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Salinity tolerance of the tilapias Oreochromis aureus, O. niloticus and an O. mossambicus x O. niloticus hybrid

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Cover: ICLARM research assistant Mei-Chan Huang at the National Sun Yat-Sen University, Kaohsiung, Taiwan, examining tilapia eggs to determine fertilization rate. Photo by Ching-Ming Kuo.

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Salinity Tolerance of the Tilapias Oreochromis aureus, O. niloticus and an O. mossambicus X O. niloticus Hybrid

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Abstract

The ontogeny of salinity tolerance was studied in the tilapias *Oreochromis aureus*, *O. niloticus* and an *O. mossambicus* ($^{\circ}$) X *O. niloticus* ($^{\circ}$) (M X N) hybrid, spawned and reared in freshwater. Several indices were employed as practical measures of salinity tolerance: (1) median lethal salinity-96 hours (MLS-96), defined as the salinity at which survival falls to 50%, 96 hours following direct transfer from freshwater to varying salinities; (2) mean survival time (MST), defined as the mean survival time over a 96-hour period, following direct transfer from freshwater to seawater (32 ppt); and (3) median survival time (ST₅₀), defined as the time at which survival falls to 50% following direct transfer from freshwater to seawater.

No significant age-specific differences in salinity tolerance were observed in either O. aureus or O. niloticus on the basis of the MLS-96 index. Mean MLS-96 values over all ages from 7 to 120 days post-hatching were 18.9 ppt for O. niloticus, and 19.2 ppt for O. aureus. In contrast, the M X N hybrid exhibited relatively greater changes in salinity tolerance with age, MLS-96 ranging from 17.2 ppt at 30 days post-hatching to 26.7 ppt at 60 days post-hatching. Distinct age-specific differences in salinity tolerance were observed in all three on the basis of the MST index.

These ontogenetic changes in salinity tolerance were determined to be more closely related to body size than to chronological age. No consistent relationship was observed between salinity tolerance and condition factor. The practical implications of these findings for the culture of tilapias in brackishwater and seawater are discussed.

Introduction

The tilapias are economically important food fishes which are cultured primarily in freshwater ponds in tropical countries. They exhibit many qualities which suit them for culture, including resistance to handling and disease, efficient conversion of low protein diets, ease of breeding, and

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high palatability. The tilapias also display varying degrees of salt tolerance, a trait which has suggested the possibility for the expansion of their culture into brackishwater or marine systems.

A decreased reliance on freshwater and the increased use of brackishwater and marine systems for fish culture have some important advantages. They allow the utilization of areas which are otherwise unsuitable for agricultural purposes, such as arid regions where freshwater is severely limited, or coastal land areas having soils too saline for crop production (Loya and Fishelson 1969; Payne 1983). They also permit the expansion of culture activities into coastal waters, which further reduces competition between agricultural and aquacultural use of arable lands and limited freshwater (Kuo and Neal 1982).

Despite the reputed salt tolerance of tilapias, their culture is still limited primarily to freshwater. There have been a few preliminary studies on the growth and survival of some commercially important tilapias in concrete tanks, aquaria and earth ponds at various salinities (Chervinski 1961a, 1961b, 1966; Fishelson and Popper 1968; Chervinski and Yashouv 1971; Chervinski and Zorn 1974; Liao and Chang 1983). These results appear to suggest that although tilapias can be acclimated and grown at varying salinities, normal growth and reproduction are constrained by increasing salinity. It is evident that more basic studies are required in order to assess accurately the culture potential of each species under saline conditions.

The generally used approach to the problem of saltwater tilapia culture is to produce seedstock from adults spawned in freshwater, followed by the transfer of these stocks to seawater for growout. At present, the optimum age or size for transfer of freshwater-reared stocks to seawater has not been standardized, nor is there any information to suggest that time of transfer may influence growth or survival in seawater.

It is well established for the anadromous salmonid fishes that hypoosmoregulatory ability and salinity tolerance, or the ability to survive in seawater, develop during the early freshwater phase of their life cycle, and preadapt them to subsequent seawater existence (Hoar 1976; Folmar and Dickhoff 1980; Wederneyer et al. 1980). These ontogenetic changes in salinity tolerance are known to be closely related to size, which is therefore an important criterion for determining the optimum time for release of hatcherv-reared juveniles or for their transfer to seawater pens (Mahnken et al. 1982). Failure to attain a critical size before transfer results in mortality and stunting in seawater.

The objectives of the present study were to evaluate several indices as actical measures of salinity tolerance in the tilapias, to determine and compare ontogenetic changes in salinity tolerance in some commercially important tilapias spawned and reared in freshwater, and to evaluate these changes with respect to age, size and condition factor.

Materials and Methods

The experiments described in this report were conducted at the Institute of Marine Biology, National Sun Yat-Sen University, Kaohsiung, Taiwan from September 1982 to January 1984.

The adult broodstocks of *Oreochromis aureus* (Steindachner) and *O. niloticus* (Linnaeus) employed in this study originated from captive experimental stocks held in freshwater at the Taiwan Fisheries Research Institute (Lukang and Tainan Branches). Individuals were examined to ensure conformity with known species specific morphological characteristics including head configuration, coloration and caudal fin barring (Lee 1979). Breeders ranged in size from 117 g to 208 g body weight for *O. aureus* and from 99 g to 277 g body weight for *O. niloticus*.

SPAWNING AND REARING IN FRESHWATER AQUARIA

Spawnings were conducted in freshwater in indoor 120-I glass aquaria ($60 \times 60 \times 40$ cm) at an ambient temperature range of 24-31°C, under natural photoperiod conditions. Diffuse sunlight entered through several laboratory windows. Semi-closed system conditions were employed: water

was constantly recirculated by airlift through several box-type gravel filters situated inside each aquarium. Feces were siphoned periodically and approximately one-half of the tank volume was replaced with tap water each week.

Fish were fed twice daily *ad libitum* a pelletized commercial tilapia diet (Tong Bao Company, Tainan, Taiwan) containing 24% protein.

