5 parts per thousand (ppt), whereas Assem (1981) (cited by Jurrs et al, 1984) reported that it was lowest under such conditions. Job (1969a, 1969b) suggested that the salinity of 12.5 ppt (= isosmoticity) reduced the osmotic load and the cost of regulation on the fish, permitting fishes to achieve greater " scope for activity ". Consequently, fish became more active increasing their oxygen consumption. He did not report any hyperactivity of fish that can be associated with this increase in oxygen consumption. His respirometer and experiment should have been designed to minimize the hyperactivity which increased oxygen consumption.

Furthermore, he did not measure oxygen consumption for individual fish. These factors confounded estimating the routine metabolism of the fish. Lotan (1966) reported that the lowest oxygen consumption rate in O. aureus was obtained when the blood was isosmotic with the medium. She attributed this to the low energy required for osmoregulation. Farmer and Beamish (1969) supported this assumption by their investigation of oxygen consumption of O. niloticus at different salinities and various swimming speeds. They reported that the blood is isosmotic at 11.6 ppt (using plasma freezing point depression) where energy expenditure associated with osmoregulation is lowest, hence energy required to maintain homeostasis is minimal (Farmer and Beamish, 1969).

Bashamohideen and Parvatheswararao (1976a) measured oxygen consumption for O. mossambicus. They reported two categories of physiological responses to osmotic stress. Immediate or short-term response, and stabilized or long-term response. They assumed that the sudden increase in oxygen consumption, the short-term response, indicated the time-course or the sequence of events leading to the stabilized long-term response, and the completion of adaptation. In their experiment they obtained the short-term response by measuring oxygen consumption rate within 15 minutes after introducing the fish into the respirometer containing seawater. The validity of this

short-term response is highly questionable. It is highly unlikely that the high initial "overshoot" in oxygen consumption was a response to the experimental salinity alone. The initial overshoot was probably caused by other factors such as netting the fish, transportation and placing the fish inside the respirometers. The experimental procedures used, make the estimations of energy costs for acclimation meaningless. Fish must be handled with minimum possible stress, and left for some time in the respirometer to acclimate prior to starting oxygen measurements (Black et al, 1939; Keys, 1930 and Wells, 1932). Handling of fish during the experiment creates a heightened oxygen consumption which subsides gradually. Fish need to be maintained for several hours in their respirometers before collecting any sample. Hickman (1959) reported that his fish sojourned for 18 to 22 h in their respirometers before collecting any samples.

Bashamohideen and Parvatheswararao (1976a) fed fish inside the respirometers, but there was no description of how oxygen consumption of feed remains and feces were accounted for. Similarly, there was no description of any precautions to neutralize Biological Oxygen Demands (BOD) so as to avoid incorporating it with the oxygen consumption of the experimental fish. Oxygen consumption increases with feeding and it may reach a peak, depending on the species, within 10 - 12 h after feeding (Muir and Niimi, 1972).

Feeding fish in respirometers makes it difficult to separate any increase in oxygen consumption due to salinity, from that attributable to Specific Dynamic Action (SDA).

SIGNIFICANCE OF RESEARCH

The literature available on energy expenditures during salinity acclimation is relatively poor and somewhat contradictory. This investigation was undertaken to develop a better understanding of the biology of O. niloticus and the physiological changes occurring in the transition from freshwater to seawater. In-depth investigations of energetic requirements of fish during acclimation to brackish or seawater provide the understanding necessary to design efficient aquaculture facilities. If primary physical and physiological requirements of an animal are not understood, aquaculture practices will always be exaggerated to avoid potential mishaps. Generally such approaches involve wastes of resources.

MATERIALS AND METHODS

I. The respirometer

Six respirometers made exclusively of a non-toxic acrylic were constructed. The volume of each was approximately 2.8 liters (Figure 1). The respirometers were basically cylinders with a diameter of 10 cm and a length of 32 cm mounted horizontally on a table. One end of the cylinder was permanently sealed. The other end was fitted with a removable cap for introducing the fish. The respirometer had an inlet and an outlet to permit the flow of water through the chamber. A small funnel was fitted at the bottom of the respirometer to collect any feces, to avoid confounding oxygen uptake with Biological Oxygen Demand. A plugged opening at the top of the respirometer served as a bubble-trap. Excitement of fish was greatly reduced by covering about 90 % of the respirometer with a black sheath (Figure 2).

II. Water supply system

Two identical recirculating flow-through systems were constructed. One supplied water for the freshwater control fish (FW) and other supplied the salinity treatment fish (SW). Each system was composed of a Nalgene collection tank

Figure 1. Components of a 2.8 l respirometer. A= water supply through PVC pipes, B= overhead cylinder, C= three-way T-connection entering the respirometer, D= sealed end of the respirometer, E= bubble trap, F= plastic tubing exiting the respirometer, G= plastic funnel for feces collection, X= removable clamps. Arrows indicate the direction of water flow. Water samples were obtained by changing the place of the clamp on F. Water sample from the respirometer was obtained by removing the clamp from F and fixing it on the PVC plastic tube that by-passes the respirometer. Water sample from the water source was obtained by removing the clamp from the by-pass and fixing it on F.

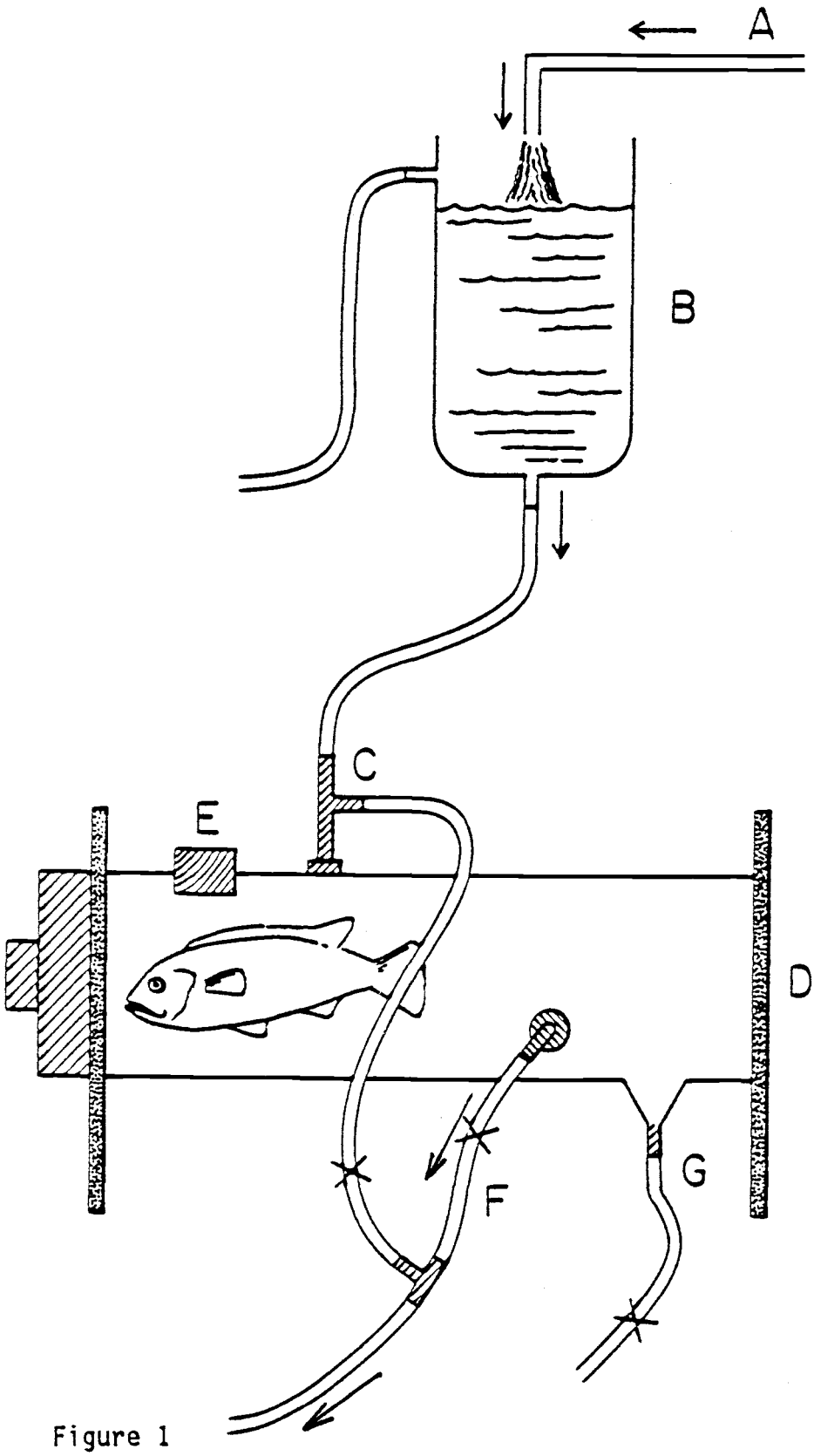

Figure 1

Figure 2. A respirometer, covered by a plastic sheath, mounted horizontally on a table. The overhead cylinder appears in the background.

Figure 3. The collection tank (120 l) heated by two vycor immersion heaters supported by a styrofoam float. The airlift aerated and mixed the water. Water was pumped to the head tank (40 l) then flowed by gravity to the respirometers.

