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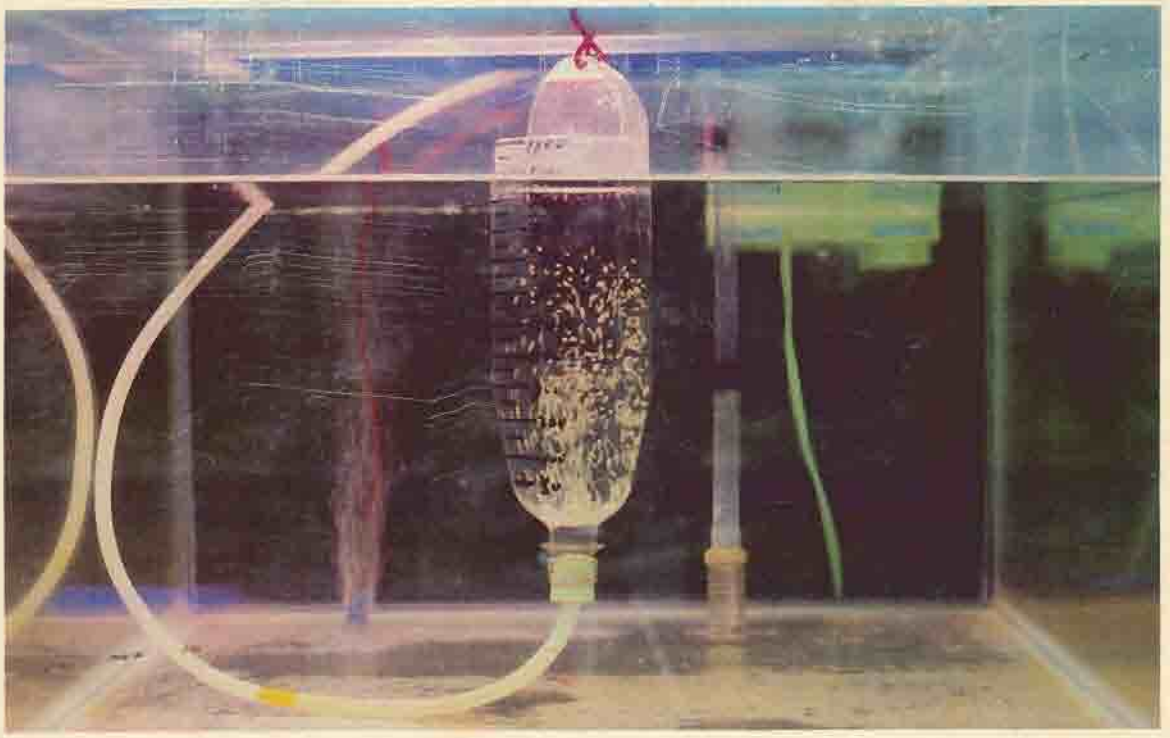

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Cover: An incubator of the type used for experimental rearing of *O. niloticus* in various salinities.

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Experimental Rearing of Nile Tilapia Fry *(Oreochromis niloticus)* for Saltwater Culture

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Abstract

Fertilized eggs of the Nile tilapia (*Oreochromis niloticus* L.) spawned in freshwater, were removed from mouthbrooding females one day post-spawning and artificially incubated at elevated salinities. Mortality during artificial incubation occurred primarily during early development and generally increased with increasing incubation salinity. At six days post-hatching, mean survivals of 85.5, 84.4, 82.5, 56.3, 37.9, 20.0 and 0% were recorded for broods incubated at salinities of 0, 5, 10, 15, 20, 25 and 32 ppt, respectively. Fertilized eggs exhibited a 96-hour median lethal salinity (MLS-96) of 18.9 ppt, identical to that of 7 to 120-day old fry and fingerlings. Fertilized eggs, however, exhibited a much higher median survival time (ST_{50} = 978 min) than 7 to 395-day old fry and fingerlings (ST_{50} = 28.8 – 179.0 min), reflecting the ability of eggs to survive direct seawater transfer for longer periods of time than fry or fingerlings.

