



# PD/A CRSP NINETEENTH ANNUAL TECHNICAL REPORT

## STUDIES ON POTENTIAL USE OF SALINITY TO INCREASE GROWTH OF TILAPIA IN AQUACULTURE IN MALAWI

*Ninth Work Plan, Adoption/Diffusion Research 4B (9ADR4B)  
Final Report*

Jeremy S. Likongwe  
Aquaculture and Fisheries Science Department  
Bunda College of Agriculture  
University of Malawi  
Lilongwe, Malawi

### ABSTRACT

In a series of studies conducted in Malawi to determine the effects of different salinity concentrations on survival, growth, feed conversion, reproduction, and whole-body composition of five taxonomic groups of tilapia—*Oreochromis shiranus chilwae* (Lake Chilwa strain), *O. shiranus chilwae* (Bunda College strain), *O. karongae*, *O. shiranus shiranus*, and *Tilapia rendalli*—it was observed that the first three species grew faster in 10‰ salinity and would be recommended as potential candidates for brackishwater aquaculture in Malawi. *T. rendalli* and *O. shiranus shiranus* grew faster in fresh water (0‰ salinity) and are unsuitable for brackishwater aquaculture. With the exception of *O. shiranus chilwae* (Lake Chilwa strain) and *O. shiranus chilwae* (Bunda College strain), all species had lost carcass protein at the end of the study, suggesting that they used tissue protein as an additional energy source for osmoregulation and homeostasis. Salinity tolerance varied ontogenetically in almost all the above taxonomic groups, with younger individuals tolerating salinity longer than larger individuals. This study has also shown that the range of *T. rendalli* and *O. shiranus shiranus* would effectively be limited by salinity. The interactive effect of salinity and water temperature was not investigated in this study since all experiments were conducted at room temperature and ambient photoperiod. Temperature, however, has an influence on salinity tolerance, and in that light, we strongly recommend further investigations on the combined influence of the two abiotic factors (salinity and temperature) since they fluctuate together in nature, and their fluctuations may positively or negatively influence growth and reproductive performance of the above cichlids.

### INTRODUCTION

Use of natural resources, including those of seemingly marginal value, is an important human activity designed to increase food production and income. In agriculture, soils may be too saline to support profitable crop husbandry, yet such soils may be used alternatively for productive aquaculture if a salinity-tolerant fish species is used. The cichlids of the genera *Tilapia* and *Oreochromis*, known to have evolved from the marine environment, are euryhaline, as they have the genes for salinity tolerance and can adapt, grow, and even breed in seawater. Morgan and Iwama (1991) provided a comprehensive classification of fishes based on differences in their metabolic rates in seawater and fresh water, but a similar classification is totally unknown for the cultured tilapias in Malawi. Reports and reviews of tilapia salinity tolerance have been published by several researchers (Fryer and Iles, 1972; Whitfield and Blaber, 1976; Chervinski, 1982; Payne and Collinson, 1983; Wohlfarth and Hulata, 1983; Watanabe et al., 1985, 1993; Zale and Gregory, 1989; Likongwe et al., 1996) among others. With the exception of *Tilapia rendalli*, none of the species reported by the above authors is one of the tilapia species currently cultured in Malawi. The present study was conducted to investigate how salinity tolerance in tilapias may be used to culture these fish in marginal areas of Malawi where the soils may be too saline for productive crop husbandry.

### METHODS AND MATERIALS

The study was conducted in a wet laboratory at Bunda College of Agriculture in central Malawi. Five taxonomic groups of

tilapia were used: *Oreochromis shiranus shiranus*, *O. shiranus chilwae* (from the fish ponds at Bunda College and Lake Chilwa), *O. karongae*, and *T. rendalli*. Approximately 900 fingerlings of *O. shiranus chilwae* were collected from Lake Chilwa and transported to Bunda College on 15 January 2001 in plastic bags under oxygen. On arrival at Bunda College, the fish were introduced into a fiberglass tank containing fresh water (salinity range 0.10 to 0.20‰) in the hatchery. The fish were maintained and acclimated in this tank for a week. The rest of the taxonomic groups above were collected from the fish ponds at Bunda College.

Four salinity concentrations—0, 10, 20, and 30‰—were originally proposed as treatments in the experiments, but the failure of all five taxonomic groups to adapt to 30‰ forced us to drop that treatment. The growth experiments, each lasting approximately seven to eight weeks, were designed to study the effects of salinity on growth, feed utilization, reproduction, and whole-body composition of the fish. The other five short-duration supporting experiments, conducted over variable periods of time depending on species and fish size, were designed to study the effect of salinity stress on survival of the fish after their direct transfer into 30‰ salinity.

All experiments were conducted in plastic tanks in a wet laboratory supplied by well water. In the various experiments that were conducted, nine 100-l, twelve 30-l, twelve 200-l, and twelve 50-l plastic tanks were used. Each tank was adequately aerated using a blower, and fish were cultured at room temperature under ambient photoperiod. The fish were fed twice a day on a pelleted diet containing 30% crude protein.

The fish were fed at 5% body weight per day (BWD). Fishmeal and soybeans were the main sources of dietary protein, while wheat offal and rice bran were the main carbohydrate energy sources in the formulated diet.

During the experimental periods the fish were checked every day for mortalities. Salinity, temperature, and pH were also checked daily. Dissolved oxygen (DO), ammonia, conductivity, and turbidity were checked three times a week (Monday, Wednesday, and Friday). Fish were sampled every two weeks to monitor changes in weight. All species ranged in average weight from 5.5 to 10.0 g. In each experiment only one statistically uniform size of fish was used.