In each aquarium, one male was paired with one to three individually tagged females. The pre-maxilla was removed from all males in order to reduce female mortality due to aggressive nipping (Lee 1979). Aquaria were observed daily for spawning activity; spawner and date of spawn were recorded whenever a female was found to be mouthbrooding eggs. In order to establish the exact ages of individual broods, eggs were usually removed from the mouth of the female 1-2 days post-spawning and incubated artificially in 1.2-I bottles fitted with a perforated disc bottom, through which was provided a continuous current of filtered, recirculated water from an externally situated aquarium filter. At an incubation temperature range of 24-31°C, hatching occurred 3-5 days following spawning. Each incubator was observed daily in order to establish date of hatching (age 0 days). For broods incubated naturally in the mouth of the parent female, date of hatching was estimated as occurring three days following date of spawn. Newly-hatched larvae were allowed to remain in incubators until yolk sac absorption was completed at approximately seven days post-hatching, at which time they were transferred to aquaria (120-400 I) for rearing.

Rearing was conducted in freshwater under conditions similar to those described earlier for spawning aquaria. Individual broods were reared separately to ensure age uniformity. Fish were fed twice daily *ad libitum* the pelletized commercial tilapia diet. Salinity tolerance tests were performed as described below on broods of known ages. During tolerance testing, feeding was discontinued beginning on the day of the experiment.

SALINITY TOLERANCE INDICES

Seawater was filtered by recirculation through a 6-cm bed of crushed oyster shells for several days prior to use. Water of varying salinities was prepared by diluting filtered seawater with tap water similarly conditioned by recirculation through oyster shells.

All salinity tolerance tests were conducted in white 20-I plastic aquaria, under closed-system conditions. In each aquarium, water was recirculated by airlift through an internal box-type gravel filter.

Several tests were employed as practical indices of salinity tolerance:

(1) Median Lethal Salinity-96 hours (MLS-96), defined as the salinity at which survival falls to 50%, 96 hours following direct transfer from freshwater to various salinities (0, 7.5, 15, 17.5, 20, 22.5, 25, 27.5, 30 and 32 ppt). A sample of 25-30 individuals was weighed and measured in order to establish mean body length, weight and condition factor of the experimental brood. Individual fish were blotted with tissue paper before weighing. Condition factor (K) was calculated from the formula ($K = W/L^3 \times 100$), where W denotes weight in grams and L denotes total length in centimeters. Ten to twenty individuals were transferred from freshwater directly into each of the experimental salinities. Dead individuals were counted and removed daily over a period of four days (96 hours). Final survival (percent) in each experimental salinity was calculated as the sum of the number of days each individual survived, divided by the product of total experimental days (4) and initial number of fish. Percentage survival was then plotted against the salinity of transfer and MLS-96 determined as the salinity at which survival fell to 50% (Fig. 1).

(2) Mean Survival Time (MST), defined as the mean survival time for all individuals in an experimental group over a 96-hour period following direct transfer from freshwater to full seawater (Fig. 2). Twenty-five individuals were used for each trial. Dead individuals were removed as soon as they succumbed to salinity stress and time of death; body length and body weight were recorded.

Cessation of opercular movements and failure to respond to gentle prodding were the criteria used for death.

(3) Median Survival Time (ST₅₀), defined as the time at which survival falls to 50% following direct transfer from freshwater to full seawater (Fig. 2).

Direct transfer between freshwater and the experimental salinities was performed under isothermal conditions. Most experiments were conducted at an ambient temperature range of 24-31°C.



Fig. 1a. Representative examples of survivorship patterns for 7- to 120-day old freshwater-spawned and reared *O. niloticus*, 96 hours following direct transfer to various salinities. (\bigcirc 7 days, MLS-96 = 18.5 ppt; • 15 days, MLS-96 = 17 ppt; \triangle 30 days, MLS-96 = 18 ppt; • 45 days, 1LS-96 = 18.5 ppt; \square 60 days, MLS-96 = 19 ppt; \blacksquare 90 days, MLS-96 = 17 ppt; X 120 days, MLS-96 = 21 ppt).

Fig. 1b. Generalized survivorship patterns for 7- to 120-day old freshwater-spawned and reared *O. niloticus* (\odot) and *O. aureus* (\bullet), 96 hours following direct transfer to various salinities. Plotted points represent mean values of 20 determinations for *O. niloticus* and 16 determinations for *O. aureus*. Vertical bars represent \pm S.E.M. MLS-96 for each species is indicated by the value beneath ecch dotted vertical line.





Fig. 1c. Survivorship patterns for 7- to 60-day old ireshwater-spawned and reared *O. mossambicus* (Q) X *O. niloticus* (\overline{O}) hybrids, 96 hours following direct transfer to various salinities (\bigcirc 7 days, \bullet 15 days, \triangle 30 days, \blacktriangle 45 days, \square 60 days). MLS-96 for each age is indicated by the value beneath each dotted vertical line.

When environmental temperature declined below this range, aquarium heaters were used to elevate rearing tank water temperature to 28°C. Experimental fish were preacclimatized to this temperature for at least five days prior to tolerance testing. Temperatures were recorded daily during tolerance testing. Dissolved oxygen levels were maintained near air saturation (6.0-8.6 ppm) at all temperatures and salinities.

Limited data are presented in this report for *O. mossambicus* (?) X *O. niloticus* (d) (M X N) hybrids, which were produced in outdoor 250-I fiberglass aquaria at the Taiwan Fisheries Research Institute (Tainan Branch). Salinity tolerance tests on M X N hybrid progeny were performed as described above.

Results and Discussion

EVALUATION OF SALINITY TOLERANCE INDICES (MST, ST₅₀, AND MLS-96)

Fig. 1a provides examples of survivorship patterns for 7- to 120-day old freshwater-spawned and reared *O. niloticus* at 96 hours following direct transfer to various salinities. These closely

Table 1. Mean survival time (MST), median survival time (ST₅₀), and median lethal salinity (MLS-96) of freshwater-spawned and reared *Oreochromis niloticus* at various ages: means \pm S.E.M. (Standard Error of the Mean). The age-specific means for MST and ST₅₀ are significantly different (p <0.001, ANOVA). Temperature ranges for MST and ST₅₀ determinations were identical.