The reproductive performance of yearling *O. niloticus* broodstock was monitored under laboratory conditions at various salinities and results compared with the performance of an older (two to three-year) broodstock in freshwater. Spawning was observed in salinities ranging from freshwater to full seawater (32 ppt). Mean hatching successes were similar for eggs spawned by yearling females in freshwater (30.9%), 10 ppt (32.7%) and 15 ppt (36.9%). Extremely poor hatching success was obtained with eggs spawned in full seawater. Mean hatching success was considerably higher for eggs spawned at 5 ppt (51.6%) and compared with that obtained with eggs spawned by older females in freshwater (54.2%). Seasonal egg and fry production per female was much greater in the older broodstock in freshwater than in yearling females in any salinity. However, seasonal egg and fry production per unit weight was greater in yearling females in salinities of 5 to 15 ppt than in older females in freshwater.

The salinity tolerance of fry spawned at various salinities and fry spawned in freshwater but hatched at various salinities, was determined using the median survival time (ST_{50}), mean survival time (MST) and 96-hour median lethal salinity (MLS-96) indices. For comparative purposes, fry spawned and hatched in freshwater were acclimatized to various salinities and their salinity tolerances likewise determined. Fry salinity tolerance progressively increased with increasing salinity of spawning, hatching, or acclimatization. However, at equivalent salinity, early exposure (spawning) produced progeny of comparatively higher salinity tolerance than those spawned in freshwater and hatched at elevated salinity. Similarly, at equivalent salinity, progeny spawned in freshwater but hatched at elevated salinity exhibited higher salinity tolerance than those spawned and hatched in freshwater, then acclimatized to an elevated salinity.

The utility of these methods of early salinity exposure toward the saltwater culture of tilapias is discussed.

Introduction

Although tilapia culture is limited primarily to freshwater and low salinity brackishwater at present, it has been widely suggested that euryhaline tilapias could be cultured in higher salinity brackishwater and marine systems, thereby enabling their exploitation in arid lands and coastal areas. The realization of these important objectives has been impeded by an inadequate research base on their biology and culture with respect to salinity tolerance. For recent reviews see Chervinski (1982) and Payne (1983).

The general approach to saltwater tilapia culture has been to produce seed and juveniles in freshwater, followed by growout in brackishwater or seawater. In an earlier study (Watanabe et al., 1984), ontogenic changes in salinity tolerance were observed in several tilapia species. In *Oreochromis niloticus* and *O. aureus,* salinity tolerance increased from relatively low values over the initial 45 to 60 days post-hatching, to maximal values from 150 to 180 days post-hatching. Hybrid progeny of *O. mossambicus* (♀) X *O. niloticus* (♂) exhibited a comparatively faster rate of increase in tolerance with age. These ontogenic changes in salinity tolerance were determined to be more closely related to body size than to chronological age. Assuming that maximal survival and growth in seawater would result if transfer from freshwater was implemented at the size of maximum salinity tolerance, these results have provided a rational basis for selecting the optimal time for transfer of freshwater-spawned and reared stocks to seawater for growout.

A knowledge of optimal transfer size also minimizes freshwater requirements by allowing the culturist to implement transfer at the earliest possible time. However, when freshwater is severely limited, costs associated with spawning and early rearing in freshwater may still outweigh the benefits of improved survival and growth attributable to this approach. Furthermore, the amount of fish that can be produced in freshwater can limit total production, as has been demonstrated for salmon (Landless and Jackson 1976).

An alternative approach to the problem of saltwater tilapia culture is to expose the fish to low concentrations of seawater at very early stages of their life cycle in order to pre-adapt them to subsequent rearing at higher salinities. This approach may involve, for example, the exposure of freshwater-spawned and hatched progeny to elevated salinities soon after hatching. Exposure may be performed at an even earlier stage of development by removing fertilized eggs from the mouth of the parent female for artificial incubation and hatching at elevated salinities. Alternatively, if successful spawning is achieved at elevated salinities, the eggs are exposed to a saline environment immediately after oviposition when they leave the ovarian fluid. A knowledge of the relative effects of these various methods of early exposure on the salinity tolerance of resultant progeny would be of considerable practical importance to the saltwater tilapia culturist.

The present study represents a preliminary evaluation of the utility of these various approaches of early salinity exposure for saltwater culture of tilapias. The reproductive performance of the Nile tilapia *(Oreochromis niloticus* L.*)* was monitored under laboratory conditions at various salinities, and the salinity tolerance of progeny determined. Survivorship of fertilized eggs, spawned

in freshwater but removed from the mouth of the parent female and artificially incubated at various salinities, was also evaluated, and the salinity tolerance of resultant fry determined. The salinity tolerance of fry spawned and hatched in freshwater but subsequently acclimatized to various salinities was also determined. Finally, the salinity tolerances of fry subjected to these various methods of early exposure were compared.