The effect of salinity on whole-body composition of fish (dry matter, crude protein, fat, and ash) was determined by taking initial fish samples before starting the experiments. Final fish samples were taken at the end of the experiments to determine salinity effects on whole-body composition. There was an overlap in the experimental periods for the five experiments, but each had different stocking and sampling dates. In the next series of five supporting experiments, selected individuals of each species were introduced into 30‰ salinity water to determine their response to salinity stress. Specific growth rate (SGR), expressed as percent body weight per day, was calculated from:

$$\text{SGR} = 100 (\ln W_f - \ln W_i) / t$$

where

$W_f$  = final mean weight,

$W_i$  = initial mean weight, and

$t$  = experimental time in days.

Feed conversion efficiency (FCE) was expressed as the ratio of growth (weight gain) to total feed consumed (dry weight). The mean concentration of salinity across treatments ranged from 0.10 to 20.6‰. DO ranged from 7.89 to 9.21 mg l<sup>-1</sup>, water temperature ranged from 22.9 to 27.0°C, and pH ranged from 6.18 to 7.99. Ammonia concentration ranged from 0.70 to 2.60 mg l<sup>-1</sup>, conductivity ranged from 0.292 to 38.40 mS cm<sup>-1</sup>, and turbidity ranged from 2.0 to 48.0 mg l<sup>-1</sup>.

### Experiment 1: Growth and Whole-body Composition of *Oreochromis shiranus chilwae* (Lake Chilwa Strain) Cultured in Four Salinity Concentrations

After a week of acclimation in a fiberglass tank, juveniles of *O. shiranus chilwae* (Lake Chilwa strain) were transferred into twelve 50-l rectangular plastic tanks. Four salinity levels (0, 10, 20, and 30‰) were assigned to these tanks in triplicate. Each tank was filled with well water and was stocked with ten fingerlings (average weight = 8.7 to 9.6 g) at 1.58 fish cm l<sup>-1</sup>. Fish were acclimated to their respective experimental salinity concentrations by adding salt at 2.5‰ d<sup>-1</sup>. Fish were fed twice a day on a diet formulated from fish meal, soybean meal, wheat offal, and rice bran and containing 30% protein. Fish were fed at about 5% BWD. Fish mortality, salinity, and temperature were checked daily, while DO, ammonia, pH, conductivity, and turbidity were monitored three times weekly. Fish started dying within two weeks in the highest (30‰) salinity treatment. Between 20 January and 17 February 2001 (28 d), all fish in 30‰ salinity died, and that treatment was withdrawn from the study. We then continued with three treatments (0, 10, and 20‰ salinity). The effect of salinity on reproduction was evaluated by determining gonadosomal indices (GSI) of the experimental fish.

### Experiment 2: Response of *O. shiranus chilwae* (Lake Chilwa Strain) to Salinity Stress

This experiment was conducted in two parts. In the first part of the experiment, smaller fish (average weight = 2.36 g) were subjected to a sudden transfer into an equivalent of 86‰ seawater (30‰ salinity). In the second part, larger individuals of the same species (average weight = 15.22 g) were also transferred to the same environment (30‰ salinity). The objective of this experiment was to determine differences in the response of *O. shiranus chilwae* to acute stress following direct transfer to 30‰ salinity. Thirty-seven fingerlings (average weight = 2.36 g) were collected from a fiberglass tank in the hatchery where they were maintained. Ten of these individuals were transferred into a 30-l circular tank half-filled with well water containing 30‰ salinity. The experiment was started at 1030 h and was timed. The water and fish in the tank were adequately aerated with a blower while the experiment was in progress. Cessation of opercular movement and the fish's failure to respond to physical touch or gentle prodding were used as the criteria for death. The opercular and jaw movements in the fish were therefore closely monitored. Dead individuals were instantly removed from the tank. The experiment was conducted under ambient photoperiod. The second part of this experiment started at 1045 h when ten individuals of the above species (average weight = 15.22 g) were transferred into another 30-l tank half-filled with well water containing 30‰ salinity.

### Experiment 3: Growth and Whole-body Composition of *O. shiranus chilwae* (Bunda College Strain) Cultured under Laboratory Tank Conditions in Three Salinity Concentrations.

The cichlid *O. shiranus chilwae* (Bunda College strain) was collected locally from a breeding pond at Bunda College of Agriculture. After a week of acclimation to their respective experimental salinity concentrations in tanks, the fish were stocked in nine 100-l plastic tanks in a wet laboratory. Three salinity levels (0, 10, and 20‰) were assigned to the tanks in triplicate. The highest salinity concentration (30‰) was not included in the experiment due to a shortage of experimental tanks. Fish were acclimated to their respective salinity concentrations by adding salt at 2.5‰ d<sup>-1</sup> as in the first experiment. Each tank was stocked with ten fingerlings (average weight = 5.42 to 5.60 g) at 0.66 fish cm l<sup>-1</sup>. Fish were fed a formulated diet containing 30% protein, two times a day at 5% body weight. Fish mortality, salinity, pH, and temperature were checked every day, while DO, ammonia, conductivity, and turbidity were monitored three times weekly.

### Experiment 4: Response of *O. shiranus chilwae* (Bunda College Strain) to Salinity Stress

At 1535 h, ten juveniles of *O. shiranus chilwae* (Bunda College strain) (average weight = 6.00 g) were placed in a 50-l plastic tank half-filled with well water of 30‰ salinity. The experiment was timed and the tank was aerated. The pH and the DO concentration of the water were both normal (refer to range above). In the second part of this experiment, we used slightly larger fish (average weight = 10.0 g). At 1515 h, ten of these individuals were again placed in a 50-l plastic tank containing well water at 30‰ salinity, and the experiment was again timed.