Age (days)	MST (min.)				<u></u>	ST ₅₀ (min.)				MLS-96 (ppt)			
7	50.2 (25.5	± 	4.5 29.8) ^b	(10) ^a	49.7	±	4.4	(10)	19.7 (25.9	± 	0.6 29.8)	(7)	
15	29.8 (25.8	± 	1.9 30.2)	(4)	28.8	±	1.8	(4)	17.0 (25.6)			(1)	
30	37.5 (26.8	± 	6.9 28.7)	(3)	37.3	±	7.7	(3)	17.3 (26.3	± _	0.8 27.5)	(2)	
45	42.2 (26.0	1 	5.8 28.3)	(3)	39.0	±	5.6	(3)	18.5 (25.1	± -	0.0 28.6)	(2)	
60	84.5 (29.4	± 	12.3 29.5)	(2)	79.0	±	11,0	(2)	19.3 (29.6	± 	0,3 30,3)	(2)	
90	92.8 (27.6	± _	19.6 29.0)	(4)	89.3	±	21.3	(4)	18,1 (28,4	± 	0.4 29.5)	(4)	
120	102.9 (28.5	± _	13.3 28.8)	(2)	96.0	±	11.0	(2)	20.2 (28.0	± -	2.0 28.5)	(2)	
150	180.5 (26.8	± -	11.0 27.1)	(2)	179.0	±	5.0	(2)		no	data		
180	153.9 (25.1	± -	7.7 28.0)	(3)	155.0	±	7.6	(3)		no	data		
210	184.6 (28.0)			(1)	178.0			(1)		no	data		
395	148.4 (25.0	± 	20.5 29.8)	(2)	147.0	±	22.0	(2)		no	data		

^aNumber of determinations.

^bTemperature range (°C).

similar survivorship patterns resulted in MLS-96 values which fell within a narrow range of salinities from 17 to 21 ppt. Age-specific differences in salinity tolerances were not easily distinguished on the basis of this index. Mean MLS-96 values determined for broods of various ages are presented in Table 1 for *O. niloticus* and Table 2 for *O. aureus*. A statistical comparison of mean MLS-96 values by analysis of variance revealed no significant age-specific differences in either *O. aureus* or *O. niloticus*. Therefore, data for all ages were combined and generalized survivorship patterns computed for each species. These patterns are superimposed in Fig. 1b. The close similarity of these patterns is evident. In general, they showed near maximum (85-100%) survival up to 15 ppt, and slight reduction at 17.5 ppt, although survival still exceeded 80%. A steep decline in survival occurred between 17.5 and 20 ppt, and near complete mortality occurred at 22.5 ppt. No individual survived direct transfer to 25 ppt. The mean MLS-96 over all ages from 7 to 120 days was 18.9 ppt (n = 20, range = 16.5-22 ppt) for *O. niloticus*, and 19.2 ppt (n = 16, range = 17.5-22 ppt) for *O. aureus*. These mean values were not found to be significantly different (P > 0.05, t-test).

Fig. 1c presents survivorship patterns for the *O. mossambicus* (?) X *O. niloticus* (d) (M X N) hybrids from 7 to 60 days of age. The M X N hybrid MLS-96 values exhibited relatively greater age-specific variation than *O. aureus* or *O. niloticus*, ranging from a minimum of 17.2 ppt at 30 days to a maximum of 26.7 ppt at 60 days (Table 2). As only single determinations were available for each age, data were not treated statistically. Fig. 1c shows that the M X N hybrid was unable to survive direct transfer to full seawater (32 ppt) at any age. However, at 60 days, 10% survived direct transfer to a relatively high salinity of 30 ppt.

Fig. 2 illustrates survival as a function of time for freshwater-spawned and reared *O. niloticus* from 7 to 120 days of age, following direct transfer to full seawater (32 ppt). Data presented in Fig. 2 are the results of tests employing broods identical to those in Fig. 1a. As Fig. 2 shows, freshwater-spawned and reared *O. niloticus* expired rapidly following direct transfer to full seawater, with complete mortality occurring approximately 200 min. post-transfer for individuals of all ages. However, distinct age-specific differences in survival patterns were evident, with time of death



Fig. 2. Representative examples of survivorship patterns as a function of time for 7- to 120-day old freshwater-spawned and reared *O. niloticus*, following direct transfer to full seawater (32 ppt). The results presented in this figure were obtained from the identical broods shown in Fig. 1. (\circ 7 days, ST₅₀ = 27 min., MST = 29.9 min.; \bullet 15 days, ST₅₀ = 23 min., MST = 26.9 min.; Δ 30 days, ST₅₀ = 52 min., MST = 50.9 min.; \blacktriangle 45 days, ST₅₀ = 52 min., MST = 53.8 min.; \Box 60 days, ST₅₀ = 66 min., MST = 72.2 min.; \blacksquare 90 days, ST₅₀ = 79 min., MST = 81.0 min.; X 120 days, ST₅₀ = 105 min., MST = 116.1 min.). Median survival times (ST₅₀) for each age are also indicated by the values beneath each dotted vertical line.

generally increasing with age of brood. For example, in the 7-day old brood, initial mortality occurred at 11 min. post-transfer and final mortality occurred at 54 min. post-transfer (ST₅₀ = 27 min., MST = 29.9 min.). In comparison, initial and final mortalities in the 120-day old brood occurred at 75 min., and 197 min. post-transfer, respectively (ST₅₀ = 105 min., MST = 116.1 min.). Hence, whereas age-specific differences in salinity tolerance were not clearly defined by the MLS-96 index, the MST and ST₅₀ indices exhibited comparatively greater sensitivity in detecting such differences.

Age (days)		MST (min.)				ST ₅₀ (min.)				MLS-96 (ppt)			
. O. aureus													
7	43.9 (25.0	± —	9.0 28.4) ^b	(3) ⁸	44.3	±	9.4	(3)	18.5 (25.7	± 0.03 - 28.2)	(3)		
15	51.8 (24.4	± 	3.3 29.5)	(3)	54.3	±	5.4	(3)	18.3 (24.9	± 0.4 30.4)	(3)		
30	42.3 (24.8	± _	11,4 26,6)	(3)	41,5	±	12,5	(2)	19.0 (26.1)		(1)		
45	54.2 (27.8	± ~	2.6 29.2)	(2)	50.5	±	0.5	(2)	20.3 (28.3	± 1.3 29,1)	(2)		
60	63.9 (27.6	± _	5.1 31.0)	(4)	62.0	±	5.1	(4)	19.7 (28.1	± 0.7 - 30.2)	(3)		
90	122.8 (28.1)			(1)	128			(1)	18.5 (28.7)		(1)		
120	136.3 (27.7	± _	15.9 28.6)	. (3)	130.3	±	18.2	(3)	20.0 (27.7	± 1.0 - 28.8)	(3)		
150		no	data			no data			no data				
180	151.4 (28.0)			(1)	148			(1)		no data			
2. O. mossambicus	(♀) X O. niloti	cus (c	ð) hybrid					<u> </u>					
7	53 (31.1)			(1)	55			(1)	21.5 (26.5)		(1)		
15	139 (30.0)			(1)	132			(1)	24.0 (29.5)		(1)		
30	129 (30.1)			(1)	120			(1)	17.2 (30.3)		(1)		
45	157 (30.0)			(1)	152			(1)	25.2 (30.2)		(1)		

Table 2. Mzan survival time (MST), median survival time (ST50), and median lethal salinity (MLS-96) of freshwater-spewned and reared Oreochromis aureus and O. mossambicus (?) X O. niloticus (d) hybrids of various ages: means ± S.E.M. (Standard Error of the Mean). Age-specific means for MST and ST 50 are significantly different (p < 0.001, ANOVA). Temperature ranges for MST and ST_{50} determinations were identical.