Materials and Methods

The experiments described in this report were conducted at the National Sun Yat-Sen University, Institute of Marine Biology, Kaohsiung, Taiwan, from March to December 1983.

SPAWNING IN FRESHWATER LABORATORY AQUARIA

The adult Nile tilapia *(Oreochromis niloticus)* broodstock used 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, mature coloration, and caudal fin barring (Lee 1979). Fuller descriptions have now been published in Trewavas (1983). Breeders ranged in size from 99 to 277 g in initial body weight.

Spawnings were conducted in freshwater in indoor 120-liter glass aquaria (60 x 60 x 40 cm) at 24 to 31°C, under natural photoperiod with diffused sunlight through several laboratory windows. A semi-closed recirculation system was employed with water constantly recirculated by airlift through box-type gravel filters situated inside each aquarium. Feces were siphoned out 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 stocked 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 checked daily for spawning activity and the spawner and date of observation of each spawn were recorded whenever mouthbrooding was observed.

HATCHING SUCCESS OF FERTILIZED EGGS SPAWNED IN FRESHWATER BUT ARTIFICIALLY INCUBATED AT VARIOUS SALINITIES

Eggs were removed from female mouthbrooders approximately one day post-spawning, counted, then evenly distributed into artificial incubators in various salinities (0, 5, 10, 15, 20, 25, and 32 ppt). The number of eggs ranged from 164 to 224 per incubator. Eggs were transferred directly from freshwater to the salinity of incubation. Water of varying salinities was prepared by diluting seawater obtained from the Hsitzewan Beach in Kaohsiung with tap water. Both seawater and tap water were conditioned by airlift recirculation through a 6-cm bed of oyster shells for several days prior to use.

Artificial incubators were fashioned from 1.2-l clear plastic bottles having a cylindrical base and a cone-shaped section near the spout (see Plate 1). A half section was removed from the bottle base to create an opening for placement and removal of eggs and fry and a concave, perforated disc was fitted near the spout to serve as a shower device. The incubator was suspended (spout down) from a PVC pipe support in a 100-l tank containing approximately 80 l of water of appropriate salinity. A continuous current of filtered, recirculated water from an electrically operated external aquarium filter was provided to the incubator through a tygon tube connected to the bottle spout by means of a rubber stopper. Flow rate of water through the incubator was adjusted to approximately 1.75 l/min by adjusting the degree of incubator submergence, thereby regulating the amount of water vented through ports preceding the incubator inlet point. This was achieved either by adjusting the length of the suspension wire or by adjusting the tank water level. Incubated eggs were maintained in suspension by the constant flow of water through the incubator.

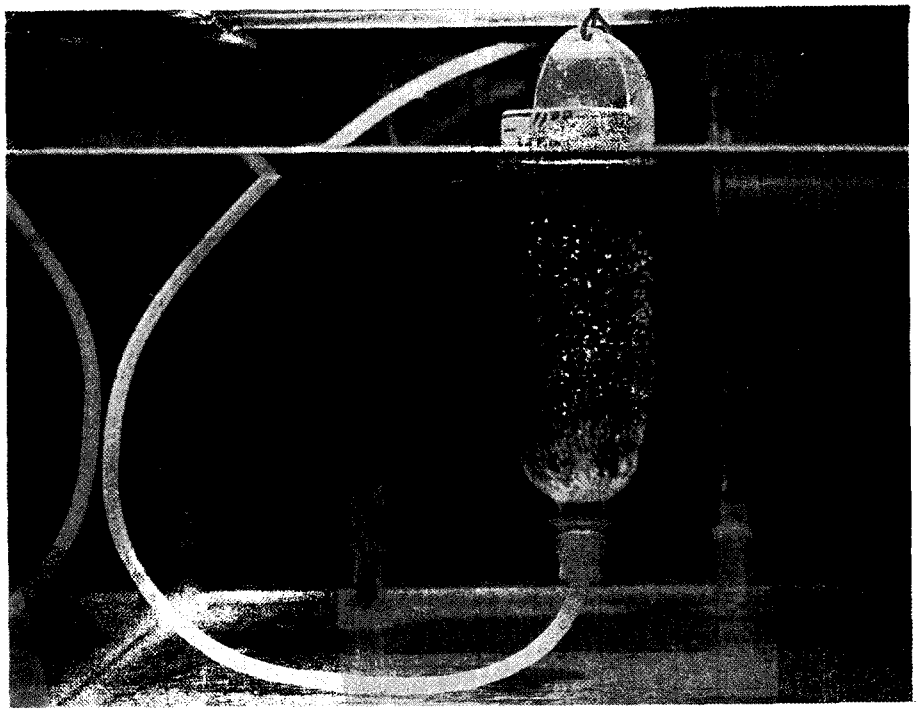

Plate 1. Incubator used for experimental breeding of *O. niloticus*, as described in the text.