### Experiment 5: Growth and Whole-body Composition of *Tilapia rendalli* Reared under Laboratory Tank Conditions at Two Salinity Concentrations

*Tilapia rendalli* fingerlings (average weight = 6.9 to 9.0 g) were collected from a breeding pond at Bunda College and introduced into nine 200-l circular plastic tanks filled with well water. Each tank was stocked with 14 fingerlings at 0.53 fish cm<sup>-1</sup>. Initially, three treatments (0, 10, and 20‰ salinity) were assigned in triplicate (the highest salinity concentration (30‰) proposed earlier in this study was not included). Fish succumbed to 20‰ very early (within two to three weeks), and this treatment had to be withdrawn from the study. The treatment of 20‰ salinity may not have been necessary since *T. rendalli* has been reported to tolerate a maximum salinity level of 19‰. We therefore continued the study using 0 and 10‰ salinity as treatments. The general maintenance of the experimental animals (in relation to water quality, feeding, and sampling) was the same as that outlined above for other experiments.

### Experiment 6: Response of *T. rendalli* to Salinity Stress

In this experiment only juveniles of one size were used. It was not possible to get another size for this study. Ten juveniles of *T. rendalli* (average weight = 11.4 g) were introduced into a 50-l plastic tank containing well water at 30‰ and pH 7.46. The tank was adequately aerated. The experiment started at 1510 h and was also timed.

### Experiment 7: Growth and Whole-body Composition of *O. karongae* Cultured under Laboratory Tank Conditions in Two Salinity Concentrations

Fingerlings of *O. karongae* (average weight = 6.9 to 9.0 g) were collected from a breeding pond at Bunda College and introduced into nine 200-l circular plastic tanks filled with well water. For this experiment each tank was stocked with 14 fingerlings at 0.53 fish cm<sup>-1</sup>. Initially, three treatments (0, 10, and 20‰ salinity) were assigned, but lack of adequate tanks forced us to investigate two salinity levels, 0 and 10‰. Salinity was increased in the experimental tanks by adding salt at 2.5‰ d<sup>-1</sup>. The general maintenance of the experimental animals (in relation to water quality, feeding, and sampling) was carried out as outlined in the above experiments.

### Experiment 8: Response of *O. karongae* to Salinity Stress

The method used was similar to that used for the other species above. The tank was stocked using *O. karongae* (average weight = 6.89 g). The experiment was started at 1540 h and was timed. The experiment was carried out using one size range of fish, as larger sizes were not available for the experiment.

### Experiment 9: Growth and Whole-body Composition of *O. shiranus shiranus* Cultured under Laboratory Tank Conditions in Three Salinity Concentrations

Juvenile *O. shiranus shiranus* (average weight = 7.03 to 7.09 g) were collected from a nursery pond at Bunda College of Agriculture and placed in a concrete tank for five days acclimation. Twelve 30-l plastic tanks were cleaned and set up in the wet laboratory. Three treatments (0, 10, and 20‰

salinity) were assigned to the plastic tanks in triplicate. Each tank contained aerated well water. The fish were collected from the concrete tank and transferred into the experimental tanks at a stocking density of 8 fish tank<sup>-1</sup> (1.88 fish cm<sup>-1</sup>). Salinity was increased in the experimental tanks by adding salt at 2.5‰ d<sup>-1</sup>. The fish were fed on 30% protein diet, twice a day at 5% body weight. Fish were sampled every two weeks to monitor changes in weight. Fish mortality, salinity, pH, and temperature were monitored every day, while DO, ammonia, conductivity, and turbidity were checked three times a week.

### Experiment 10: Response of *O. shiranus shiranus* to Salinity Stress

At 1428 h, ten juveniles (average weight = 6.52 g) and ten others (average weight = 11.0 g) were simultaneously introduced into two separate 30-l plastic tanks containing water at 30‰ salinity.

## RESULTS

### Experiment 1: Growth and Whole-body Composition of *O. shiranus chilwae* (Lake Chilwa Strain)

#### Survival

Survival was highest in both the 0 and 10‰ salinity treatments; it was lowest (53.33%) in the highest-salinity treatment (20‰).

#### Growth

Growth of *O. shiranus chilwae* (Lake Chilwa strain) differed significantly ( $P < 0.05$ ) among treatments (Table 1). Final mean weight was highest in 10‰ salinity water, where the fish gained weight by 15.10%. Growth was negative in fresh water (0‰), where fish lost about 4.20% of their initial weight. There was no significant difference ( $P > 0.05$ ) in the final mean weights of fish in 10 and 20‰. Similarly, there was no significant difference in the final mean weights of fish in 0 and 20‰ salinity. In 20‰ water, fish gained weight by 7.91% (Table 1).

#### Whole-body Composition

Dry matter increased in proportion to an increase in salinity. In fresh water, where fish lost weight, there was a 13.80% decrease in whole-body protein (Table 2). In 10‰ salinity, where growth was highest, there was a 21.8% decrease in whole-body fat. Whole-body ash decreased in all treatments, with a maximum drop of 20.40% from the initial value in the 20‰ treatment.

Table 1. Growth of *O. shiranus chilwae* (Lake Chilwa strain) cultured under laboratory tank conditions in three salinity concentrations.