217

(30.0)

(1)

195

(1)

26.7

(30.0)

(1)

^aNumber of determinations.

^bTemperature range (°C).

60

The relationship between the MLS-96 and MST indices is shown in Fig. 3. When only data from tolerance tests involving freshwater-spawned and reared broods were employed, no consistent relationship between these indices was observed. However, when data from tests employing broods pre-acclimatized by spawning or hatching at elevated salinities were included, a curvilinear relationship between these indices was evident. A basic difference between the two tolerance measures is that the MST index readily distinguishes age-specific differences, while the MLS-96 index is unable to detect such differences. According to the relationship in Fig. 3, a brood which has an MLS-96 equivalent to full seawater (32 ppt) will have a mean survival time of 3,291 min. following direct transfer to full seawater. This relationship also predicts that a brood which has an MLS-96 of at least 33.7 ppt will exhibit full survival following direct transfer to full seawater.

The median survival time index (ST_{50}) provided a value closely similar to MST in the lower tolerance ranges. However, it was limited by the fact that it could greatly underestimate tolerance when survivorship patterns involved a few relatively tolerant individuals tending to elevate MST.



Fig. 3. Relationship between MST and MLS-96 in *O. niloticus*. Data in this figure are the results obtained with freshwater-spawned and reared progeny (\bigcirc), freshwater-spawned, saline water-hatchad progeny (\square), and saline water-spawned and reared progeny (\triangle). Tolerance tests involving broods spawned or incubated at elevated salinities involved direct transfer from pre-acclimation salinity to full seawater. Regression analysis produced the following relationship: In MST = -2.53 + 0.33 MLS-96, n = 40, R² = 0.73. Data for which survival exceeded 50% at 96 hours in all salinities (MLS-96 > 32 ppt) were excluded from the regression analysis.

The MLS-96 and ST₅₀ indices had limited comparative utility under high tolerance conditions when survival did not fall to 50% by 96 hours (MLS-96 > 32 ppt; ST₅₀ > 96 hours). The MST index was similarly limited when there was full survival by 96 hours so that MST attained a ceiling value of 5,760 min. However, as shown in Fig. 3, when MLS-96 was greater than 32 ppt, the MST index still provided a relative tolerance value as long as survival remained less than 100%. The MST index was generally the most reliable for assessing tolerance due to its sensitivity and accuracy under high

and low tolerance conditions, and because it provided relative tolerance values over the widest range of tolerance conditions.

SALINITY TOLERANCE OF FRESHWATER-SPAWNED AND REARED TILAPIAS OF VARIOUS AGES

Mean MST and ST₅₀ values determined for freshwater-spawned and reared tilapias of various ages are presented in Table 1 for *O. niloticus*, and in Table 2 for *O. aureus*. Table 2 also presents limited data for the M X N hybrid. For both *O. aureus* and *O. niloticus*, statistical comparison of mean MST values by analysis of variance revealed highly significant age-specific differences in salinity tolerance. Mean MST values were further analyzed using the Student-Newman-Keuls Test (SNK) for samples of unequal sizes, in order to compute the significance of these differences (Fig. 4). Level of significance in all tests was P < 0.05. Only single determinations were available for certain ages and could not be included in the analysis of variance. These values were omitted during significance testing by SNK.

Mean MST values for *O. aureus* from 7 to 180 days, for *O. niloticus* from 7 to 395 days, and for the M X N hybrid from 7 to 60 days are illustrated in Fig. 4. In *O. aureus*, MST values remained relatively low over the initial 45 days then increased to a maximum of 151.4 min. at 180 days. Mean MST values remained statistically unchanged over the initial 60 days, but were significantly elevated at 120 days, relative to all initial 60-day values. Similarly, in *O. niloticus*, MST values remained low



Fig. 4. Salinity tolerance (MST) of freshwater-spawned and reared *O. aureus* (\bullet), *O. niloticus* (\circ), and an *O. mossambicus* (?) x *O. niloticus* (\circ) hybrid (Δ) at various ages. Plotted points represent mean values and vertical bars represent \ddagger S.E.M. Numbers in parentheses indicate number of determinations (25 fish/determination). Mean MST values are shown below ranked in order of magnitude. Lines beneath MST figures link values that are not significantly different. Means not within the range of any one line are significantly different (P < 0.05, SNK).

			O. i	aureus						
Rank	1	2	3	4	5	6				
Age	30	7	15	45	60	120				
MST	42.3	43.9	51.8	54.2	63.9	136.3				
					O. nil	oticus				
Rank	1	2	3	4	5	6	7	8	9	10
Age	15	30	45	7	60	90	120	395	180	150
MST	29.8	37.5	42.2	50.2	84.5	92.8	102.9	148.4	153. 9	180.5

over the initial 45 days, then increased to a near maximum value of 180.5 min. at 150 days. In *O. niloticus*, a conspicuous decline in MST occurred between 7 and 15 days which was not observed in *O. aureus*. However, mean MST values did not differ significantly over the initial 45 days. At 90 days and older, MST values were significantly elevated relative to all initial 45-day values. *O. niloticus* MST values did not progressively increase with age past 150 days. Instead, they fluctuated near maximum values through 180, 210 and 395 days. MST values at 150 days and older were significantly greater than those for all ages from 7 to 120 days. However, from 150 through 395 days, MST values did not differ significantly. These results suggest that in *O. niloticus*, salinity tolerance attains a maximum at 150 days and remains relatively unchanged thereafter.