Incubation was conducted at 27.2 to 31.5°C. Dead embryos and larvae were counted and removed daily. Dissolved oxygen, pH, total NH_4-N and total NO_2-N were monitored in each incubator on alternate days. Dissolved oxygen (DO) levels were maintained near air saturation (6.3 to 8.5 ppm) and pH ranged from 8.0 to 8.5 during incubation in all salinities. Total NH_4-N and NO_2-N concentrations did not exceed 0.06 and 0.02 mg/l, respectively, in any salinity during incubation.

Hatching occurred approximately three days post-spawning. Hatched larvae were allowed to remain in the incubators until yolk sac absorption was completed at approximately six to seven days post-hatching. The salinity tolerance of seven-day old fry was determined using the MST, ST_{50} and MLS-96 indices described below.

REPRODUCTIVE PERFORMANCE OF YEARLING *O. NILOTICUS* IN LABORATORY AQUARIA AT VARIOUS SALINITIES

Twenty-five *O. niloticus* individuals (mean initial length, weight: 7.2 cm, 6.2 g) from a single brood, spawned and reared in freshwater until 232 days post-hatching, were acclimatized to seawater (32 ppt) over a period of six days at a rate of approximately 5 ppt per day. A control group consisting of 25 individuals (mean initial length, weight: 6.9 cm, 5.3 g) of the same brood was retained in freshwater. Seawater-acclimatized and control groups were both held in white 100-l plastic aquaria under semi-closed system conditions as described earlier.

At 40 days following initiation of acclimatization (272 days post-hatching), one female in the seawater-acclimatized group (length, weight: 9.6 cm, 12.6 g) was observed to be mouthbrooding eggs. The eggs were removed from the mouth of the female and artificially incubated in seawater (32 ppt) although no embryonic development was observed. At 118 days, following initiation of acclimatization (350 days post-hatching), another female in the seawater-acclimatized group (length, weight: 10.0 cm, 16.0 g) was observed mouthbrooding. No embryonic development was observed during artificial incubation. One female in the freshwater control group (length, weight: 11.0 cm, 18.8 g) was observed to be mouthbrooding eggs at 312 days post-hatching, although eggs were not removed for artificial incubation. Such reproductive activity in full seawater suggested the feasibility

of comparing the reproductive performance of seawater-acclimatized individuals following re-acclimatization to reduced salinities.

Seawater-acclimatized individuals (mean initial length, weight: 9.9 cm, 16.6 g) were subsequently distributed to salinities of 32, 15, 10, and 5 ppt and their reproductive performance monitored parallel to that of the freshwater controls. Spawnings were conducted in indoor 120-l glass aquaria at 27.0-30.2°C, under natural photoperiod conditions. Semi-closed system conditions were employed as described earlier. Approximately one-third of the tank volume was replaced with conditioned water of equivalent salinity each week. Three individually-tagged females and three males were maintained in each aquarium. Each tank was observed daily for spawning activity. Whenever a female was observed to be mouthbrooding, spawner and spawn date were recorded. Eggs were removed from the mouth approximately one day post-spawning and artificially incubated at equivalent salinity. Incubators were monitored daily in order to establish date of hatching (age 0 days). Hatched larvae were allowed to remain in the incubators until yolk sac absorption was completed at approximately six to seven days post-hatching. The salinity tolerance of six to nine-day old fry was determined using the MST, ST_{50} and MLS-96 indices described below.

SALINITY TOLERANCE OF FRESHWATER-SPAWNED AND HATCHED FRY ACCLIMATIZED TO VARIOUS SALINITIES

O. niloticus fry (4 to 10 days post-hatching) of a single brood spawned and hatched in freshwater were transferred directly to acclimatization salinities of 5, 10 and 15 ppt. Seven to eight days following transfer, the salinity tolerance of the fry was determined using the MST and ST_{50} indices described below. Fish were fed daily *ad libitum* the pelletized commercial tilapia diet during the acclimatization period. Feeding was discontinued beginning on the day of tolerance testing.