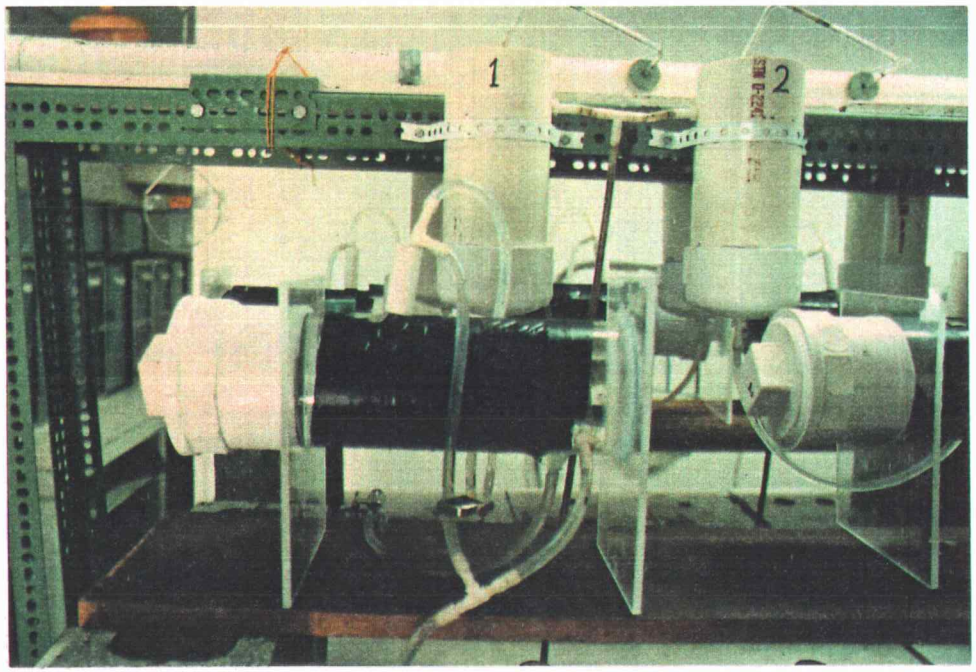

Figure 2

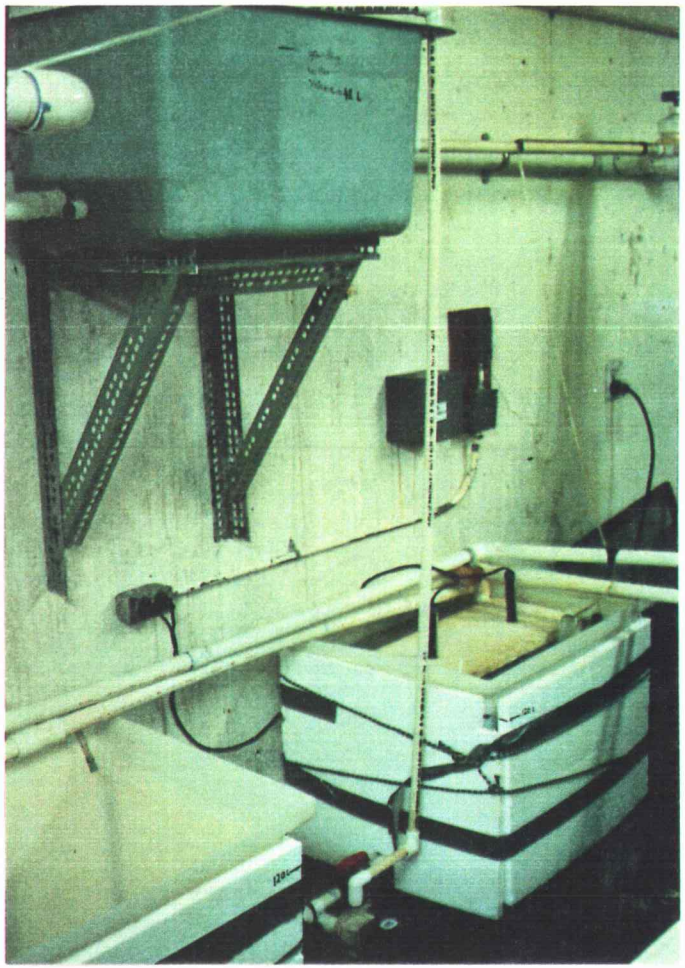

Figure 3

supported by blocks on the ground level with an operating volume of 120 l. The collection tank was connected to a head tank 2 m above it by means of a small pump (Figure 3). The operating volume of the head tank was 40 l. The head tank delivered water to the respective respirometers through plastic PVC pipes by gravity. Water collected in 1.8 l overhead cylinders positioned over each respirometer (Figure 1). These cylinders served two functions. First, it prevented the formation of air bubbles inside the respirometer by degassing the supersaturated water in the overhead cylinder before flowing into the respirometer. Secondarily, it ensured a consistent flow rate through the respirometers. Each overhead cylinder had an over-flow outlet, hence the extra water collected, along with the water that was leaving the respirometers, in pipes that returned water to the collection tank by gravity. The temperature in the system was controlled by two 1000 watt Vycor immersion heaters that were supported by styrofoam floats in the collection tank (Figure 3). The temperature was adjusted to 27^{o} C in the collection tank, consequently it was 26^{o} C in the respirometers due to losses to the ambient room temperature. Airlifts placed inside the collection tank mixed and aerated the water and prevented any thermal stratification due to the heaters.

III. Experimental design

Two respirometers were assigned for the control (FW) fish and four were assigned for the treatments (SW). Well water at the Oak Creek Lab of Biology served as a freshwater source. Seawater was obtained by mixing synthetic salt (Marine Environment Salt, Marine Environment Products, San Francisco, CA) with freshwater to the desired salinity. The salinity increments tested were 9, 18, 27 and 36 ppt which corresponded to 25, 50, 75 and 100 % seawater, respectively. Salinity was monitored daily by a salinity refractometer (Atago co., Ltd.). Any change in salinity was adjusted by adding salts or freshwater to the system. The flow rates in FW and SW respirometers were adjusted to 80 ml min^{-1} throughout the experiment.

Based on trial and error, five days were assumed to be adequate to complete the acclimation process to progressive increases in salinity. In measuring oxygen consumption at any given salinity, all six fish sojourned for five consecutive days in their respective respirometers. Two water samples were taken from each respirometer, 7-8 hours apart, each day including the FW control respirometers. The first sample was always collected at least 3 h subsequent to transfer of fish into the respirometer to avoid any increases in oxygen consumption attributable to excitement. After five days fish were transferred to aquaria of the same

salinities. Transfers were conducted in total darkness to minimize stress. The respirometer was disconnected and immersed in the aquarium, and the fish could freely leave the respirometer. The introduction of the fish into the respirometer was carried out in the same way. Respirometers were placed inside the aquaria two nights before the experiment, thus fish sought shelter by going inside the respirometers. Each fish was placed in a separate aquarium with the proper salinity, including FW. Fish stayed five days in the aquaria, and were fed ad libitum on Oregon Moist Pellet daily. The amount of feed consumed was determined and food remains were siphoned out daily. Fish were starved on the fifth day, before the transfer to their respirometers, for two reasons. The first was to avoid feces excretion inside the respirometers and the elevation of oxygen consumption due to BOD. Secondarily, starvation minimized the possibility of involving SDA in any increases in oxygen consumption (Muir and Niimi, 1972). Photoperiod was adjusted to 12 hours day time from 0600 to 1800 hrs. To avoid the buildup of metabolites, twenty percent of the water was renewed daily in FW and SW. The respirometers and aquaria were thoroughly cleaned with warm water before the introduction of fish.

IV. Data collection

Two sets of experiments were conducted between June 23, and September 12, 1989 using 4 experimental fish and two control fish for each experiment. The experimental fish were from the same hatch, and were brought from a stock at Auburn University, Alabama. Wet weight and length of fish were measured before and after each experiment. The weight of fish in experiment 1 ranged from 43.50 to 117.80 g (Table 4) with an average weight of 71.53 $\pm$ 26 g. In experiment 2, fish ranged from 44.40 to 78.80 g (Table 5) with an average of 56.50 $\pm$ 12.60 g. The fish which served as FW control in experiment 1 had an average weight of 90.65 $\pm$ 38.40 g, while the SW treatment had an average weight of 61.97 $\pm$ 16.27 g. In experiment 2, average weight of FW was 54.30 $\pm$ 11.17 g and the SW was 57.60 $\pm$ 14.78 g (Table 1).

Four fish, not involved in experiment 1, were selected from the stock tank (St.F). They were also used as an additional control for comparisons of plasma electrolytes and dry weights for FW and SW fish in experiment 1 (Table 1).

Fish were anesthetized prior to weighing (75 mg l^{-1} of MS 222, tricaine methanesulfonate), while a concentration of 250 mg l^{-1} was used to kill fish to obtain blood samples. Water samples were collected in 300 ml BOD bottles

Table 1. Average measurements of fish used in experiment 1 and 2 to measure oxygen consumption of O. niloticus. St.F.= Stock fish (n=4). FW= Freshwater control fish (n=2). SW= Seawater treatment fish (n=4).

	Experiment 1			Experiment 2	
	St.F.	FW	SW	FW	SW
Initial length (cm)	19.32 ± 1.34	17.35 ± 2.90	15.35 ± 0.88	14.10 ± 0.85	14.67 ± 0.96
Final length (cm)	--	--	--	14.70 ± 0.56	15.25 ± 0.62
Initial wet weight (g)	129.72 ± 28.75	90.65 ± 38.40	61.97 ± 16.27	54.30 ± 11.17	57.60 ± 14.78
Final wet weight (g)	--	92.75 ± 41.51	60.12 ± 12.04	53.10 ± 8.20	57.70 ± 10.15
Dry weight (g)	41.47 ± 8.45	27.20 ± 11.17	18.52 ± 4.28	15.90 ± 3.39	17.12 ± 2.93
%	32.07 ± 1.48	29.59 ± 1.19	30.70 ± 1.80	29.80 ± 1.79	29.70 ± 0.96

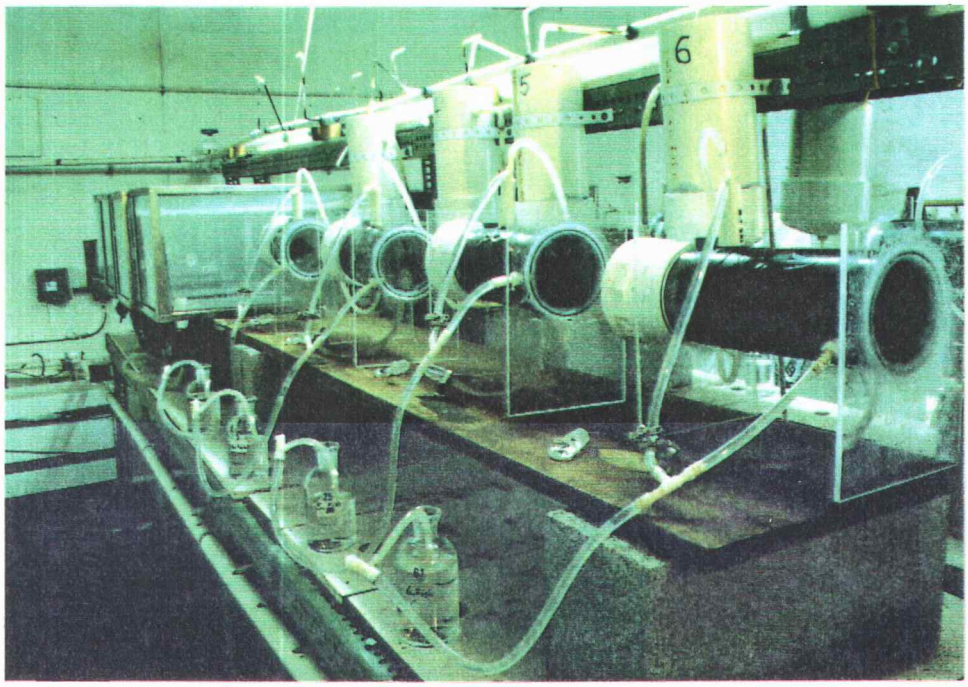

Figure 4. Two water samples were collected daily, 7-8 apart, by 300 ml BOD bottles. Dissolved oxygen was measured following the azide modification of Winkler method.

(Figure 4) and dissolved oxygen (DO) was measured following the azide modification of Winkler method (APHA, 1985). Oxygen consumption for each fish in the respirometer was determined from two water samples collected daily for five days. Blood samples were obtained at the end of first experiment, by severing the caudal fin. Blood was collected in ammonium heparinized capillary tubes. The samples were centrifuged immediately to isolate plasma for 10 - 13 min at a speed of 11500 RPM (Microcapillary centrifuge, model MB, International Equipment Company, USA). A Sodium-Potassium Analyzer, was used to measure the plasma Na^+ and K^+ concentrations. Dry weight of fish was obtained at the end of each experiment after splitting the fish and placing in a drying oven at 75° C for 5 days, and cooling in a desiccator for 24 h prior to weighing.