As Fig. 4 shows, *O. aureus* and *O. niloticus* exhibit very similar age-specific changes in salinity tolerance. MST values for *O. aureus* slightly exceeded those of *O. niloticus* at most ages up through 120 days. However, interspecific differences were not statistically significant at any age except 15 days where *O. aureus* fry were found to have a mean MST significantly greater (P < 0.01, t-test) than *O. niloticus* fry of identical age. This difference resulted mainly from a decline in tolerance of *O. niloticus* fry between 7 and 15 days as described earlier. The scarcity of data for *O. aureus* past 120 days precluded precise comparison between these species, as it is unknown what maximum values *O. aureus* MST would attain.

Fig. 4 also shows that the M X N hybrid MST is similar to that of *O. aureus* or *O. niloticus* at age 7 days. However, the M X N hybrid MST was relatively elevated by 15 to 30 days, increasing to a maximum value at 60 days. The MST of the 60-day M X N hybrid (217 min.) was well above that recorded for either *O. aureus* (63.9 min.) or *O. niloticus* (84.5 min.). The M X N hybrid MST values at various ages were significantly greater (P < 0.05, t-test) than those for corresponding ages for *O. aureus* at 15, 45 and 60 days, and for *O. niloticus* at 15, 30 and 45 days.

The increase in salinity tolerance with age appears to be closely related to body size. Mean body length, body weight, condition factor, and MST of the experimental broods of various ages employed in this study are illustrated in Figs. 5, 6 and 7 for *O. aureus, O. niloticus* and M X N



Fig. 5. Mean body length (O), body weight (\bullet), condition factor (\triangle), and MST (D) of the *O. aureus* broods of various ages employed in this stand. Plotted points represent mean values of 2-4 determinations (25 fish/determination) and vertical bars represent ± S.E.M. Absence of vertical bars indicates that the S.E.M. lies within the area of the plotted point. Data for 30 and 180 days represent single determinations.



Fig. 6. Mean body length (\odot), body weight (\bullet), condition factor (Δ) and MST (\Box) of the *O. niloticus* broods of various ages employed in this study. Plotted points represent mean values of 2-10 determinations (25 fish/determination) and vertical bars represent ± S.E.M. Absence of vertical bars indicates that the S.E.M. lies within the area of the plotted point. Data for 210 days represent a single determination.



Fig. 7. Mean body length (O), body weight (\bullet), condition factor (Δ) and MST (\Box) of *O. mossambicus* X *O. niloticus* hybrid broods of various ages employed in this study. Data for all ages represent single determinations (25 fish/determination).

hybrid, respectively. In *O. aureus* and *O. niloticus* the patterns of change in MST with age paralleled the patterns of change in body length and body weight. Although data presented in Fig. 7 for the M X N hybrid represent the results of only single determinations for each age, an increase in MST with age which roughly paralleled the increase in body length and body weight was similarly observed. In contrast, changes in MST did not closely parallel changes in condition factor in any group.

The relationships between mean body length and mean MST are shown in Fig. 8a for *O. aureus*, and Fig. 8b for *O. niloticus*. In *O. aureus*, when data for all ages from 7 to 180 days were



Fig. 8b. Relationship between salinity tolerance (MST) and body length in *O. niloticus.* Plotted points represent mean values for 2-10 determinations (25 fish/determination) on individuals of uniform age. Numbers above each point indicate age. Vertical and horizontal bars represent \pm S.E.M. Data for 210 days represent a single determination. The straight linu best fitting these data is: Y = -0.72 + 3.38X; n = 10, R² = 0.88. Data for 395 days were excluded from the regression analysis.

Fig. 8a. Relationship between salinity tolerance (MST) and body length in *O. aureus.* Plotted points represent mean values for 2-4 determinations (25 fish/determination) on ind⁻ iduals of uniform age. Numbers near each point indicate age. Vertical and horizontal bars represent \pm S.E.M. Data for 90 and 180 days represent single determinations. The straight line best fitting these data is: Y = 14.28 + 2.56X; n = 8, R² = 0.96.





Fig. 8c. Comparison of the relationships between salinity tolerance (MST) and body length in *O. aureus* (\bullet), *O. niloticus* (O), and an *O. mossambicus* X *O. niloticus* hybrid (Δ). For M X N hybrid, plotted points represent the results of single determinations (25 fish/determination). The straight line best fitting these data is: Y = 29.86 + 6.22X; n = 5, R² = 0.66.

used, a linear increase in MST with body length was observed. Similarly, in *O. niloticus*, when data for ages from 7 to 210 days were employed, MST was observed to increase linearly with body length. However, the MST value for the oldest (395 days) broods was well below that expected on the basis of the defined relationship between MST and body length for 7- to 210-day broods. In Fig. 8c, data for both *O. aureus* and *O. niloticus* are superimposed and compared with data for the M X N hybrid. These results show that the relationships between body length and MST were very similar in *O. aureus* and *O. niloticus*. In comparison, the rate of increase in MST with body length was greater in the M X N hybrid.

During high density rearing in small aquaria, experimental fish were found to attain maximum body lengths of only approximately 40-50 mm, after which growth virtually ceased. Consequently, in Figs. 5 and 6, mean body lengths and mean body weights of experimental broods older than 90 days (*O. aureus*) and 150 days (*O. niloticus*) did not necessarily show the expected increase with age. The 180-day *O. niloticus* broods were particularly stunted, having a mean body length of only 40 mm. Mean MST for these stunted 180-day broods was 153.9 min., a value which is in general agreement with the defined relationship between body length and MST for 7- to 210-day broods. Conversely, much younger (150 days) but relatively larger individuals with a mean body length of 51.7 mm had a comparatively higher MST of 180.5 min.

These results demonstrate that salinity tolerance is more closely related to size than to chronological age. Therefore, in *O. niloticus*, the stabilization of MST past 150 days (Fig. 4) is related to size rather than to age. If the stabilization of tolerance past 150 days was the result of stunting, it follows that salinity tolerance would continue to increase if size progressively increased with age. As shown in Fig. 8b, the 395-day *O. niloticus* broods employed in this study had attained a relatively large mean body length of 70.4 mm through rearing in large concrete tanks. As described earlier, despite the relatively large size of these individuals, mean MST retained a value of 148.4 min., which was similar to those characterizing 150 to 210-day broods, and which was less than expected on the basis of the defined relationship between body length and MST. These results suggest that in *O. niloticus*, although salinity tolerance was more closely related to size than to chronological age, maximum tolerance may be acquired at a body length of approximately 51.7 mm and further size increases may not result in greater salinity tolerance.