Salinity (‰)	Initial Weight ± SD (g)	Final Weight ± SD (g)	Specific Growth Rate (%)	Survival (%)
0	8.34 ± 1.63 <sup>a</sup>	7.99 ± 2.78 <sup>b</sup>	---	96.67
10	8.41 ± 1.52 <sup>a</sup>	9.68 ± 1.85 <sup>a</sup>	0.254	96.67
20	8.34 ± 1.42 <sup>a</sup>	9.00 ± 1.78 <sup>ab</sup>	0.140	53.33

<sup>a,b</sup> Means in a column followed by the same letter are not significantly ( $P > 0.05$ ) different (Duncan's multiple range test).

### Reproduction

In all treatment groups, the gonads (ovaries and testes) were too small to give accurate GSI values. In addition, visual examination of the gonads did not show any differences in gonadal development among treatments.

### Experiment 2: Response of *O. shiranus chilwae* (Lake Chilwa Strain) to Salinity Stress

#### Survival of Younger Fish

On being introduced into the tank, almost all the fish floated. At 1245 h all fish stopped breathing and were pronounced dead. It took 2 h 15 min for them to succumb to the salinity challenge.

#### Survival of Older Fish

On being introduced into 30‰ salinity, the fish started swimming vigorously, and they did not float. At 1228 h (103 min after introduction), the fish were still alive. At 1232 h they died after exactly 1 h 47 min.

### Experiment 3: Growth and Whole-body Composition of *O. shiranus chilwae* (Bunda College Strain)

#### Survival

Survival was inversely proportional to salinity. Survival was highest (93.33%) in fresh water, followed by 90% in 10‰, and finally 66.67% in 20‰ water (Table 3).

#### Growth

Growth did not differ significantly ( $P > 0.05$ ) across treatments (Table 3). Specific growth rate was highest (0.81%) in 10‰ and lowest (0.71%) in 0‰ water.

#### Whole-body Composition

Whole-body dry matter increased in proportion to an increase in salinity. Final values for all treatments were higher than the initial values. The increase ranged from 6.25% (in 0‰) to 6.97% (in 20‰). Whole-body protein also increased in proportion to an increase in salinity. The increase ranged from 11.90% (in 0‰) to 22.89% (in 20‰). All treatments showed far lower final whole-body fat levels than the initial values. Whole-body fat and experimental salinity were inversely proportional. Whole-body fat decreased most (by 12.73%) in 20‰ water (Table 4).

Table 2. Effect of salinity on whole-body composition of *O. shiranus chilwae* (Lake Chilwa strain).

Treatment	Dry Matter (%)	Crude Protein (%)	Fat (%)	Ash (%)
Initial	91.20	63.47	19.18	18.35
0‰	85.80 (-5.4)	54.70 (-8.70)	17.00 (-2.18)	15.80 (-2.55)
10‰	89.20 (-2.0)	63.47 (0)	15.00 (-4.18)	15.80 (-2.55)
20‰	93.40 (2.20)	63.47 (0)	27.07 (7.89)	14.60 (-3.75)

Note: Numbers within parentheses are differences between initial and final values of each observed parameter.

### Experiment 4: Response of *O. shiranus chilwae* (Bunda College Strain) to Salinity Stress

#### Survival of Younger Fish

On direct transfer to the 30‰ salinity, fish maintained their normal swimming postures. At 1600 h (25 min later) the fish still responded to touch by swimming away from the point of stimulus. A little while later one fish floated on the surface but resumed swimming. At 1620 h (45 min after introduction) five individuals died. At 1631 h (56 min after introduction) only one individual was swimming in the tank while showing clear signs of salinity stress. At 1642 h (67 minutes after introduction) the last surviving fish was still able to use its fins for locomotion. The last fish died at 1650 h, after exactly 1 h 15 min.

#### Survival of Older Fish

On their direct transfer to this medium, the fish swam in their normal positions and did not float on the surface as was the case with the younger individuals. At 1600 h none of the fish floated on the surface. They were able to respond to touch. At 1607 h one fish was seen migrating to the bottom of the tank. At 1609 h (54 min after introduction) one fish died, and it was removed from the water. By 1620 h (65 min after introduction) all fish died. When they were withdrawn from the water, only two of them made some reflex movements, but they had already stopped breathing. This group died after exactly 1 h 5 min, showing a slight difference of 10 min sooner than the younger individuals.

Table 3. Growth of *O. shiranus chilwae* (Bunda College strain) cultured under laboratory tank conditions in three salinity concentrations.

Salinity (%)	Initial Weight ± SD (g)	Final Weight ± SD (g)	Specific Growth Rate (%)	Survival (%)
0	5.596 ± 0.447 <sup>a</sup>	8.833 ± 1.297 <sup>a</sup>	0.71 <sup>a</sup>	93.33
10	5.479 ± 0.800 <sup>a</sup>	8.639 ± 1.482 <sup>a</sup>	0.81 <sup>a</sup>	90.00
20	5.418 ± 0.685 <sup>a</sup>	8.285 ± 1.779 <sup>a</sup>	0.76 <sup>a</sup>	66.67

<sup>a</sup> Means in a column followed by the same letter are not significantly ( $P > 0.05$ ) different (Duncan's multiple range test).

Table 4. Effect of salinity on whole-body composition of *O. shiranus chilwae* (Bunda College strain) reared under laboratory tank conditions in three salinity concentrations.

Treatment	Dry Matter (%)	Crude Protein (%)	Fat (%)	Ash (%)
Initial	83.20	50.09	24.47	21.20
0‰	88.40 (5.20)	55.80 (5.71)	16.00 (-8.47)	15.50 (-5.70)
10‰	88.60 (5.40)	61.28 (11.19)	16.00 (-8.47)	16.40 (-4.80)
20‰	89.00 (5.80)	62.37 (11.47)	15.00 (-9.47)	18.50 (-2.70)

Note: Numbers within parentheses are differences between initial and final values of each observed parameter.