V. Statistical analysis

The rate of oxygen consumption of each fish was regressed on the time-course of the experiment ($y = a + bx$), with y as the rate of oxygen consumption and x as the acclimation time (5 days) at each salinity. The average slope of all regression lines for any given salinity was compared to its control using one way analysis of variance test (e.g., the slopes of 9 ppt for all fish in SW were averaged then compared with average slope for all fish in FW

that played control for that specific salinity). The range test applied for this method was the Least Significant Difference. After establishing this relationship, a Two-Sample analysis (Student's t-test) was performed to compare the average slope of all fish in SW of any given salinity with the average slope of any other salinity (e.g., the slopes of 9 ppt for all fish in SW were averaged then compared with average slope of 18 ppt for all fish in SW).

RESULTS

Fish were calm inside the respirometers, and only spontaneous movements of the opercula or the pectoral fins were noticed. Conversely, fish were active when placed in aquaria. Feeding was slow on the first day in the aquaria, and was slowest at the beginning of the experiment. Feeding increased with time in the aquaria. Fecal material was initially white and became denser and darker as feeding progressed. In both experiments fish consumed between 0.5 to 1.5 % of their body weight of the presented ration. There was a trend, although statistically not significant, to consume less ration at low salinity (9 ppt). Consumption increased noticeably at moderate salinities (18 ppt), then declined at the high salinity (27 ppt). The decreased feeding rate at low salinities was probably caused by stress of the new physical environment. At higher salinities (27 ppt) osmotic constraints imposed on the fish probably resulted in a change of fish behavior and a low feeding rate.

The plasma Na^+ and K^+ concentrations were determined for the fish in experiment 1. The concentrations of Na^+ in the FW control, SW treatment and stock fish (St.F) were 145.50 ± 7.35; 169.02 ± 10.26 and 140.40 ± 12.74 mMol l^{-1}, respectively, while the concentrations of K^+ were 19.12 ± 6.06; 23.52 ± 3.11 and 15.13 ± 5.78 mMol l^{-1},

respectively. There was no significant difference ($P > 0.05$) in the percentages of dry weight between FW control and SW treatment (Table 1).

The actual oxygen consumption, expressed as micrograms of oxygen consumed per gram of fish per minute (ug g^{-1} min^{-1}), was determined for individual fish in both experiments (Figures 5 and 6). Oxygen consumption of FW fish was almost constant during the two experiments (35 days each) with some slight fluctuation ranging from 1.4 to 2.7 ug g^{-1} min^{-1} (Figures 5a,b and 6a,b). The average oxygen consumption in freshwater was 2.04 ± 0.18 ug g^{-1} min^{-1} (based on the pooled data of both experiments), and it was considered as a baseline for any comparisons with oxygen consumption of SW fish. Fluctuations of oxygen consumption showed no distinct pattern, except that attributed to the excitement of fish during transfers to and from the respirometers. Oxygen consumption of SW fish showed distinct patterns corresponding to the different salinity increments. An **"initial overshoot"** in oxygen consumption was evident in all SW fish at almost all salinities (Figures 5c,d,e,f and 6c,d,e,f). The magnitude of this initial overshoot varied from one salinity to another and between fish. The highest values were at 27 ppt, and the lowest were at 9 ppt.

I investigated oxygen consumption of empty respirometers which held fish for 5 days. Four out of six trials showed no detectable oxygen consumption by

Figure 5. The actual oxygen consumption measurements for 6 fish (O. niloticus) in experiment 1, during acclimation to progressive salinity increases to 9, 18, 27 and 36 ppt. Each 5 days represent one experimental salinity.

Figures 5a and b. Oxygen consumption of the control fish 1 and 2 which sojourned in freshwater throughout the course of experiment 1.

Figures 5c, d, e and f. Oxygen consumption of the treatment fish 3, 4, 5 and 6 which sojourned for 5 days in each acclimating salinity of 9, 18, 27 and 36 ppt in experiment 1.

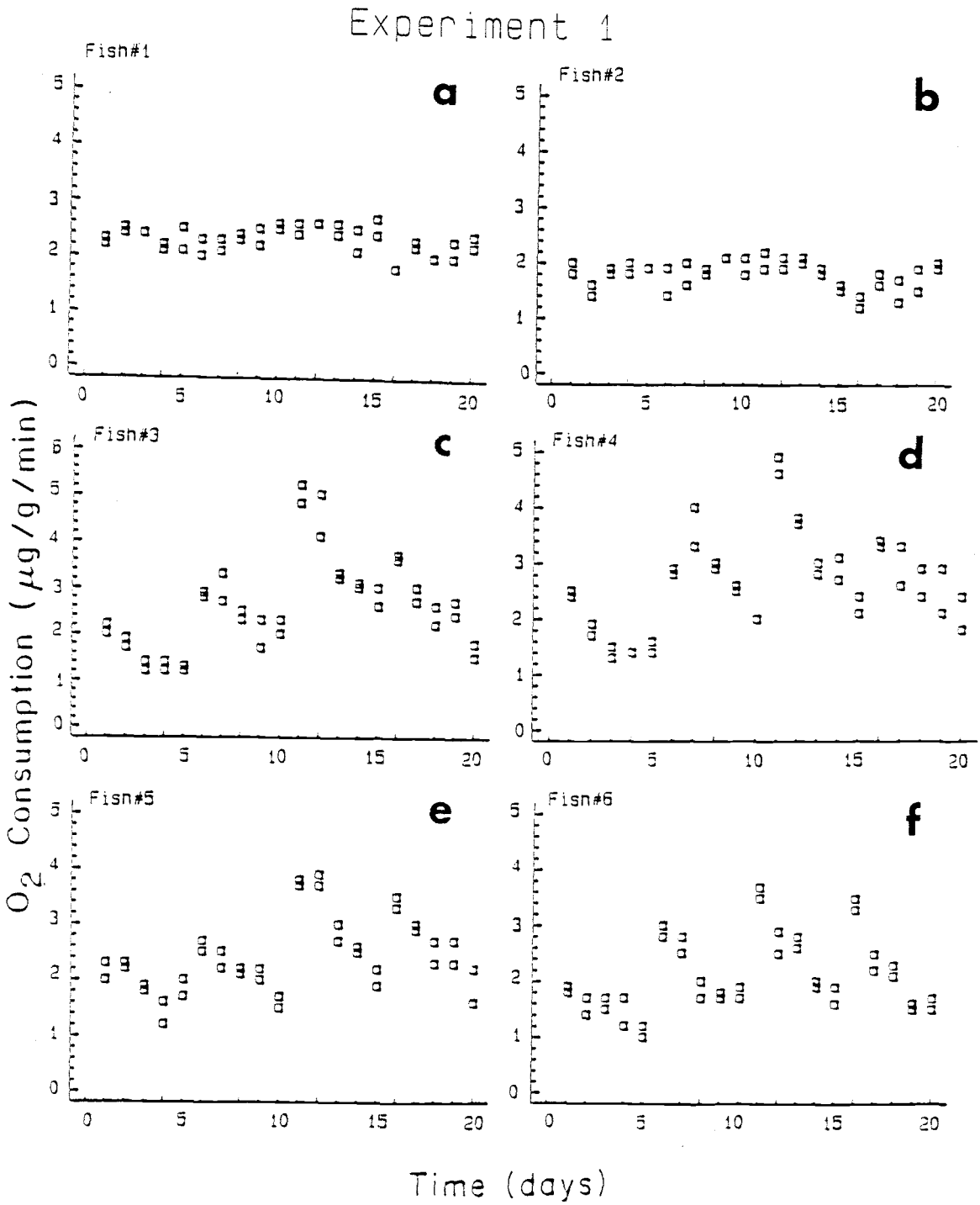
Experiment 1
Fish#1
a
Fish#2
b
Fish#3
c
Fish#4
d
Fish#5
e
Fish#6
f
O_2 Consumption (μg / g / min)
Time (days)

Figure 5

Figure 6. The actual oxygen consumption measurements for 6 fish (O. niloticus) in experiment 2, during acclimation to progressive salinity of 9, 18, 27 and 36 ppt. Each 5 days represent one experimental salinity.

Figures 6a and b. Oxygen consumption of the control fish 1 and 2 which sojourned in freshwater throughout the course of experiment 2.

Figures 6c, d, e and f. Oxygen consumption of the treatment fish 3, 4, 5 and 6 which sojourned for 5 days in each acclimating salinity of 9, 18, 27 and 36 ppt in experiment 2.

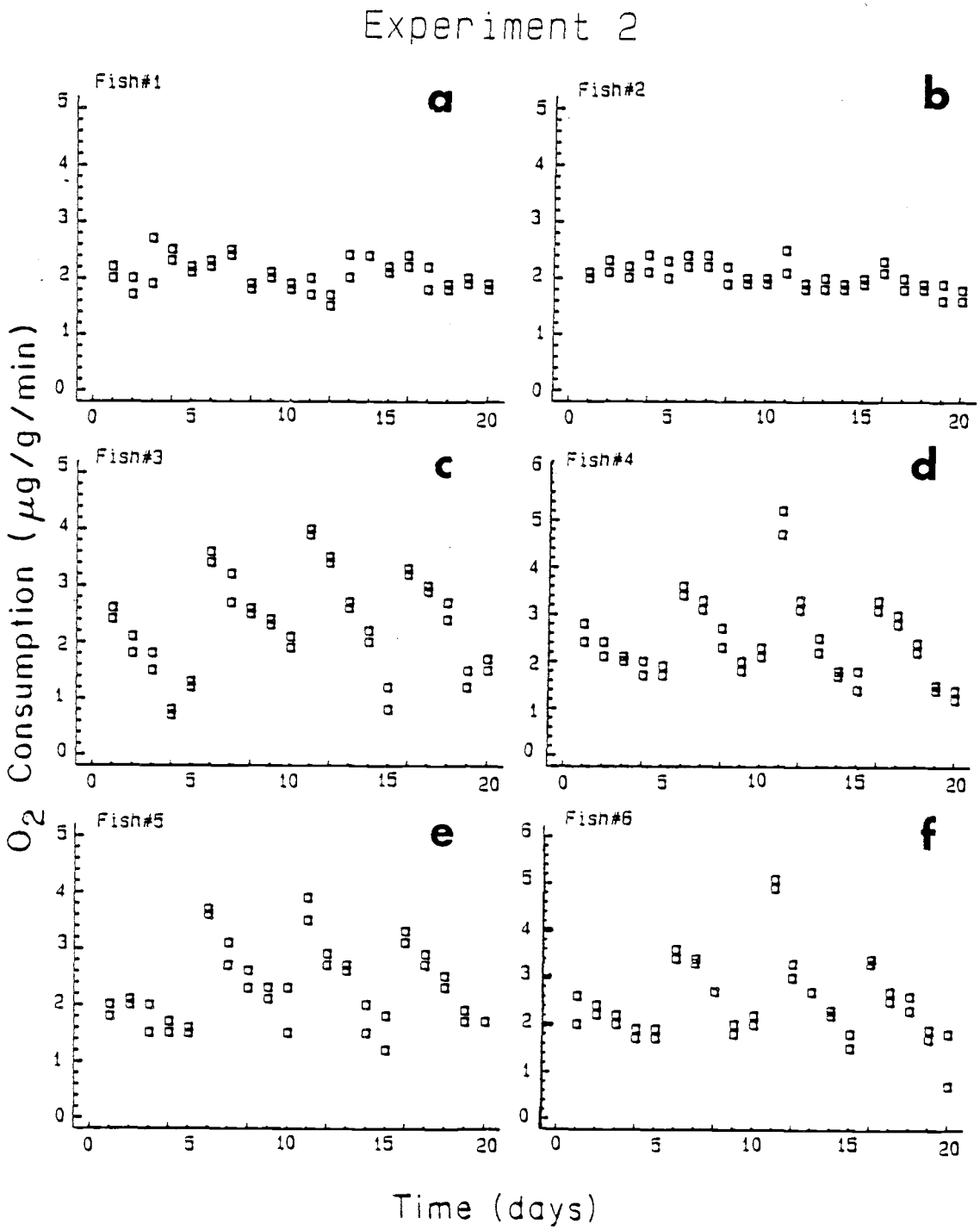
Experiment 2
Fish#1
a
Fish#2
b
Fish#3
c
Fish#4
d
Fish#5
e
Fish#6
f
O_2 Consumption ($\mu g/g/min$)
Time (days)

Figure 6

respirometers. Two trials showed a negligible consumption of 0.05 mg l^{-1}. Thus respirometers alone represented little interference with oxygen consumption determinations for fish.