Available data for *O. aureus* also appear to agree with the premise that salinity tolerance is more closely related to size than to chronological age. Thus, close similarities in MST values for broods from 90 to 180 days (Fig. 8a) result from the fact that these individuals were stunted and had achieved mean body lengths of only 46.5 and 48.2 mm at 120 and 180 days, respectively, still very similar to that of the 90-day old broods (47.3 mm). MST, therefore, remained related to body length rather than to age. As the maximum mean body length of *O. aureus* broods employed in this study was 48.2 mm, no inference can be made with respect to the salinity tolerance of larger individuals. However, the similarities in patterns of MST with age for both *O. niloticus* and *O. aureus* suggest that a similar stabilization in tolerance would be expected with further increases in size.

Since salinity tolerance is closely related to size, it follows that a relatively rapid increase in hybrid tolerance with age may have resulted from the larger sizes (faster growth) of the M X N hybrid used. Fig. 9 compares the mean body lengths and weights of the *O. aureus*, *O. niloticus* and M X N hybrid broods of various ages employed for tolerance testing in this study. As Fig. 9 shows, 7-day M X N hybrids were very similar in size to their *O. aureus* and *O. niloticus* counterparts. Salinity tolerance in all three species was very similar at this age (Fig. 4). The M X N hybrid initially exhibited faster growth so that by 30 days M X N hybrid progeny were noticeably larger than their *O. aureus* or *O. niloticus* counterparts. Hence, faster growth may possibly have contributed to faster acquisition of salinity tolerance with age. By 60 days, however, all three species were nearly equivalent in size, yet M X N hybrid tolerance remained much greater than either *O. aureus* or *O. niloticus* or *O. niloticus* and and tolerance remained much greater than either *O. aureus* or *O. niloticus* or *O. niloticus* and and and a store growth may accelerate the increase in tolerance with age,



Fig. 9. Comparison of mean body lengths (open symbols) and mean body weights (closed symbols) of the *O. aureus* (O), *O. niloticus* (\triangle) and *O. mossambicus* X *O. niloticus* hybrid (\Box) broods of various ages employed in this study. See Figs. 5, 6 and 7 for details.

tolerance was nevertheless greater in M X N hybrids at equivalent size. Fig. 8c clearly illustrates greater M X N hybrid salinity tolerance at equivalent body lengths.

The relationship between body weight and MST is illustrated in Fig. 10a for O. aureus and Fig. 10b for O. niloticus. Log transformed variables have been used in Fig. 10. The curvilinear relationship between MST and body weight reflects the non-linear relationship between body length and body weight. From these results, it may be similarly inferred that salinity tolerance in both species is more closely related to size, in this case, body weight, than to chronological age. Stabilization of tolerance past 90 days (O. aureus) and 150 days (O. niloticus) can be explained in terms of size: tolerance relationships. As discussed earlier, salinity tolerance in O. niloticus attained a plateau of 150 days at a mean body length of 51.7 mm. These broods had a mean body weight of 3.07 g. That a further increase in body weight did not result in increased tolerance is suggested by the observation that 395-day old fish, which had a much larger mean body weight of 6.0 g, still maintained a mean MST value similar to those of 15- to 210-day old broods. In Fig. 10b, data for 395-day old broods were excluded from the regression analysis since, as was observed for body length (Fig. 8b), these individuals appeared to have exceeded the maximum body size below which MST increased with size. Lack of data for larger individuals in O. aureus precludes establishment of body weight at maximum salinity tolerance. In Fig. 10c, data for O. aureus and O. niloticus are superimposed and compared with data for the M X N hybrid. As was observed for body length, the relationships between MST and body weight were very similar in O. aureus and O. niloticus. In comparison, rate of increase in salinity tolerance with weight was much faster in the M X N hybrid.

No consistent relationship between condition factor and MST was evident (Fig. 11). In *O. niloticus*, the conspicuous decline in MST from 7 to 15 days was associated with a corresponding decline in condition factor to a minimum value of 1.03 (Fig. 6). It was often observed that at 15 days post-hatching, *O. niloticus* fry were in relatively poor physical condition. It is possible that, under the rearing conditions employed, *O. niloticus* fry experienced difficulties in the transition from endotrophic to exotrophic feeding, following exhaustion of yolk reserves around seven days. The exceptionally weakened condition of the fish at 15 days could thereby result in decreased resistance



Fig. 10b. Relationship between salinity tolerance (MST) and body weight in *O. niloticus.* See Fig. 8b for explanation. The straight line best fitting these data is: In Y = 4.79 + 0.28In X; n = 10, R² = 0.86. Data for the 395-day broods were excluded from the regression analysis for reasons explained in the text.







Fig. 10c. Comparison of the relationships between salinity tolerance (MST) and body weight in *O. aureus* (\bullet), *O. niloticus* (O), and an *O. mossambicus* X *O. niloticus* hybrid (Δ). For the hybrid, plotted points represent the results of single determinations (25 fish/determination). The straight line best fitting these data is: ln Y = 5.79 + 0.40 ln X; n = 5, R² = 0.95.



Fig. 11. Relationship between salinity tolerance (MST) and condition factor in *O. niloticus* (O), *O. aureus* (\bullet) and an *O. mossambicus* ($\stackrel{Q}{\rightarrow}$) X *O. niloticus* ($\stackrel{D}{\circ}$) hybrid (\triangle). See Figs. 5, 6 and 7 for details.



Fig. 12. Relationship between survival time and body length (\bullet), body weight (O), and condition factor (X) following direct transfer of 60-day freshwater-spawned and reared *O. aureus* to seawater (32 ppt). Survival time is time till death following transfer from freshwater to seawater (each plotted point represents a single individual). The straight line best fitting these data is: In survival time = 4.68 + 0.45 In body weight; n = 25, R² = 0.82; survival time = -9.20 + 2.86 body length; n = 25, R² = 0.91.

to environmental stress. However, in view of the overall lack of correlation between condition factor and MST in all three species, it is inferred that, although salinity tolerance may have been lowered under conditions of poor health, size was nevertheless the primary factor determining tolerance. Hence, salinity tolerance was acquired concomitant with growth (Fig. 12) and tolerance could not be predicted solely on the basis of fish health as indicated by condition factor.