### Experiment 5: Growth and Whole-body Composition of *T. rendalli*

#### Survival

Survival was higher (97.60%) in fresh water than in 10‰ salinity (80.90%) (Table 5).

#### Growth

Growth differed significantly ( $P < 0.05$ ) between the two treatment groups in 0 and 10‰ salinity. Growth was significantly ( $P < 0.05$ ) higher (by 26.3%) in fresh water (0‰) than in 10‰ water (Table 5).

#### Whole-body Composition

There was a decrease in tissue dry matter, crude protein, and ash in both treatments at the end of the experiment. In fresh water (0‰), fish had a lower fat content than those raised in 10‰ salinity. The initial fat content was very low in the fingerlings at the time of stocking, but values increased significantly ( $P < 0.05$ ) by 354.15 to 395.10%. Ash was lower in 0‰, whereas growth was higher than in 10‰ (Table 6).

### Experiment 6: Response of *T. rendalli* to Salinity Stress

#### Survival of Fish (One Size Group)

In this trial, fish of only one size (11.40 g) were used. On direct transfer to the tank, all fish floated, while some of them made jerking movements. At 1530 h (20 min after introduction) most of the fish failed to maintain their normal posture. At 1545 h (35 min after introduction) two fish died. At 1548 h (38 min after introduction) two more fish succumbed to the salt concentration. The remaining fish continued making jerking movements. At 1558 h (48 min after introduction) three more fish died. The remaining fish stopped breathing but continued

Table 5. Growth of *T. rendalli* cultured under laboratory tank conditions in two salinity concentrations.

Salinity (‰)	Initial Weight (g)	Final Weight (g)	Specific Growth Rate (%)	Survival (%)
0	8.12 <sup>a</sup>	11.05 <sup>a</sup>	0.56	97.6
10	8.02 <sup>a</sup>	8.60 <sup>b</sup>	0.127	80.9

<sup>a,b</sup> Means in a column followed by the same letter are not significantly ( $P > 0.05$ ) different (Duncan's multiple range test).

Table 6. Effect of salinity on whole-body composition of *T. rendalli* reared under laboratory tank condition in two salinity concentrations.

Treatment	Dry Matter (%)	Crude Protein (%)	Fat (%)	Ash (%)
Initial	91.00	56.90	5.30	29.40
0‰	88.10 (-2.90)	52.52 (-4.38)	24.07 (18.77)	13.13 (-16.27)
10‰	87.00 (-4.0)	50.34 (-6.56)	26.24 (20.94)	15.27 (-14.13)

Note: Numbers within parentheses are differences between initial and final values of each observed parameter.

showing reflex action in their muscles. At 1600 h the remaining fish died. This group took 50 min to succumb to 30‰ salinity.

### Experiment 7: Growth and Whole-body Composition of *O. karongae*

#### Survival

Survival was much lower in this species than in the other taxonomic groups. Survival was about 71% in each of the two treatments (Table 7).

#### Growth

Growth differed significantly ( $P < 0.05$ ) between the two treatment groups in 0‰ and 10‰ salinity. Growth was significantly ( $P < 0.05$ ) higher (by 26.3%) in 10‰ salinity than in fresh water (0‰) (Table 7).

#### Whole-body Composition

Salinity changed the dry matter values of *O. karongae*. There was a slight increase in dry matter in fresh water, while in 10‰ salinity there was a reduction in dry matter. Whole-body protein decreased in both treatments, with more protein lost in 10‰ water. Fish lost about 7.9% of their carcass protein in 10‰ water compared with 3.1% loss of protein in 0‰ water. Carcass fat increased significantly by 88.07 and 96.88% in 0 and 10‰ salinity, respectively. There was an inverse relationship between carcass ash and salinity. As salinity increased, ash levels decreased. Ash decreased by 39.15% in 0‰ and 44.60% in 10‰ (Table 8).

### Experiment 8: Response of *O. karongae* to Salinity Stress

#### Survival (One Size Group)

On being introduced into the tank, the fish swam in all directions initially, and 8 min later (1548 h) most of them were

Table 7. Growth of *O. karongae* cultured under laboratory tank conditions in two salinity concentrations.

Salinity (‰)	Initial Weight ± SD (g)	Final Weight ± SD (g)	Specific Growth Rate (%)	Survival (%)
0	5.58 ± 1.02	6.58 ± 1.09	0.308 <sup>a</sup>	71.42
10	5.27 ± 1.09	6.86 ± 1.19	0.508 <sup>b</sup>	71.42

<sup>a,b</sup> Means in a column followed by the same letter are not significantly ( $P > 0.05$ ) different (Duncan's multiple range test).

Table 8. Changes in the whole-body composition of *O. karongae* cultured under laboratory tank conditions in two salinity concentrations.

Treatment	Dry Matter (%)	Crude Protein (%)	Fat (%)	Ash (%)
Initial	90.00	61.28	10.90	21.20
0‰	90.20 (0.20)	59.38 (-1.90)	20.50 (9.60)	12.84 (-8.30)
10‰	89.04 (-0.40)	56.44 (-4.84)	21.46 (10.56)	11.74 (-9.46)

Note: Numbers within parentheses are differences between initial and final values of each observed parameter.

swimming near the bottom of the tank. They moved to the surface at about 1610 h (30 min after introduction). At 1625 h (45 min after introduction) two fish died, settled at the bottom, and were instantly removed. At 1629 h (49 min after introduction) most fish started swimming horizontally with their heads oriented slightly upwards. At 1700 h (80 min after introduction) five more fish died. At 1705 h, another fish died. At 1710 h (90 min after introduction) one more fish died. The remaining fish died by 1722 h (1 h 42 min after introduction).