I. Percentage initial overshoot

After the initial overshoot, average oxygen consumption declined with time at all salinities. A baseline that was equivalent to or lower, in some salinities, than that of FW occurred within 5 days. The initial overshoots in experiment 1 were: 2.14, 2.80, 4.27 and 3.45 ug g^{-1} min^{-1}, while values were 2.32, 3.54, 4.40 and 3.25 ug g^{-1} min^{-1} in experiment 2 (Figures 7 and 8) for salinities 9, 18, 27 and 36 ppt, respectively.

The average oxygen consumption of fish in 9 ppt was not different than that of FW during the first two days (Figures 7a and 8a). Subsequently, it became lower than the average oxygen consumption in FW in both experiments. In salinity 18 ppt, average oxygen consumption of fish in SW was higher than that of FW for the first two days in experiment 1 (Figure 7b), and for the first three days in experiment 2 (Figure 8b). It declined afterwards and was nearly equivalent to that of FW by the fourth and the fifth day. In salinities 27 and 36 ppt, the average oxygen consumption of fish in SW was higher, in both experiments, for the first

Figure 7. The difference in the average oxygen consumption of O. niloticus between seawater-acclimated fish (n=4) and freshwater control fish (n=2) during acclimation to various experimental salinities. Each point represents the mean ± 1 SE of 8 and 4 measurements per day for the treatment and the control, respectively.

Figure 7a, b, c and d. The average oxygen consumption of treatment fish and their freshwater control, during acclimation to salinities 9, 18, 27 and 36 ppt in experiment 1.

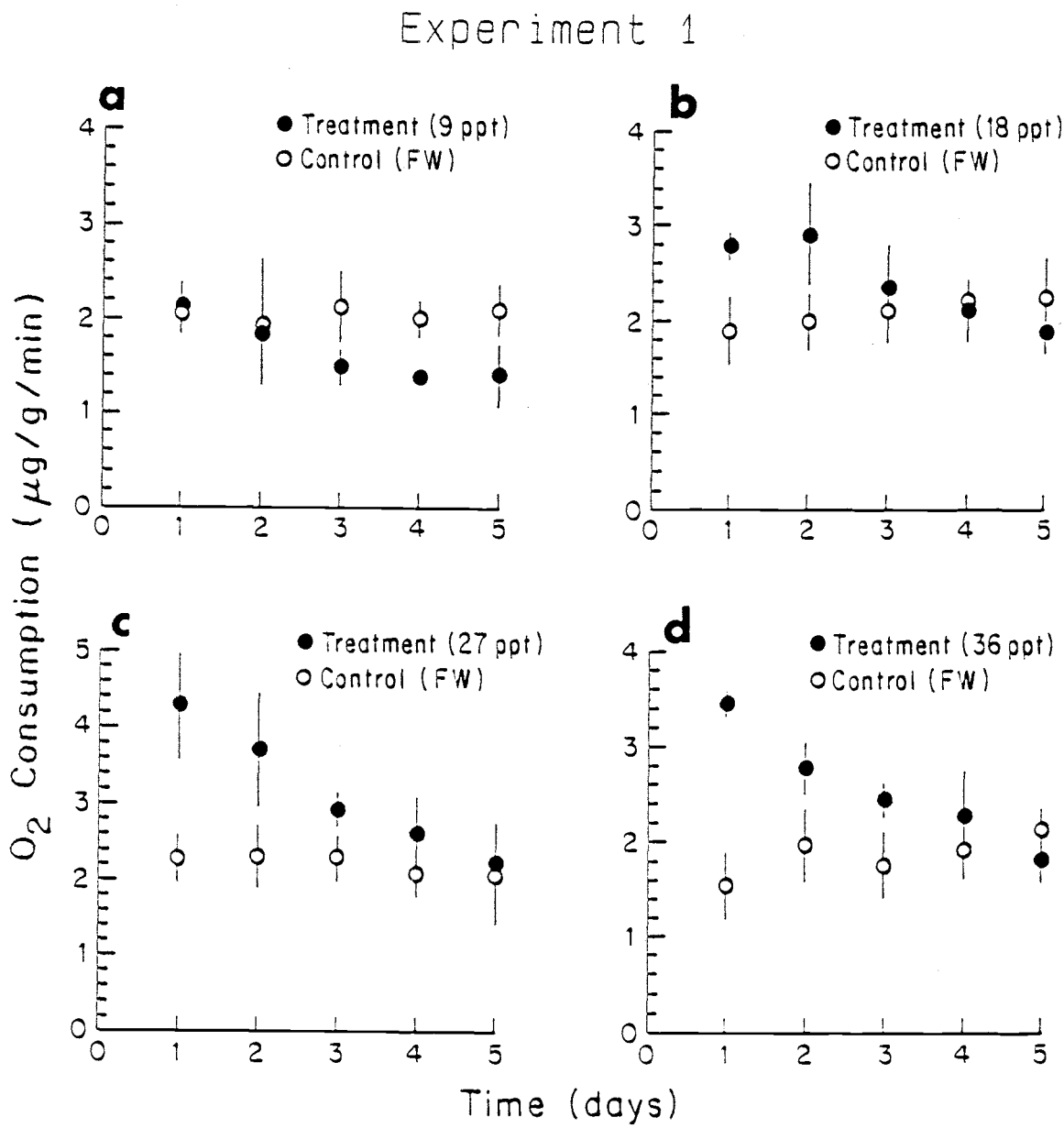
Experiment 1
a
Treatment (9 ppt)
Control (FW)
b
Treatment (18 ppt)
Control (FW)
c
Treatment (27 ppt)
Control (FW)
d
Treatment (36 ppt)
Control (FW)
O2 Consumption (μg/g/min)
Time (days)

Figure 7

Figure 8. The difference in the average oxygen consumption of O. niloticus between seawater-acclimated fish (n=4) and freshwater control fish (n=2) during acclimation to various experimental salinities. Each point represents the mean ± 1 SE of 8 and 4 measurements per day for the treatment and the control, respectively.

Figure 8a, b, c and d. The average oxygen consumption of treatment fish and their freshwater control, during acclimation to salinities 9, 18, 27 and 36 ppt in experiment 2.

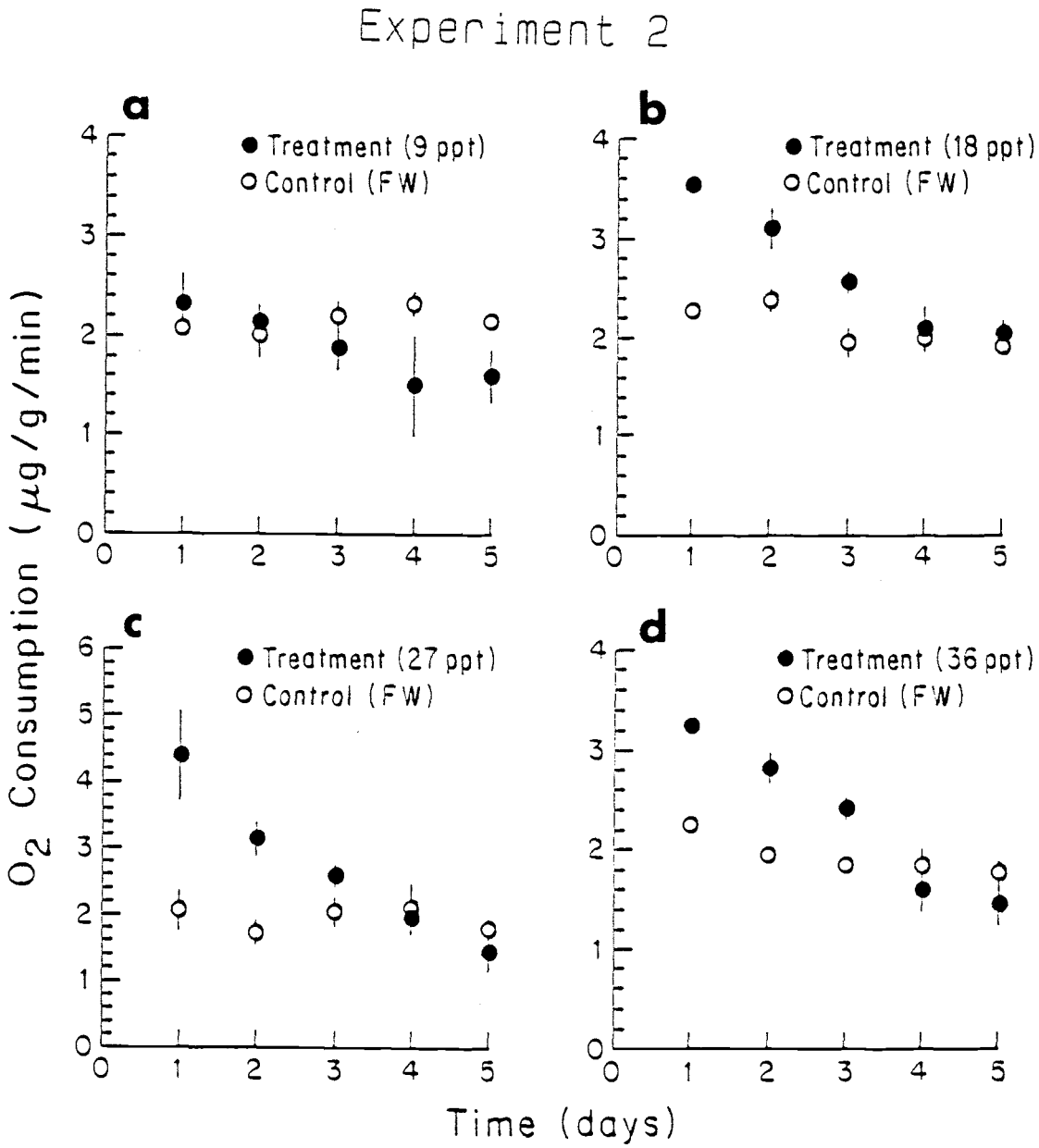
Experiment 2
a
Treatment (9 ppt)
Control (FW)
b
Treatment (18 ppt)
Control (FW)
c
Treatment (27 ppt)
Control (FW)
d
Treatment (36 ppt)
Control (FW)
O2 Consumption (μg/g/min)
Time (days)