SALINITY TOLERANCE INDICES

Seawater was obtained from Hsitzewan Beach in Kaohsiung and filtered by recirculation through a 6-cm bed of 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-l 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 the salinity to which the brood had been pre-exposed (during spawning, hatching, or acclimatization) to varying test 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 before weighing. Total length was determined to the nearest 0.01 cm. 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 salinity of pre-exposure directly into each of the test salinities. Dead individuals were counted and removed daily over a period of four days (96 hours). Final survival (percent) in each test 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%.

(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 salinity of pre-exposure to full seawater (32 ppt). Twenty-five individuals were employed 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 (ST_{50}) defined as the time at which survival falls to 50% following direct transfer from salinity of pre-exposure to full seawater.

Direct transfers between salinity of pre-exposure and the test salinities were performed under isothermal conditions. Experiments were conducted at temperatures of 27 to 30.2°C. 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.

Results and Discussion

HATCHING SUCCESS OF FERTILIZED EGGS SPAWNED IN FRESHWATER BUT ARTIFICIALLY INCUBATED AT VARIOUS SALINITIES

Fig. 1 presents a generalized survivorship pattern for fertilized *O. niloticus* eggs 96 hours following direct transfer from the freshwater spawning medium to artificial incubators in various salinities. Eggs were removed from the mouth of the parent female approximately one day post-spawning. This survivorship pattern resulted in a MLS-96 of 18.9 ppt (range = 16.0 – 27.6 ppt, n = 4). For comparative purposes, a generalized survivorship pattern for 7 to 120-day old freshwater-spawned and reared *O. niloticus* fry and fingerlings, 96 hours following direct transfer to various salinities, is superimposed in Fig. 1.

Although both patterns showed an identical MLS-96 value of 18.9 ppt, some important differences between these patterns are evident. Variations between broods with respect to survival in any given salinity were more pronounced for developing embryos (i.e., fertilized eggs) than for fry and fingerlings. Appreciable embryo mortality occurred in all salinities, including freshwater. However, embryos were better able to tolerate direct transfer to salinities of 20 ppt and above. For example, no fry or fingerlings from 7 to 120 days old survived 96 hours following direct transfer to 25 ppt. In comparison, a mean survival of 20.5% was recorded for embryos 96 hours following direct transfer to this salinity. No embryos survived 96 hours following direct transfer to full seawater (32 ppt). However, it is evident from the daily survivorship pattern of embryos transferred directly to full seawater (Fig. 2) that there was some survival for as long as 48 hours following transfer. Embryos survived direct seawater transfer for much longer periods of time than 7 to 395-day old fry and fingerlings, which survived for a maximum of five hours following direct seawater transfer at comparable temperatures. The survivorship pattern for embryos transferred directly to full seawater (Fig. 2) results in a median survival time (ST_{50}) of 978 min, a value far greater than those observed for 7 to 395-day old *O. niloticus* fry and fingerlings, which ranged from 28.8 to 179.0 min (Watanabe et al. 1984).

Daily survival of freshwater-spawned *O. niloticus* eggs during artificial incubation in various salinities is presented in Table 1. These survivorship patterns are illustrated in Fig. 2. As these results show, mortality during artificial incubation occurred primarily during early development from day of removal through one day post-hatching. Mortality generally increased with increasing salinity and was particularly heavy in the higher salinities of 20 to 32 ppt. In full seawater, (32 ppt), there was no survival during this period. After the first day of incubation (age one day), survival remained

relatively high (range 85.4-94.0%) in all salinities up to 25 ppt. In full seawater, however, mean survival fell to 25.5% during this period. Differential mortality in the various salinities became gradually more pronounced over the second and third day of incubation so that distinct differences