### Experiment 9: Growth and Whole-body Composition of *O. shiranus shiranus*

#### Survival

Survival ranged from 83.33% in both 0 and 10‰ treatment groups to 87.50% in 20‰ salinity (Table 9).

#### Growth

Growth was significantly ( $P < 0.05$ ) influenced by salinity. Specific growth rate was highest ( $P < 0.05$ ) in fresh water (0‰ salinity), followed by growth rate in 10‰, but differences in weight in the two treatments did not differ significantly ( $P > 0.05$ ). Specific growth rate was lowest ( $P < 0.05$ ) in the highest salinity (20‰) treatment where growth was significantly different ( $P < 0.05$ ) from the growth rate in fresh water (0‰) (Table 9).

#### Whole-body Composition

There was a decrease in dry matter in all treatment groups at the end of the experiment. Similarly, there was a decrease in both carcass protein and ash, but there was a slight increase in carcass fat in the 0 and 10‰ salinity treatments (Table 10).

### Experiment 10: Response of *O. shiranus shiranus* to Salinity Stress

#### Survival of Younger Fish

On transfer into the tank, juvenile *O. shiranus shiranus* swam in different directions, but they did not float. One individual swam up to the surface and started gasping for air. At 1508 h (40 min after introduction) the fish had not yet started dying. At 1517 h (49 min after introduction) six fish died and settled at the bottom. A minute later another fish also died. The remaining fish died by 1730 h (after 182 min).

#### Survival of Older Fish

The first fish died after 82 min from the time they were introduced into 30‰ salinity water. The last fish died after 135 min.

Table 9. Growth of *O. shiranus shiranus* cultured under laboratory tank conditions in three salinity concentrations.

Salinity (%)	Initial Weight ± SD (g)	Final Weight ± SD (g)	Specific Growth Rate (%)	Survival (%)
0	7.03 ± 0.48 <sup>a</sup>	8.92 ± 1.58 <sup>a</sup>	0.444 <sup>a</sup>	83.33
10	7.07 ± 0.52 <sup>a</sup>	8.14 ± 1.46 <sup>ab</sup>	0.272 <sup>ab</sup>	83.33
20	7.09 ± 0.69 <sup>a</sup>	7.46 ± 1.37 <sup>b</sup>	0.094 <sup>b</sup>	87.50

<sup>a, b</sup> Means in a column followed by the same letter are not significantly ( $P > 0.05$ ) different (Duncan's multiple range test).

## DISCUSSION

### Experiment 1: Growth and Whole-body Composition of *O. shiranus chilwae* (Lake Chilwa Strain)

*O. shiranus chilwae* (Lake Chilwa strain) required adequate time to convincingly demonstrate the effects of salinity on growth. Long-term exposure to fresh water (0‰ salinity) did not seem to result in their positive adaptation to that environment. Growth of fish in 10 and 20‰ salinity did not differ significantly ( $P > 0.05$ ). From this study it appeared that 10‰ salinity concentration is isotonic to the blood of this species. Negative growth in fresh water (0‰) may be a reflection of the fish's earlier adaptation to the brackishwater environment of Lake Chilwa. The lowest whole-body fat, in 10‰, where growth was highest, may suggest that body fat was efficiently burned to provide fuel energy for growth, and this may have helped to spare body protein, which did not change at the end of this experiment. The effect of salinity on reproduction was not conclusive in all the experiments conducted in this study. Two possible reasons for this may be cited: either the experimental period was too short, or the sizes of the experimental animals were still very small. Visual observation of the reproductive products clearly showed that the eggs were still very small. It was difficult to distinguish ovarian size differences due to treatment effect. The most important observation in this experiment was that *O. shiranus chilwae* (Lake Chilwa strain) should be recommended as one of the most ideal candidate species for stocking brackishwater ponds that may be developed in saline soils. Raising this fish in fresh water would be analogous to subjecting it to a reverse acclimatory process, from saline water to fresh water.

### Experiment 2: Response of *O. shiranus chilwae* (Lake Chilwa Strain) to Salinity Stress

The results of this study suggested that if the fingerlings of *O. shiranus chilwae* (weight = 2.0 to 16.0 g) are introduced into 86‰ seawater, they will most likely die within a maximum period of 3 h at the experimental temperature recorded in this experiment. Since the water in the experimental tanks was adequately aerated, anoxic conditions could not be implicated in fish mortality. The pH of the water was about 7.5 and was considered normal for cichlids. Floating of the younger fish on direct transfer into 30‰ salinity could not be considered as an early indicator of mortality, but simply the failure of the fish to

Table 10. Changes in whole-body composition of *Oreochromis shiranus shiranus* cultured under laboratory tank conditions in three salinity concentrations.

Treatment	Dry Matter (%)	Crude Protein (%)	Fat (%)	Ash (%)
Initial	89.80	61.28	14.40	21.30
0‰	66.68	49.18	16.44	16.40
	(-23.22)	(-12.10)	(2.04)	(-4.90)
10‰	87.60	55.23	15.92	16.00
	(-2.20)	(-6.05)	(1.52)	(-5.30)
20‰	88.60	52.39	14.08	16.91
	(-1.20)	(-8.89)	(-0.32)	(-4.39)

Note: Numbers within parentheses are differences between initial and final values of each observed parameter.

swim in their normal position in a dense saline environment (30‰). Earlier studies have shown that premature transfer of red tilapia juveniles to seawater can impair their survival (Watanabe et al., 1990). In the present study, salinity tolerance also varied ontogenetically in *O. shiranus chilwae* (Lake Chilwa strain) with larger individuals (average weight = 15.22 g) dying much earlier than smaller individuals (average weight = 2.36 g) following direct transfer to the same 30‰ salinity concentration. This disparity in salinity tolerance between the two sizes may be due to a larger surface:volume ratio in the smaller fish. It is possible that the younger individuals took comparatively small quantities of ions through their normal respiration. Their higher metabolic rates may have helped them to process the ingested salt faster than in larger fish.