General Discussion

There are numerous reports in the literature which describe salinity tolerance characteristics in various species of tilapias. These include descriptive accounts of the ranges of salinities over which a species is found to grow and reproduce naturally, reports on small-scale experiments in brackishwater aquaria, concrete tanks and earth ponds, on production trials in seawater ponds, and on survival studies performed under laboratory conditions. These have been summarized in several recent reviews (Balarin and Hatton 1979; Chervinski 1982; Philippart and Ruwet 1982; Wohlfarth and Hulata 1983). Interpretation of these results, representing a combination of descriptive and experimental evidence, is difficult for a number of reasons. Descriptive data on natural distributions are often ambiguous since the distribution of a species depends upon a number of interacting factors (e.g., temperature, depth, oxygen, current velocity), only one of which is salinity. Interspecific comparisons are often confounded by the fact that the salinity limits for survival, reproduction and growth may be quite different in a given species, and that these limits have not been accurately defined for most species. Furthermore, the salinity tolerance limits exhibited by unacclimatized fish upon transfer from freshwater may be very different from the tolerance limits displayed following gradual acclimatization. This is exemplified by O. aureus which reproduces at a maximum salinity of 19 ppt (Chervinski and Yashouv 1971), survives direct transfer from freshwater to 20-25 ppt (Lotan 1960), grows well at salinities as high as 36-45 ppt (Chervinski and Yashouv 1971), and can withstand a salinity of 53.5 ppt if gradually acclimatized (Lotan 1960).

Provided that the assay conditions employed by an investigator are defined, the use of salinity tolerance tests in which survival is monitored at predetermined salinities under laboratory conditions should provide a better basis for accurate interspecific comparisons of tolerance limits. Unfortunately, experimental determinations of salinity tolerance in the tilapias have so far employed different test criteria under environmental conditions which have sometimes remained unspecified.

In the present study, it was determined that laboratory-reared O. aureus from 7 to 120 days of age tolerate (50% survival at 96 hours) a maximum salinity of 19.2 ppt (range = 17.5-22 ppt) following direct transfer from freshwater, at a temperature range of 24.4 to 31°C. These results appear to be lower than the maximum tolerance range of 20-25 ppt previously reported for this species (Lotan 1960). Differences in assay conditions may have influenced these results. Tilapia rendalli (8-12 cm) was experimentally determined to tolerate (100% survival at 24 hours) a maximum salinity of 19 ppt following direct transfer from freshwater, at a temperature range of 20-28°C. Tolerance was observed to decline outside this optimum temperature range (Whitfield and Blaber 1976). In O. niloticus and O. aureus, salinity tolerance is influenced by temperature, being markedly higher at a temperature range of 19-24°C than at 24-31°C (Watanabe, unpublished data). T. zillii (1-3 g) was reported to have a maximum salinity tolerance range of 23.4 to 27.3 ppt (50-70% survival at 24 hours) following direct transfer from freshwater at an unspecified temperature range (Chervinski and Hering 1973). The unacclimatized salinity tolerance limits for an O. niloticus X O. aureus hybrid were set at 20 ppt (Payne 1983), although assay criteria were not specified. The need to standardize procedures for salinity tolerance indexing in order that more precise interspecific comparisons can be made is evident. In Taiwan, present efforts toward the development of strains or hybrids suitable for culture under saline conditions have further emphasized the importance of a reliable index for routine monitoring of improved tolerance in experimental stocks.

In view of the commercial importance of *O. niloticus* in many areas of the world, it is surprising that there is a lack of detailed investigation of the salinity tolerance of this species. *O. niloticus* is reputed to be less salt tolerant than *O. aureus*. However, apart from a single report in which a salinity of 12.5 ppt was experimentally determined to be lethal in this species (Fukusho 1969, in Balarin and Hatton 1979), there has been little evidence to support any firm conclusions regarding the relative tolerance of these two species. It is noteworthy, therefore, that in the present study, MLS-96 values for combined ages from 7 to 120 days were found to be virtually identical in *O. aureus* and *O. niloticus*, under identical assay conditions. Body size:salinity tolerance relationships were also very similar in these species. Due to a lack of data for *O. aureus* over a greater range of sizes, the possibility that *O. aureus* may attain relatively higher tolerance levels in individuals of larger sizes cannot be excluded.

Chervinski (1961b) noted that following transfer of *O. niloticus* from freshwater to 60% seawater "big fish survived better than young" from which he concluded that large fish may be better able to survive in brackishwater than the young. Increasing salinity tolerance with size is a well documented phenomenon in the anadromous salmonid fishes (Hoar 1976; Folmar and Dickhoff 1980; Wedemeyer et al. 1980). Parry (1960) demonstrated that in the Atlantic salmon (*Salmo salar* L.), salinity tolerance (median survival time) in various dilutions of seawater gradually increased with age from fry to smolt stage. This increase was determined to be closely related to size; in a given age group, larger fish were found to have higher survival values than smaller ones. These results were further demonstrated in several salmonid species (Conte and Wagner 1965; Conte et al. 1966; Wagner et al. 1969; Wagner 1974).

The subtle increases in salinity tolerance with size observed in O. aureus and O. niloticus in the present study are less dramatic than those associated with parr-smolt transformation in salmonids. For example, in a study of ontogenetic changes in salinity tolerance in chinook salmon (Oncorhynchus tshawytscha), maximum salinity tolerated following direct transfer from freshwater increased from 15 ppt at hatching to 30 ppt by three months following absorption of the yolk sac (Wagner et al. 1969). In contrast, generalized survivorship curves for O. aureus and O. niloticus in the present study show that survival declined rapidly at salinities exceeding 17.5 ppt in broods of all ages from seven days (yolk sac absorbed) through 120 days. In both species, no significant changes in MLS-96 with age were observed. The M X N hybrid, however, exhibited more pronounced changes in tolerance with age, with MLS-96 ranging from a minimum of 17.5 ppt at 30 days to a maximum of 26.7 ppt at 60 days. It is noteworthy that at 60 days, a survival rate of 10% was observed following direct transfer to a relatively high salinity of 30 ppt. These results suggest that considerable variability may exist between tilapia species in the acquisition of salinity tolerance with size. Speciesspecific variations in the acquisition of salinity tolerance with size are well known among salmonids ranging from those able to tolerate full seawater only at relatively large sizes, such as steelhead trout (S. gairdneri) (Conte and Wagner 1965), to those which are able to tolerate full seawater while still in the alevin stage, such as chum (O. keta) and pink salmon (O. gorbuscha) (Weisbart 1968). These variations have been explained in terms of phylogenetic proximity to the ancestral freshwater form (Hoar 1976). It may similarly be hypothesized that in the tilapias, variations in rate of acquisition of tolerance with size are associated with phylogenetic relationships to the ancestral marine form (Chervinski 1961a).