Figure 8

three days than that of FW, then it declined to the baseline of FW in the last two days (Figures 7c,d and 8c,d). The magnitude of this initial overshoot in the average oxygen consumption of fish varied with salinity. Each initial overshoot, represented by the average oxygen consumption of the first day of each acclimation period, was converted as a percentage of the average oxygen consumption of fish in FW, which was measured on the same day. This conversion was achieved by the following equation:

$$\% \text{ initial overshoot} = \frac{\text{treatment - control}}{\text{control}} * 100$$

This expression reflected the magnitude of initial overshoot which was directly proportional to the magnitude of osmotic stress in the acclimation medium. Consequently, % initial overshoots in experiment 1 were: 3.4, 47.4, 88 and 122 % , and in experiment 2, percentages were: 12, 56, 112.6 and 44.4 % which occurred at the first day of salinities 9, 18, 27 and 36 ppt, respectively (Figure 9). The % initial overshoot in oxygen consumption at 9 ppt was not different than that of FW in either experiments (Figures 7a, 8a and 9). The remaining % initial overshoots in SW were similar in both experiments, except for 36 ppt in experiment 1 (Figure 7d). The higher value at 36 ppt was unexpected. Although the absolute values of oxygen consumption at salinity 36 ppt for

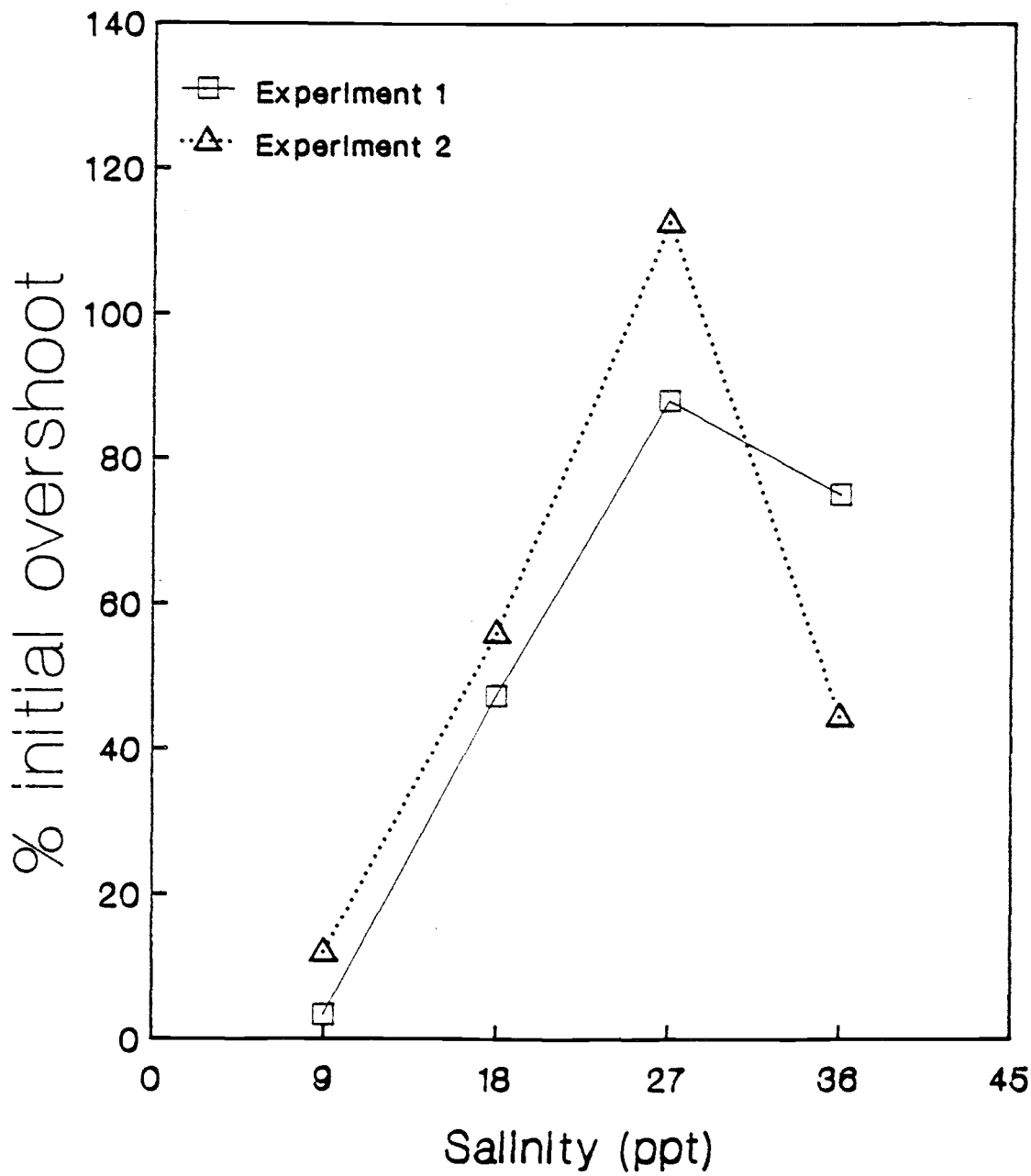

Figure 9. The initial overshoot in oxygen consumption in treatment fish that occurred at the first day of the acclimating salinities 9, 18, 27 and 36 ppt as a response to osmotic stress, was converted as percentages of the oxygen consumption of freshwater control fish, to measure the magnitude of the osmotic stress at each salinity.

both experiments were close (3.45 and 3.25 ug g^{-1} min^{-1}, respectively), the % initial overshoot in experiment 1 (122 %) was much higher than that of experiment 2 (44.4 %). The reason was probably the unusually low oxygen consumption of 1.55 ug g^{-1} min^{-1} for the control FW on that day (figure 7d). This value was significantly below 2.04 ug g^{-1} min^{-1}, the average rate of oxygen consumption in FW, suggesting that the low oxygen consumption for the FW for that day (1.55 ug g^{-1} min^{-1}) resulted from a measurement error. If the value of the second day (1.97 ug g^{-1} min^{-1}), which is around the average, is used as a point of reference instead of the first day, then the % initial overshoot would be 75 % which approximates the expected value (Figure 9).

II. Regression models for the process of acclimation

Regression lines of the rate of oxygen consumption in each acclimation salinity were fitted for individual fish in SW (Figures 10 and 11) and the slopes and intercepts for each fish were calculated (Table 2). The FW fish were not subjected to any salinity treatment, but their regression coefficients slopes were determined during each period of salinity acclimation. This neutralized any effect resulting from moving the fish to and from the respirometers. Consequently, a slope comparison between FW and SW becomes more accurate and meaningful. The slopes of oxygen

Table 2. Regression coefficients of individual fish during acclimation to 9, 18, 27 and 36 ppt in experiment 1 and 2, S= Slope, I= Intercept, FW= control fish, SW= treatment.

	Fish	Salinity							
		9ppt		18ppt		27ppt		36ppt	
		S	I	S	I	S	I	S	I
Experiment (1):	1 (FW)	-0.020	2.37	0.095	1.56	-0.020	2.75	0.090	0.48
	2 (FW)	0.040	1.69	0.090	1.15	-0.115	3.39	0.130	-0.71
	3 (SW)	-0.220	2.21	-0.240	4.40	-0.590	11.40	-0.430	10.36
	4 (SW)	-0.230	2.40	-0.280	5.04	-0.585	10.92	-0.295	8.02
	5 (SW)	-0.145	2.33	-0.225	3.98	-0.465	9.06	-0.345	8.88
	6 (SW)	-0.160	1.99	-0.310	4.67	-0.445	8.32	-0.440	10.14
Experiment (2):	1 (FW)	0.065	1.96	-0.120	3.05	0.090	0.82	-0.095	3.70
	2 (FW)	0.025	2.07	-0.105	2.95	-0.120	3.48	-0.115	3.95
	3 (SW)	-0.370	2.73	-0.360	5.55	-0.725	12.05	-0.490	11.16
	4 (SW)	-0.200	2.71	-0.390	5.78	-0.815	13.36	-0.525	11.68
	5 (SW)	-0.115	2.11	-0.420	5.92	-0.545	9.56	-0.400	9.58
	6 (SW)	-0.150	2.51	-0.425	6.11	-0.760	12.83	-0.500	11.29

Figure 10. Fitted regression lines for the oxygen consumption of treatment fish (SW) O. niloticus during acclimation to progressive salinities of 9, 18, 27 and 36 ppt in experiment 1. Each 5 days represent one experimental salinity.

Figures 10a, b, c and d, correspond to treatment fish SW: 3, 4, 5 and 6, respectively.

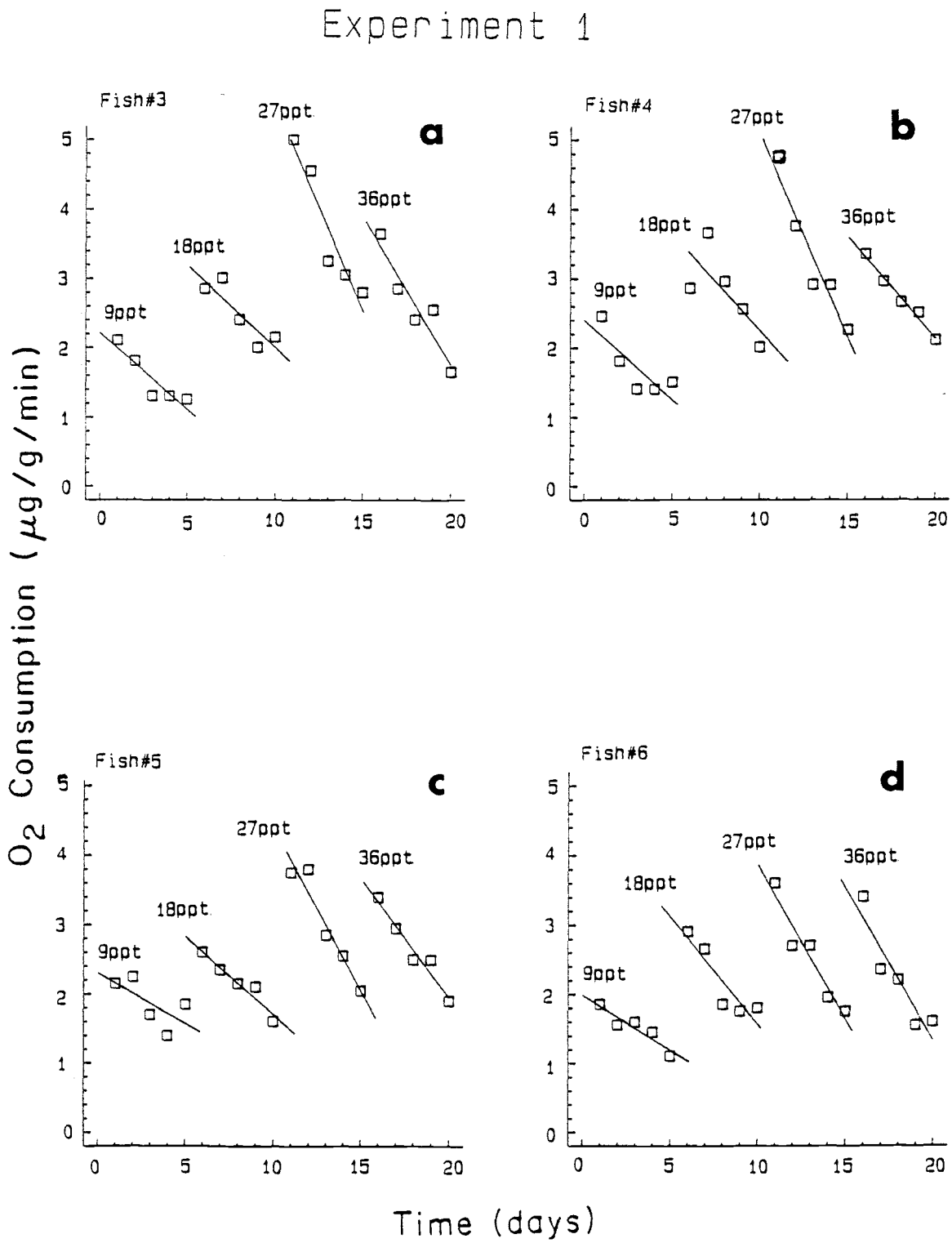
Experiment 1
Fish#3
a
27ppt
36ppt
18ppt
9ppt
Fish#4
b
27ppt
18ppt
36ppt
9ppt
Fish#5
c
27ppt
36ppt
18ppt
9ppt
Fish#6
d
27ppt
18ppt
36ppt
9ppt
O2 Consumption (µg/g/min)
Time (days)

Figure 10

Figure 11. Fitted regression lines for the oxygen consumption of treatment fish (SW) O. niloticus during acclimation to progressive salinities of 9, 18, 27 and 36 ppt in experiment 2. Each 5 days represent one experimental salinity.