In larger fish (average weight = 15.22 g), the individuals may have taken in large amounts of water through respiration and in the process flooded their systems with comparatively large amounts of salt, which needed to be cleared fast through the chloride cell intervention in the brachial epithelium. This may have demanded a lot of energy for osmoregulation at the expense of energy requirements for maintenance and homeostasis. This study therefore suggests that it would take longer to intentionally eradicate smaller fish of *O. shiranus chilwae* by taking advantage of their osmotic dysfunction. Watanabe et al. (1985) studied the effect of ontogeny on salinity tolerance in three taxonomic groups of tilapia. They used MST<sub>-96</sub> and ST<sub>-50</sub>, in which MST<sub>-96</sub> was defined as mean survival time over a 96-h period following direct transfer from fresh water to full seawater. ST<sub>-50</sub> was defined as the time at which survival fell to 50% following direct transfer from fresh water to full seawater. Based on these indicators, Watanabe et al. (1985) were able to detect distinct age-specific differences in salinity tolerance in juveniles of *Oreochromis aureus*, *O. niloticus*, and an *O. mossambicus* × *O. niloticus* hybrid. In the present study, we used ST<sub>-0</sub>, a modification of ST<sub>-50</sub>, with ST<sub>-0</sub> being defined as the time at which survival fell to 0% as was the case in the present study.

### Experiment 3: Growth and Whole-body Composition of *O. shiranus chilwae* (Bunda College Strain)

Growth of *O. shiranus chilwae* (Bunda College strain) was not significantly influenced in proportion to salinity, and growth differences were not significant ( $P > 0.05$ ). In general, whole-body fat was depleted in all treatments at the end of the experiment, suggesting that this species was able to utilize body fat as a source of energy and to spare body protein. In this study body protein increased in all treatments, suggesting that it was not used to supply energy for maintenance even in the highest salinity treatment. The highest loss in fat was observed in the highest salinity treatment (20‰), where extra energy demanded by fish in this treatment may have been met by burning the available fat to release the required energy for maintenance. This species showed a lower salinity tolerance than *O. shiranus chilwae* collected directly from Lake Chilwa. The study suggested that *O. shiranus chilwae* (Bunda College strain) could be another potential candidate for stocking any available brackishwater pond constructed on a piece of saline land.

### Experiment 4: Response of *O. shiranus chilwae* (Bunda College Strain) to Salinity Stress

In a salinity challenge test in 30‰ salinity, larger fish died a little earlier (1 h 5 min) than smaller fish (1 h 15 min). This observation is similar to the other experiments.

### Experiment 5: Growth and Whole-body Composition of *T. rendalli*

In this study, *T. rendalli* was adversely affected by salinity, suggesting that the species would not be considered as a potential candidate species for productive brackishwater aquaculture in Malawi. Although the species is reported to be isosmotic at 10‰ (Whitfield and Blaber, 1976), its failure to grow faster at this salinity concentration than at 0‰ salinity may have been caused by other factors (excluding temperature) that limit growth. Maximal salinity tolerance is also reported to be achieved by this species when the temperature range is 20 to 29°C. In the present study, the temperatures ranged from 22.90 to 27.0°C, which was within the range conducive to normal physiological reactions in *T. rendalli*. Rapid growth in 0‰ may have indicated the ability of this species to efficiently utilize body protein as a source of energy. The observation that *T. rendalli* succumbed to 20‰ salinity much earlier in this study agrees with earlier reports (Whitfield and Blaber, 1976) that the maximum salinity that *T. rendalli* tolerates is 19‰. There was a decrease in carcass protein and an increase in carcass fat at the end of the study, suggesting that *T. rendalli* derived most of its energy from protein rather than fat. This is different from *O. shiranus chilwae* (Bunda College strain), in which fat was depleted at the end of the study rather than protein, suggesting that it was fat that provided energy to *O. shiranus chilwae* for maintenance and growth. This experiment showed that salinity levels in the range of 20 to 30‰ would effectively limit the range of *T. rendalli* in the natural waters of countries in the Southern African Development Community (SADC) region. The experiment has further shown that *T. rendalli* is the least salt-tolerant among the five taxonomic groups studied and may not be recommended for brackishwater aquaculture at a given temperature range.

### Experiment 6: Response of *T. rendalli* to Salinity Stress

Death of *T. rendalli* on direct transfer to 30‰ salinity occurred after only 50 min. This is in agreement with the important observation that *T. rendalli* cannot be recommended in Malawi to stock brackishwater ponds. A comparison between salinity tolerance of *T. rendalli* and that of *O. shiranus chilwae* would only be meaningful here if individuals of both species of the same size were subjected to the same salinity. In this study the latter species succumbed to 30‰ salinity after 60 min, which may not be considered to be much different from the 50 min that elapsed before *T. rendalli* died.