In non-smolting species such as non-anadromous rainbow trout (S. gairdneri) or brown trout (S. trutta), which normally spend their entire life in freshwater, salinity tolerance is also known to increase with body size (Houston 1961; Boeuf and Harache 1982). However, following transfer to seawater, these species show alterations in blood ion concentrations requiring much longer to regulate than anadromous species. Fish size is, nevertheless, an important criterion for determining optimum time for seawater transfer in non-smolting species (Landless and Jackson 1976; Boeuf and Harache 1982).

Increasing salinity tolerance with size has often been explained in terms of body surface:volume relationships. Larger fish are subjected to less osmotic stress than smaller ones since the ratio of gill area to body weight decreases as the body weight of the fish increases (Parry 1960). Increasing tolerance with size has also been explained as being related to the functional development of the osmoregulatory system. In chinook salmon, larger fish have higher gill Na-K activated ATPase activities, supporting the idea that the osmoregulatory system is more functional in larger fish (Ewing et al. 1980). Since larger fish at a given age are faster growing, it has been proposed that growth rate is important; faster growing fish possessing a more functional or easily initiated osmoregulatory system (Wagner et al. 1969).

Clarke (1982) observed that although maximum hypoosmoregulatory ability during seawater challenge tests was similar among various salmonid species, the body size at which it developed oiffered between species. He inferred that the correlation between size and hypoosmoregulatory ability was probably related to maturational events rather than to body surface:volume relationships. Similar conclusions may be reached based on the differences in rate of acquisition of tolerance with size observed among the tilapias in this study.

Size-related ontogenetic changes in hemoglobin have been observed in salmonids. In Atlantic salmon, juvenile hemoglobin (Hb A), associated with the freshwater phase of the life cycle, becomes less predominant as the fish increases in size, while adult hemoglobin (Hb C) gradually increases in predominance (Koch 1982). Ontogenetic changes in hemoglobin have also been observed in tilapia. In *O. mossambicus*, a second hemoglobin appears at 47 days which has a higher affinity for O_2 than larval hemoglobin at higher osmotic pressure and temperature (Perez and Maclean 1976). These workers suggest that this second hemoglobin enables the adults to tolerate both warmer and more saline environments. In the present study, the increasing salinity tolerance observed in both *O. aureus* and *O. niloticus* beginning between 45 and 60 days may possibly be related to such a change.

Smoltification in salmonids is often associated with a sharp decline in condition factor due to a decrease in body lipid (Fessler and Wagner 1969). No consistent relationship between condition factor and salinity tolerance was found in the present study apart from the suggestion that in *O. niloticus*, an extremely low condition factor (< 0.011) may be associated with lowered tolerance.

In coho salmon (O. kisutch), premature transfer of fish to seawater results in poor survival and growth (Clarke and Nagahama 1977; Fryer and Bern 1979; Mahnken and Waknitz 1979; Folmar et al. 1982). Critical size in freshwater was found to be the best predictor of mortality after transfer to seawater net pens (Mahnken et al. 1982). Results of the present study demonstrate that size-related differences in salinity tolerance exist among tilapias spawned and reared in freshwater, and that these differences may vary between species. As these relationships may similarly influence survival and growth following transfer of freshwater-reared progeny to seawater, they should be considered when selecting proper time for transfer.

Assuming that growth in seawater is compromised to maintain osmoregulation and that salinity tolerance, as measured by MST, is a fair indicator of osmoregulatory ability, then maximum growth in seawater is likely to occur during periods of maximum tolerance. It follows that if freshwater-spawned and reared stocks are to be grown in seawater, best results are likely to be achieved by implementing seawater transfer at size of maximum salinity tolerance. The benefits of this approach would be expected to be greater in "species" such as the M X N hybrid, which show relatively greater changes in tolerance with size. In species showing relatively modest increases in tolerance with size (e.g., *O. aureus* and *O. niloticus*) this approach may provide an advantage during culture in brackishwater, rather than in full seawater. Experiments comparing the growth of tilapia transferred to seawater at different sizes are required to test this hypothesis.

Results of the present study suggest that hybrids of *O. mossambicus* and *O. niloticus* have higher salinity tolerance than *O. niloticus*. *O. mossambicus* is considered to be amongst the most salt tolerant of the tilapias, growing well in ponds at salinities from 32 to 40 ppt, reproducing at salinities as high as 49 ppt (Popper and Lichatowich 1975), and adapting to salinities as high as 120

ppt (Whitfield and Blaber 1979). Hybrids of *O. niloticus* and *O. mossambicus* have been reported to grow faster with better food conversion than either parent species (Avault and Shell 1967). Red tilapia, thought to be a hybrid between *O. niloticus* and *O. mossambicus*, were recently reported to grow faster in brackishwater and seawater than in freshwater during mixed-sex rearing experiments (Liao and Chang 1983). All of these results support the possibility of producing hybrids between *O. miloticus* and *O. mossambicus* and *O. niloticus* and *O. mossambicus* and *O. niloticus* which exhibit good growth and food conversion under saline conditions.

The existence of size-related differences in salinity tolerance in the tilapias has some important practical implications for saltwater tilapia culture. When freshwater is limited, it is economically advantageous to implement seawater transfer as early as possible by maximizing initial freshwater growth to size of maximum salinity tolerance. This may be achieved, for example, through temperature control (Novotny 1975) in combination with the use of recirculation and aeration systems to minimize volume (Landless and Jackson 1976). This may also be achieved through application of growth promoters such as anabolic steroids and thyroid hormones (Higgs et al. 1982; McBride et al. 1982). Hybridization experiments in salmonids have demonstrated the feasibility of producing progeny which can be transferred to seawater at very small sizes, which also reduces freshwater requirements (Novotny 1975).

A combination of hybridization (to increase salinity tolerance levels) and maximization of early freshwater growth through temperature control and hormone application (to minimize freshwater requirements) should optimize conditions for economic culture of tilapia in brackishwater or seawater. These possibilities provide a framework for future research.

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