Figures 11a, b, c and d, correspond to treatment fish SW: 3, 4, 5 and 6, respectively.

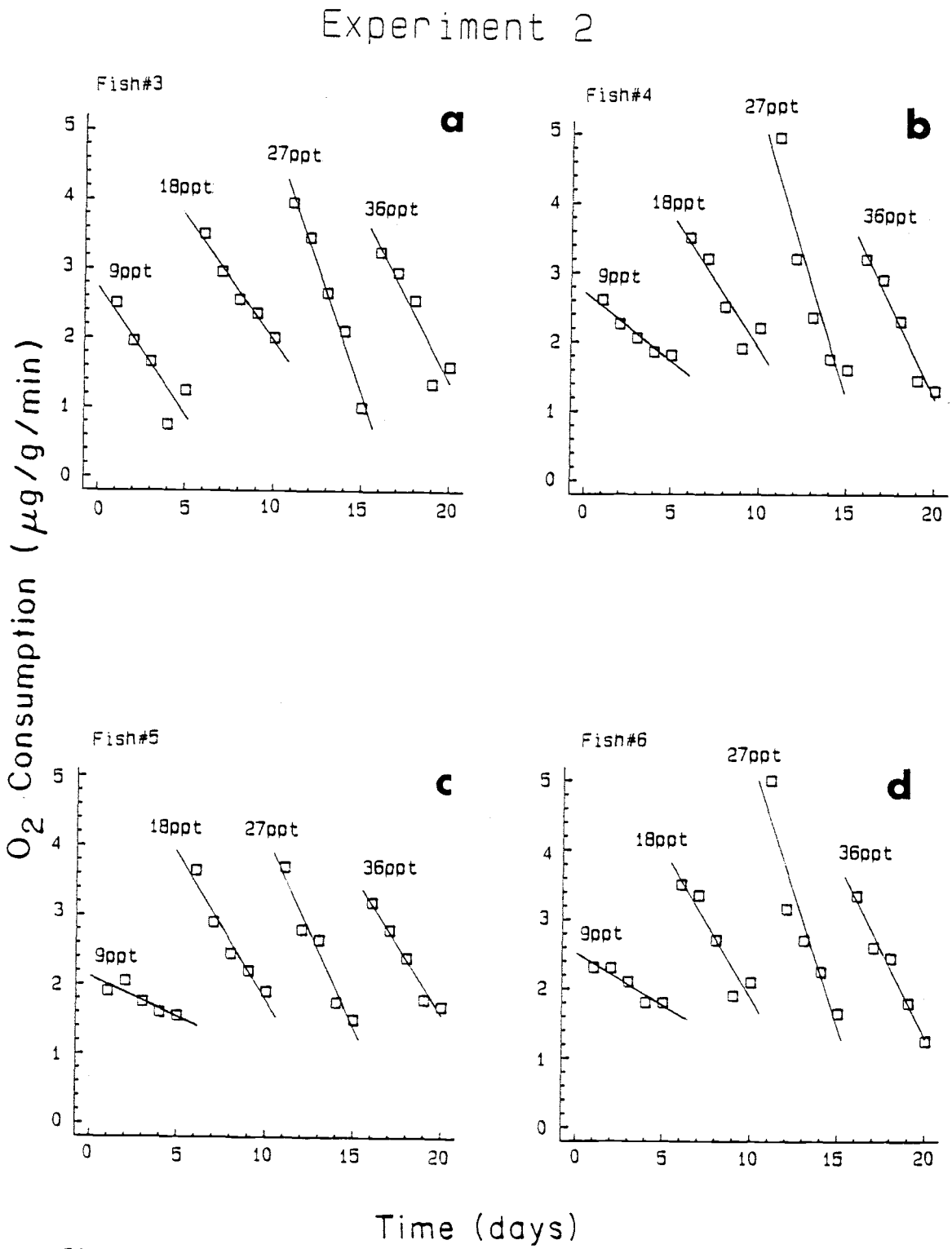
Experiment 2
Fish#3
a
9ppt
18ppt
27ppt
36ppt
Fish#4
b
9ppt
18ppt
27ppt
36ppt
Fish#5
c
9ppt
18ppt
27ppt
36ppt
Fish#6
d
9ppt
18ppt
27ppt
36ppt
O2 Consumption (μg/g/min)
Time (days)

Figure 11

consumption of FW were small, approaching zero over all "acclimation salinity" time periods. Slopes of FW control exhibited no specific pattern. Slopes ranged from -0.12 to 0.13 with an average of -0.0053 $\pm$ 0.09 ($P > 0.05$) regardless of the "acclimation salinity period". Conversely, the slopes of SW manifested a clear trend at each acclimation salinity for all fish, and were similar in both experiments. The least steep slope of the rate of oxygen consumption belonged to SW fish at 9 ppt in both experiments (Figures 10 and 11). It ranged from -0.16 to -0.23 (Table 2) with an average slope of -0.19 for experiment 1. In experiment 2, the slopes of SW fish in 9 ppt ranged from -0.115 to -0.370 with an average slope of -0.21. The steepest slope pertained to SW fish at 27 ppt, ranging from -0.445 to -0.590 (Table 2) with an average slope of -0.52 for SW fish in experiment 1. In experiment 2, slopes ranged from -0.545 to -0.815 with an average slope of -0.71.

III. Statistical differences in oxygen consumption between SW and FW; and among various salinities

To compare slopes of oxygen consumption of fish in each salinity to the slopes of their respective FW controls, the slopes of all SW fish of each acclimation salinity in both experiments were averaged (Figure 12). All average slopes of SW fish in salinities 9, 18, 27 and 36 ppt in both

Table 3. Average slopes of oxygen consumption of fish in experiment 1 and 2 during acclimation to 9, 18, 27 and 36 ppt (each number represents a mean of 4 fish).

	Salinity			
	9ppt	18ppt	27ppt	36ppt
Slopes of Experiment (1)	-0.19	-0.26	-0.52	-0.38
Slopes of Experiment (2)	-0.21	-0.40	-0.71	-0.48

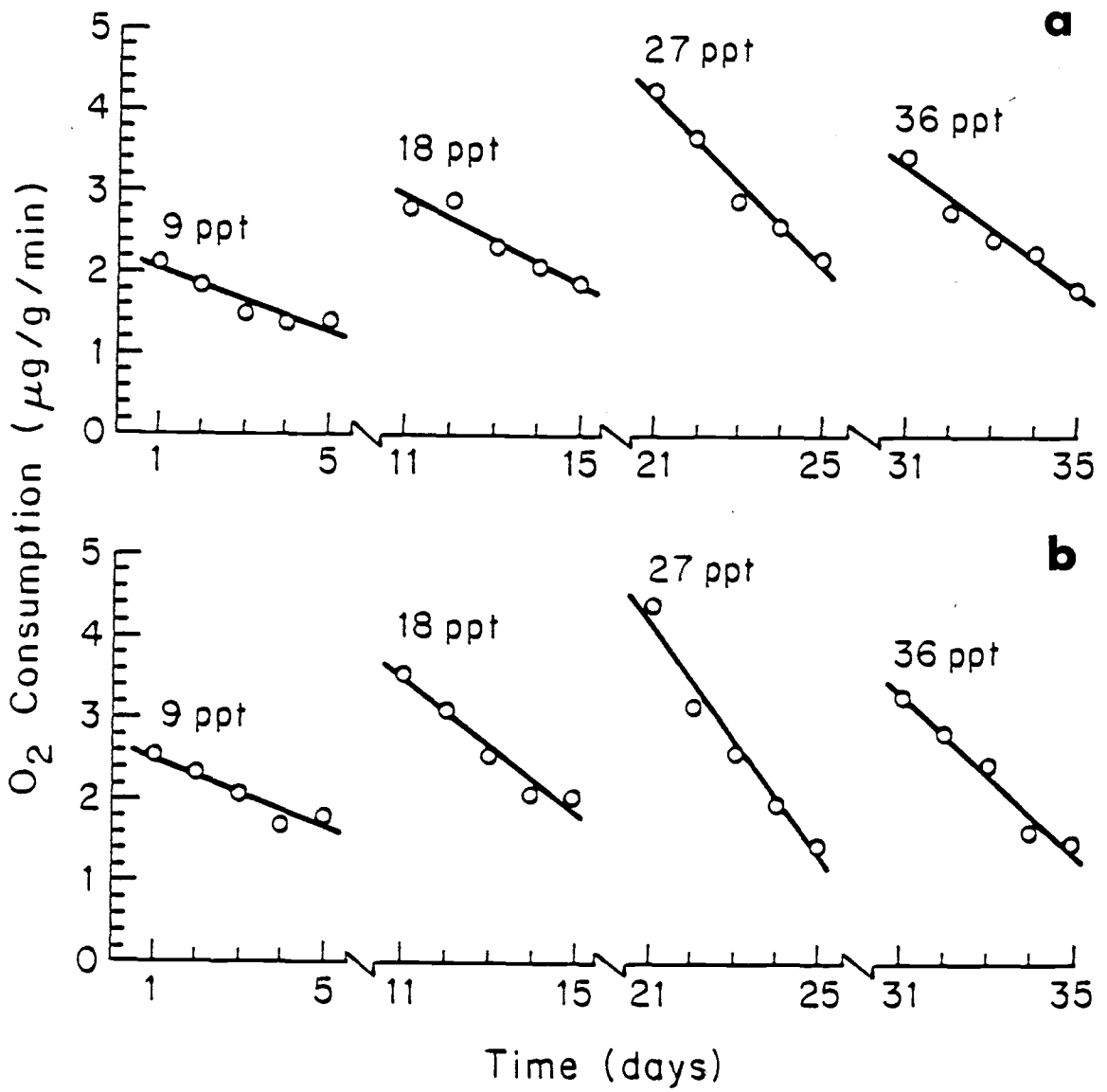

Figure 12. Regression models for oxygen consumption based on the pooled data of all treatment fish SW (n=4) for each experiment, a= Experiment 1 and b= Experiment 2.

experiments were statistically different than the average slope of controls FW ($P < 0.01$). After establishing the significant difference between the average slopes of SW and FW, the average slopes of oxygen consumption in the acclimation media were compared with each other. In experiment 1 the average slope of oxygen consumption of fish at 18 ppt (-0.26) was steeper than that at 9 ppt (-0.19) ($P < 0.05$) (Table 3). The average slope of fish at 36 ppt (-0.38) was steeper than that at 18 ppt ($P < 0.05$), and the steepest average slope of oxygen consumption of all acclimation media pertained to 27 ppt where the average slope (-0.52) was steeper than that of 36 ppt ($P < 0.05$). The same trend was observed in experiment 2, where $P < 0.01$ for 18 vs 9 ppt; $P < 0.05$ for 36 vs 18 ppt and $P < 0.01$ for 27 vs 36 ppt, with average slopes of -0.21, -0.40, -0.71 and -0.48 for the acclimated fish in salinities 9, 18, 27 and 36 ppt, respectively (Table 3).