### Experiment 7: Growth and Whole-body Composition of *O. karongae*

In this experiment growth of *O. karongae* was significantly ( $P < 0.05$ ) higher in 10‰ salinity water than in fresh water (0‰), suggesting that 10‰ may be isotonic to the blood of *O. karongae*. The results also seem to indicate that *O. karongae* quickly converted the diet into carcass fat since there was a significant accumulation (gain) of carcass fat in this species at the end of the experiment. A decrease in whole-body protein in the 10‰ treatment also suggests that the fish utilized whole-body protein as a source of energy for somatic growth, while body fat was little used for the same purpose.

### Experiment 8: Response of *O. karongae* to Salinity Stress

Salinity tolerance of *O. karongae* at two distinct sizes was inconclusive. We were unable to get fish of 15 to 20 g average

weight to compare with small size fish. In a practical situation, one would not expect *O. karongae* of the size used in this experiment to survive in 30‰ beyond two hours.

### Experiment 9: Growth and Whole-body Composition of *O. shiranus shiranus*

*O. shiranus shiranus* grew best in fresh water, where they increased carcass fat most. This is another species that may have mainly used carcass protein as a source of energy. Considering a slight increase in the fat content at the end of the study, it may be suggested that the fish also used some carcass fat as an additional source of energy for osmoregulation.

### Experiment 10: Response of *O. shiranus shiranus* to Salinity Stress

This experiment suggests that *O. shiranus shiranus* grow faster in fresh water than in a saline environment. Some of these salinity preferences may have been influenced by temperature preferences. All experiments were conducted at room temperature within a single rainy season. It has become clear that most of the fish studied cannot tolerate 30‰ salinity for a period of more than two hours at the room temperature that was recorded. The exact levels of tolerance would have to be redefined at each temperature within a species temperature tolerance range.

### ANTICIPATED BENEFITS

In landlocked Malawi, we have only freshwater aquaculture at both small-scale and semi-commercial levels with a weak base of knowledge about the environmental requirements of and best management practices for many of our cultured species. The predominant species that are cultured are the ones used in the present study. This study has contributed to our knowledge base about these species, showing that at least three of them—*O. shiranus chilwae* (both Bunda College and Lake Chilwa strains) and *O. karongae*—are potential candidates for brackishwater aquaculture in Malawi. Use of these species for productive brackishwater aquaculture would be beneficial as Malawi's marginal lands would contribute significantly to: 1) increased food production and security; 2) improved human health; 3) improved income generation; and 4) increased total national fish production from aquaculture. Both *T. rendalli* and *O. shiranus shiranus* demonstrated the best growth in fresh water, and these two species would not be recommended for brackishwater aquaculture. In this study a water body on a piece of land where the soils are saline is assumed to become

saline (brackish). The species that was found to be least salt-tolerant is *T. rendalli*, so the range of this species would effectively be limited by salinity in the natural waters of countries in the SADC.

### ACKNOWLEDGMENTS

We are very grateful to the PD/A CRSP at Oregon State University in the United States, in collaboration with James Bowman, the International Center for Living Aquatic Resources Management Project Leader in Malawi Dr. D. Jamu, and Karen L. Veverica of the Kenya Project, for funding this study.

### LITERATURE CITED

- Chervinski, J., 1982. Environmental physiology of tilapias. In: R.S.V. Pullin and R.H. Lowe-McDonnell (Editors), *The Biology and Culture of Tilapias*, ICLARM Conf. Proc., 7. International Center for Living Aquatic Resources Management, Manila, Philippines, pp. 119–128.
- Fryer, G. and T.D. Iles, 1972. *The Cichlid Fishes of the Great Lakes of Africa: Their Biology and Evolution*. T.F.H. Publications, Neptune City, New Jersey, 641 pp.
- Likongwe, J.S., T.D. Stecko, J.R. Stauffer, Jr., and R.F. Carline, 1996. Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus). *Aquaculture*, 146:37–46.
- Morgan, J.D. and G.K. Iwama, 1991. Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (*Oncorhynchus mykiss*) and fall chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.*, 48:2,083–2,094.
- Payne, A.I. and R.I. Collinson, 1983. A comparison of the biological characteristics of *Sarotherodon niloticus* (L.) with those of *S. aureus* (Steindachner) and other tilapia of the delta and lower Nile. *Aquaculture*, 30:335–351.
- Watanabe, W.O., C.-M. Kuo, and M.-C. Huang, 1985. The ontogeny of salinity tolerance in the tilapias *Oreochromis aureus*, *O. niloticus*, and an *O. mossambicus* × *O. niloticus* hybrid, spawned and reared in freshwater. *Aquaculture*, 47(4):353–367.
- Watanabe, W.O., L.J. Ellingson, B.L. Olla, D.H. Ernst, and R.I. Wicklund, 1990. Salinity tolerance and seawater survival vary ontogenetically in Florida red tilapia. *Aquaculture*, 87:311–321.
- Watanabe, W.O., D.H. Ernst, M.P. Chasar, R.I. Wicklund, and B.L. Olla, 1993. The effects of temperature and salinity on growth and feed utilization of juvenile, sex-reversed male Florida red tilapia cultured in a recirculating system. *Aquaculture*, 112(4):309–320.
- Whitfield, A.K. and S.J.M. Blaber, 1976. The effects of temperature and salinity on *Tilapia rendalli* (Boulenger 1896). *J. Fish Biol.*, 9:99–104.
- Wohlfarth, G.W. and G. Hulata, 1983. Applied genetics of tilapia. *ICLARM Stud. Rev.*, 6:26 pp.
- Zale, A.V. and R.W. Gregory, 1989. Effect of salinity on cold tolerance of juvenile blue tilapias. *Trans. Am. Fish. Soc.*, 118(6):718–720.