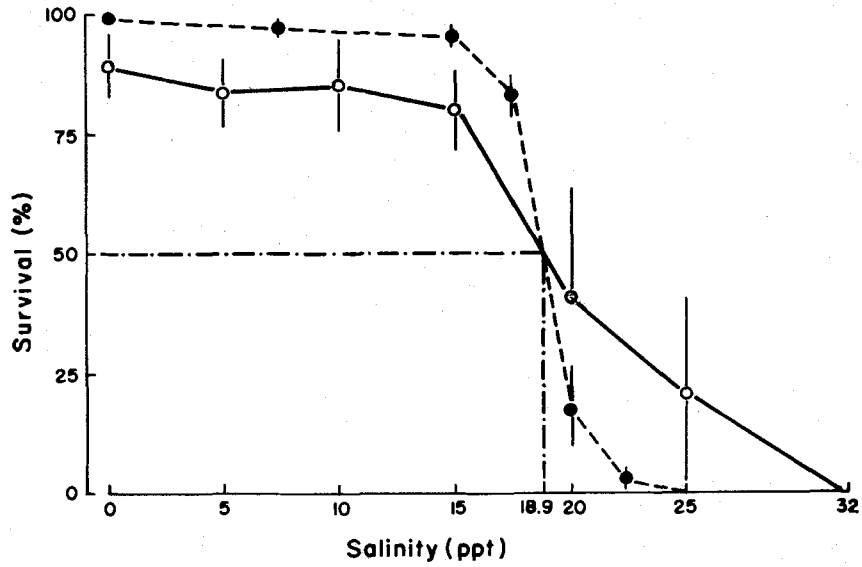

Fig. 1. Survivorship of freshwater-spawned eggs (○) and 7 to 120-day old freshwater-spawned and reared fry and fingerlings (●) of *O. niloticus*, 96 hours following direct transfer to various salinities. Eggs were removed from the mouth of the parent female approximately one day post-spawning. Each plotted point represents the mean value for four determinations for eggs and 20 determinations for fry and fingerlings. Vertical bars represent ± S.E.M. (Standard Error of the Mean). Both survivorship patterns result in a MLS-96 of 18.9 ppt.

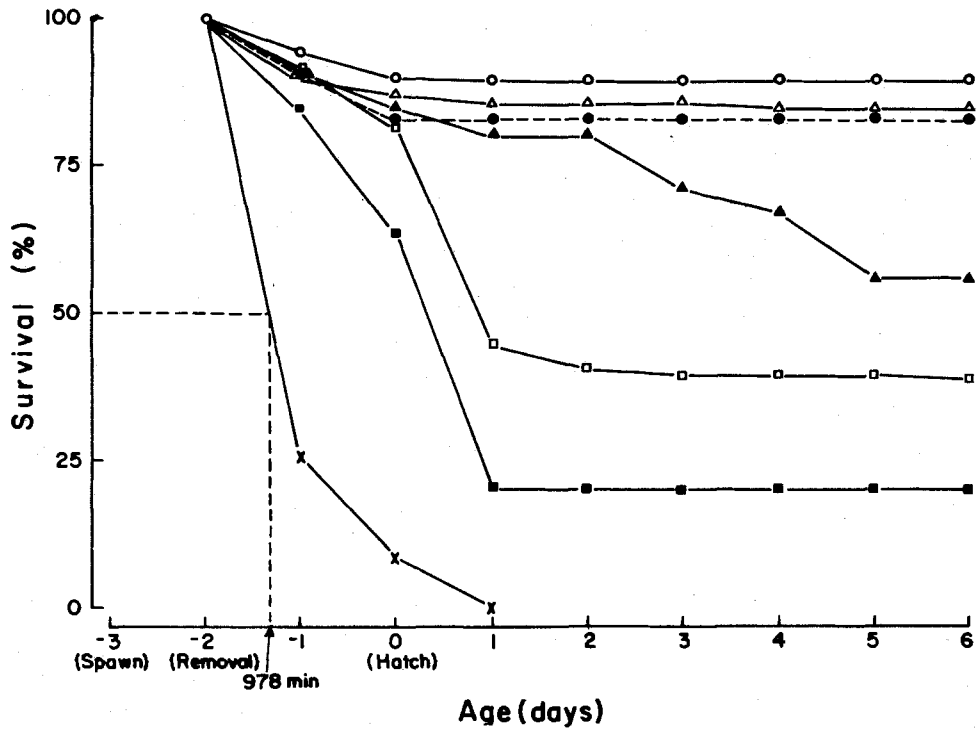

Fig. 2. Survivorship of freshwater-spawned *O. niloticus* eggs, artificially incubated at various salinities (freshwater (○), 5 ppt (●), 10 ppt (△), 15 ppt (▲), 20 ppt (□), 25 ppt (■), 32 ppt (X)). Plotted points represent mean values of Table 1.

Table 1. Daily percentage survival of freshwater-spawned *O. niloticus* eggs artificially incubated at various salinities.