DISCUSSION

I. Water content

A euryhaline teleost, like O. niloticus, becomes hypotonic when introduced to a saline medium. This osmotic stress would dehydrate fish tissues without rapid physiological adaptation. The experimental results showed no significant differences between the dry weights of FW and SW fish at the end of each experiment (Table 1). Venkatachari (1974) reported that water content of the tissues of O. mossambicus increased slightly with acclimation to progressive salinity, but declined upon reaching 100 % SW. The increase in water content became statistically insignificant at the end of his experiment, which is in agreement with my results. The initial increase in water content is achieved by drinking the medium as a response to the dehydration imposed by the hypertonicity of the medium. The degree of dehydration is dependent on the extent of salinity. Therefore, the problem of osmotic water loss is solved by drinking seawater then absorbing NaCl (monovalent salts) across the esophagus, stomach and anterior intestine to be extruded via the gills (Parry 1966; Hirano and Mayer-Gostan 1976; Kirsch 1978), then water is absorbed in the posterior intestine. Evans (1968) reported a drinking rate of 234 $\pm$ 47 ml kg^{-1} day^{-1} for a 10 - 20 g O. mossambicus

which is equivalent to 1 ± 0.2 % of body weight h^{-1}. Potts et al (1967) reported a slightly higher drinking rate of 1.54 % of body weight h^{-1} for the same species. The drinking rate of the euryhaline Tilapia is significantly higher than that of other species like the eel and sculpin (Smith 1930). The gradual decline in water content occurs as the fish becomes increasingly capable of activating its salt secreting mechanism, hence reducing the salt concentration in its tissues and bringing its osmotic condition to a balance.

The constancy of water content reflected the homeosmotic nature of O. niloticus and exhibited an adaptability to saline media without the dehydration of its tissues.

II. Plasma Na^+ and K^+ concentrations

The ionic concentration of Na^+ in the plasma at the end of experiment 1 was significantly higher in SW treatment 169.02 ± 10.26 mMol l^{-1} than in FW control 145.50 ± 7.35 mMol l^{-1}. This is in agreement with Potts et al (1967) reported for acclimated O. mossambicus. In their work Na^+ concentration in FW was 45.9 uM g^{-1} wet weight of fish, while it was 53.4 uM g^{-1} in fish acclimated 100 % seawater. Assem and Hanke (1979) reported results similar to the ones presented in this study (169.02 ± 10.26 mMol l^{-1}). After two

months of gradual acclimation, they reported 155 ± 3.34 and 121 ± 2.0 mMol l^{-1} of Na^+ concentration in plasma for 35 ppt and FW acclimated fish, respectively. Hwang et al (1989) reported a rapid increase in the O. mossambicus plasma osmolality within one hour upon transferring the fish directly from FW to 30 ppt SW, and the dehydration resulted in total mortality. There was a small increase in the gill Na-K-ATPase activity indicating an insufficient activation of chloride cells to control osmolality (Hwang et al, 1989). Consequently, the increased concentration of Na^+ in the plasma could not be lowered, hence mortality occurred. Conversely, when fish were acclimated to 20 ppt SW, plasma Na^+ increased causing dehydration, but then declined at 24 h post-transfer. Assem and Hanke (1979) reported a similar response for the same species. When fish were directly transferred from FW to 27 ppt, there was a rapid increase in Na^+ concentration peaking after 9 h. Subsequently, Na^+ declined but it was still higher than the control fish. This is in agreement with my results. A similar response occurred in rainbow trout, Salmo gairdnerii, acclimated to 30 ppt (Hegab and Hanke, 1986), and chum salmon fry, Oncorhynchus keta, transferred to 33.5 ppt SW (Iwata et al, 1982). When the anadromous ayu, Plecoglossus altivelis, was directly transferred from FW to 35 ppt SW, Na^+ concentration in the plasma increased rapidly from 144 ± 3.7 to 182 ± 19.5 mM within 3 h resulting in high mortality (Hasegawa et al,

1983). The survivors, exhibited a decrease in Na^+ to 160 - 166 mM. When the ayu were acclimated gradually, there was a slight increase in Na^+ in 50 % SW (17.5 ppt), then a significant increase in 100 % SW (35 ppt). This was followed by a decline in Na^+ concentration to a seawater-acclimated level of 165 mM 3 days post-transfer, similar to the result reported in this investigation (169 mMol l^{-1}). In my study there was no significant difference in the concentration of K^+ between FW control 19.12 $\pm$ 6.06 and SW treatment 23.52 $\pm$ 3.11 mMol l^{-1}. Similarly, Assem and Hanke (1979) reported no significant changes in the concentration of K^+ in the plasma during the course of acclimation of O. mossambicus. Hegab and Hanke (1986) reported no significant fluctuation of K^+ concentration in the plasma when acclimating S. gairdnerii to various hyperosmotic media. For the experimental O. niloticus Na^+ concentration was probably high during the course of acclimation, with K^+ concentration being relatively constant, due to dehydration and osmotic gradient. Subsequently, Na^+ concentration declined upon completion of the acclimation process at the end of the experiment. This decline was a direct consequence of the development of the leaky junctions in the branchial epithelium (Hwang, 1987) and the activation of chloride cells in the gills (Foskett et al, 1981). The gill surface is many times greater than that of the rest of the body (Parry, 1966), and is extensively vascularized, therefore it

is the site where the greater part of the Na^+ efflux occur (Potts et al, 1967).

Gradual acclimation of O. niloticus from FW to 9, 18, 27 and 36 ppt allowed sufficient time to induce an increase in Na-K-ATPase activity resulting in the activation of salt secreting mechanisms. This reduced the Na^+ concentration in the plasma and maintained the homeosmoticity of the fish.

III. Oxygen consumption in FW

Magid and Babiker (1975) showed that O. niloticus tolerated oxygen concentration of 0.1 mg l^{-1} for a few hours in freshwater. Other workers (Ahmed and Magid, 1969) reported that the rate of oxygen consumption of O. niloticus in freshwater became dependent on ambient oxygen concentration only at a critical level of 2.5 - 3 mg l^{-1}. At this level, oxygen consumption of fish declined accordingly. Farmer and Beamish (1969), however, reported a higher critical level of 4.2 mg l^{-1} for the same species during gradual acclimation from FW to 30 ppt SW, regardless of the acclimating salinity.

In the two experiments reported here, the average oxygen consumption of O. niloticus in freshwater was about 2 ug g^{-1} min^{-1}. This rate is in concordance with reports for O. mossambicus (Bashamohideen and Parvatheswararao, 1976a). A rate of 2.2 ug g^{-1} min^{-1} was interpolated from their

published data for a 10 g fish. Farmer and Beamish (1969) estimated a rate of oxygen consumption of 1.7 ug g^{-1} min^{-1} which was similar to the rates of 2 ug g^{-1} min^{-1} I measured for the species.

IV. Isosmotic salinity

Oxygen consumption measured through the stages of the process of acclimation showed some interesting patterns. In general, oxygen consumption increased when the fish was introduced to a new hyperosmotic medium (Figures 7 and 8). The increased oxygen consumption always declined to baseline after a few days, and there were no real differences between SW and FW control after acclimation. Other studies on acclimating O. mossambicus (Bashamohideen Parvatheswararao, 1976a) strongly supported this finding. The stabilization process occurring after the initial "overshoot" is fast and complete, and involves a high degree of adaptive capacity. This osmotic adaptive capacity contributes greatly to the euryhalinity of O. niloticus, and thus its likelihood of survival. The initial increase in oxygen consumption indicated the osmoregulatory burden on the fish. This burden occurred due to the osmotic gradient between the fish system and the ambient environment rendering the fish hypotonic in a saline medium. Oxygen consumption in 9 ppt exhibited no initial increase, but rather a significant decline below the

control baseline in both experiments. Such low rates of oxygen consumption indicated that the salinity of 9 ppt imposed less osmoregulatory stress on the fish than freshwater. A salinity of 9 ppt represented a concentration close to the isosmoticity of the O. niloticus blood, where minimum osmotic regulation was required to maintain a constant salt level in the blood. Farmer and Beamish (1969) reported 11.6 ppt as the salinity isosmotic to the blood of O. niloticus. This is close to the 9 ppt concentration which exhibited lowest oxygen consumption in my investigation. The 11.6 ppt was considered isosmotic, however, by the use of plasma freezing point depression technique, where a straight line relationship can be established between freezing point depression and salinity. Consequently, oxygen consumption was lowest at 11.6 ppt, during swimming exercises, compared to 0, 7.5, 22.5 and 30 ppt (Farmer and Beamish, 1969). In this study, 9 ppt represented the point closest to isosmoticity in O. niloticus where energy expenditure associated with osmoregulation is lowest, and where energy required to maintain homeostasis is minimal.

V. Oxygen consumption in SW

The sudden increase in oxygen consumption was evident in salinities 18, 27 and 36 ppt in both experiments. Two aspects, that were involved in oxygen consumption at higher

salinities, are important. The magnitude of osmotic stress imposed on the fish represented by the % initial overshoot (Figure 9), and the time required for acclimation represented by the slope of regression model for any given salinity (Figures 10 and 11). The % initial overshoot was highest in salinity 27 ppt, and the slope was steepest in both experiments. Although 36 ppt was potentially a more stressful conditions than 27 ppt due to increased osmotic gradient, but the fish initially osmoregulated with less energy at 36 ppt SW than at 27 ppt SW. Other workers reported similar trend for the same species. Farmer and Beamish (1969) reported their results during subjecting the fish to swimming exercises, nevertheless it reflected a similar response. They reported that the highest oxygen uptake during exercise was at 30 ppt which is close to the 27 ppt used here. Similarly, it was found that % initial overshoot in oxygen uptake in O. mossambicus was substantially higher in 24 ppt than in 32.5 ppt (Bashamohideen and Parvatheswararao, 1976a, 1976b) which is in agreement with the results reported in this study. Similar response occurred in O. aureus (Lotan, 1966) where oxygen consumption was highest at an acclimation salinity between 21.6 and 28.8 ppt compared to a lower oxygen consumption at 36 ppt.

The decrease of energy cost of acclimation in 100 % SW (36 ppt), relative to 75 % (27 ppt) is of interest, since

the cost of acclimation should have increased in 100 % seawater. Oxygen consumption in O. niloticus was not governed by salinity gradient per se, but rather it was the magnitude and not the direction of osmotic gradient that was important in the energy requirements for osmoregulation. The work of Bashamohideen and Parvatheswararao (1972) supports this argument. They found that blood glucose was significantly higher in 75 % than in 100 % SW. It was suggested that the increase in blood glucose level was to facilitated acetylcholine synthesis via the cholinesterase in the brain of the fish which is usually associated with increased salinity of the medium. To understand this phenomenon and explain the increased oxygen consumption at 27 ppt, it is necessary to examine the transport mechanisms involved in the excretion of excess NaCl that results from an increase in the hypertonicity of the medium. Chloride cells in O. niloticus are located in the gills and the opercular membrane (Foskett et al, 1981; Foskett and Scheffey, 1982). Foskett and Scheffey (1982) provided the first conclusive evidence that the chloride cell is the site of active chloride secretion and high ionic permeability in the euryhaline teleost O. mossambicus. They used the so-called Vibrating Probe Technique to prove that the opercular epithelium possesses a rich population of chloride cells which display ion transport properties that can be stimulated hormonally and pharmacologically. Consequently,

chloride cells are considered the only significant electrogenic and conductive elements in the tilapia opercular membrane. Chloride cells in seawater-acclimated tilapia are large, granular and mitochondria-rich, but they are small, rudimentary and poorly developed cells in freshwater fish (Foskett et al, 1981). As a euryhaline teleost acclimated to seawater, the chloride cells undergo two stages, proliferation, and hypertrophy and changes in subcellular structures (Foskett et al, 1981). It was found that the tilapia opercular membrane had an increased unidirectional chloride current within 24 h after exposing the fish to seawater, indicating the beginning of activation of the chloride cell machinery. Simultaneously, there was an increase in the number of chloride cells per cm^2 (Figure 13). This parallel increase in cell density continued for 3 days then started to decline. As cell numbers began to decline, the chloride cells started to hypertrophy rapidly, increasing the rate of active transport of chloride (electronegative current I_c). This sequence of events explains the mechanisms requiring energy as estimated by oxygen consumption in this investigation. Activation of the salt secreting mechanism and proliferation of chloride cells probably occurred when the fish were exposed to the 18 ppt SW (Figures 7b and 8b). This resulted in an increased oxygen consumption for 2 to 3 days then declined to the baseline. The decline was relatively slow with slopes -0.26 and -0.40

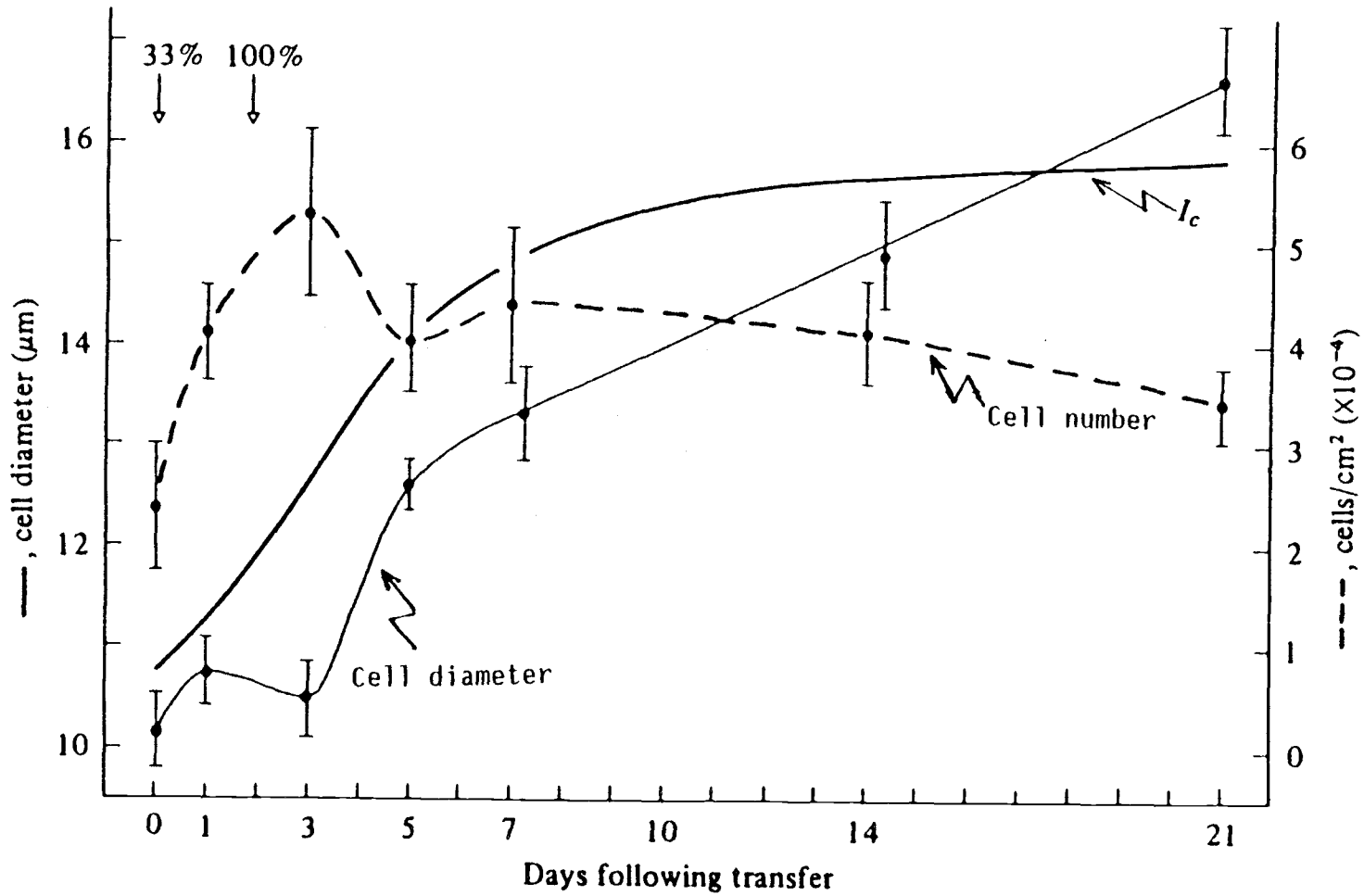

Figure 13. The sequence of events of the development of chloride cells in the opercular membrane of O. mossambicus during acclimation to seawater. I_C = electronegative current produced by active chloride cells. (After Foskett et al, 1981).

for experiments 1 and 2, respectively (Figure 12 and table 3). There was neither activation nor proliferation of the chloride cells in 9 ppt since it is the isosmoticity point. The salinity 18 ppt and freshwater (0 ppt) are osmotically equidistant from the isosmotic salinity (9 ppt). Therefore, the "magnitude" of osmotic stress at a salinity of 18 ppt was not new for the fish. Thus, it is highly unlikely that chloride cell hypertrophy contributed to the decline of oxygen consumption in 18 ppt, but rather could be attributed to the proliferation of chloride cells which preceded the hypertrophy. In contrast, 27 ppt imposed a higher osmotic stress than the chloride cells could adequately control, resulting in an increase in oxygen consumption (Figures 7c and 8c). As the chloride cells increase in size after day 3 (Figure 13) the oxygen consumption decreased to baseline levels. The steep slopes of 27 ppt, -0.52 and -0.71 for experiments 1 and 2, respectively (Figure 12 and table 3) support this conclusion. The cell size is intimately associated with its capacity to extrude chloride (Foskett et al, 1981; Hwang, 1987). Thus, a large scale hypertrophy resulted in a rapid decline in oxygen consumption. Stimulated by a salinity of 27 ppt, the chloride cells continued to hypertrophy, with no further proliferation, for at least three weeks (Foskett et al, 1981). Consequently, when the fish was acclimated to 36 ppt, the chloride cells were in a state of readiness and the increase in oxygen

consumption was less dramatic than in 27 ppt with a much lower % initial overshoot (Figure 9).

During hypertrophy chloride cells form a multicellular complex (Hwang, 1987) enhancing the formation of new leaky junctions and creating additional paracellular routes. These paracellular leaky pathways are the ones that Na^+ follows on the way to the outside medium (Hwang, 1987). It follows that Na-K-ATPase activity is maximum at 27 ppt to pump Na^+ outside the chloride cell (Hwang et al,1989). This metabolic demand requires more oxygen while creating an electro-negative gradient inside the cell necessary to actively transport Cl^- through transcellular route via the apical crypt to the outside environment (Silva et al, 1977).

Gradual acclimation allowed the fish sufficient time to activate its salt secreting mechanism, and endure dehydration associated with acclimation. O. niloticus encountered an "osmotic adaptive threshold" at a salinity of 27 ppt, where energy required for osmoregulation is maximum. Full strength seawater represented a higher osmotic gradient but less osmotic stress than 27 ppt.

During the evolution of fish, osmoregulation appears to have radiated in several directions resulting in a wide spectrum of regulatory mechanisms to solve the osmotic problems encountered in nature (Hickman, 1959). In this evolutionary context, O. niloticus was probably involved in a series of transmigrations between fresh and saline

conditions, forcing the species to adapt to diversified osmotic situations.

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APPENDIX

Table 4. Measurements of individual fish (O. niloticus) used in experiment 1 to measure oxygen consumption during gradual acclimation to increasing salinity. FW= Freshwater control, SW= Seawater treatment.

Fish	Length (cm)	Initial wet weight (g)	Final wet weight (g)	Average wet weight (g)	Gain/Loss (g)	Dry weight (g)	%
*	18.50	--	106.80	=	--	36.40	34.08
*	21.30	--	170.40	=	--	53.30	31.28
*	19.00	--	129.30	=	--	41.70	32.25
*	18.50	--	112.40	=	--	34.50	30.69
1 (FW)	15.30	63.50	63.40	63.50	-0.10	19.30	30.44
2 (FW)	19.40	117.80	122.10	120.00	+4.30	35.10	28.75
3 (SW)	14.40	43.50	47.50	45.50	+4.00	13.90	29.26
4 (SW)	14.80	54.20	52.80	53.50	-1.40	16.80	31.82
5 (SW)	16.00	70.20	66.70	68.50	-3.50	19.40	29.08
6 (SW)	16.20	80.00	73.50	76.70	-6.50	24.00	32.65

* Fish collected from stock tank (not involved in the experiment).

Table 5. Measurements of individual fish (O. niloticus) used in experiment 2 to measure oxygen consumption during gradual acclimation to increasing salinity. FW= Freshwater control, SW= Seawater treatment.

Fish	Initial length (cm)	Final length (cm)	Initial wet weight (g)	Final wet weight (g)	Average wet weight (g)	Gain/Loss (g)	Dry weight (g)	%
1 (FW)	14.7	15.1	62.2	58.9	60.5	-3.3	18.3	31.07
2 (FW)	13.5	14.3	46.4	47.3	46.8	+0.9	13.5	28.54
3 (SW)	14.0	15.3	44.4	52.5	48.4	+8.1	15.3	29.14
4 (SW)	14.2	14.7	53.6	51.1	52.3	-2.5	15.9	31.11
5 (SW)	16.1	16.1	78.8	72.8	75.8	-6.0	21.5	29.53
6 (SW)	14.4	14.9	53.6	54.4	54.0	+0.8	15.8	29.04