Incubation salinity (ppt)	Age: (days post-hatching)	−3 (spawn)	−2 (remove and incubate)	Age: (days post-hatching) −1	0 (hatch)	1	2	3	4	5	6 (yolk sac absorbed)
0			100	94.0 ± 4.7[a] (80.0 − 99.4)[b]	90.3 ± 6.5 (70.8 − 98.8)	89.4 ± 6.3 (70.8 − 98.2)	89.2 ± 6.3 (70.8 − 98.2)	89.0 ± 6.2 (70.8 − 97.6)	88.6 ± 6.3 (70.0 − 97.6)	88.6 ± 6.3 (70 − 97.6)	88.5 ± 6.3 (70 − 97.6)
5			100	92.0 ± 5.8 (74.6) − 99.4)	83.3 ± 7.5 (62.3 − 96.3)	83.1 ± 6.9 (63.9 − 94.5)	83.0 ± 6.9 (63.9 − 94.5)	82.6 ± 7.1 (63.1 − 94.5)	82.5 ± 7.0 (63.1 − 94.5)	82.5 ± 7.0 (63.1 − 94.5)	82.5 ± 7.0 (63.1 − 94.5)
10			100	91.0 ± 7.5 (68.5 − 99.4)	87.4 ± 8.9 (60.8 − 98.8)	85.9 ± 9.2 (58.5 − 98.2)	85.5 ± 9.6 (56.9 − 98.2)	85.5 ± 9.6 (56.9 − 98.2)	84.4 ± 9.3 (56.9 − 98.2)	84.4 ± 9.3 (56.9 − 98.2)	84.4 ± 9.3 (56.9 − 98.2)
15			100	90.9 ± 5.8 (74.6 − 98.8)	84.7 ± 6.4 (67.7 − 95.1)	80.8 ± 7.9 (62.3 − 95.1)	80.6 ± 8.0 (62.3 − 95.1)	72.2 ± 7.3 (61.6 − 92.9)	64.2 ± 12.9 (30.8 − 92.9)	56.3 ± 19.9 (0 − 92.9)	56.3 ± 19.9 (0 − 92.9)
20			100	92.2 ± 4.9 (78.5 − 98.8)	82.4 ± 8.7 (57.7 − 96.3)	45.2 ± 22.2 (0 − 91.6)	41.1 ± 22.4 (0 − 90.7)	39.2 ± 23.1 (0 − 89.7)	38.9 ± 23.0 (0 − 89.7)	38.9 ± 23.0 (0 − 89.7)	37.9 ± 22.6 (0 − 89.7)
25			100	85.4 ± 11.8 (50.0 − 99.4)	63.5 ± 16.4 (20.8 − 90.3)	21.5 ± 20.2 (0 − 82.1)	20.5 ± 19.8 (0 − 79.9)	20.0 ± 20.0 (0 − 79.9)	20.0 ± 20.0 (0 − 79.9)	20.0 ± 20.0 (0 − 79.9)	20.0 ± 20.0 (0 − 79.9)
32			100	25.5 ± 14.5 (0 − 51.2)	7.9 ± 4.0 (0 − 31.7)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

[a]Mean ± S.E.M. (Standard Error of the Mean) for 4 incubation trials.
[b]Range.

in survival values between salinities were observed by one day post-hatching. Following this period of early mortality, survival generally stabilized through six days post-hatching, although some mortality continued at 15 ppt. Survivorship patterns for eggs incubated at salinities of 5 and 10 ppt were closely similar to the pattern observed for eggs incubated in freshwater. At six days post-hatching, mean survival values of 82.5, 84.4 and 88.5%, respectively, were recorded in these salinities. Progressively greater mortality occurred with increasing incubation salinity. At six days post-hatching, mean survival values of 56.3, 37.9 and 20.0% were recorded for incubation salinities of 15, 20 and 25 ppt, respectively. However, as the standard errors and ranges of Table 1 indicate, survival varied considerably between broods incubated in salinities of 15 ppt and above. For example, at incubation salinities of 15 and 20 ppt, survival values at six days post-hatching ranged from 0 to 92.9% and from 0 to 89.7%, respectively.

No consistent relationship was observed between incubation salinity and time to hatching. Poor survival and hatching of eggs incubated in the higher salinities was associated with various structural abnormalities generally characterized by an underdevelopment of organs.

SALINITY TOLERANCE OF FRY HATCHED FROM FRESHWATER-SPAWNED EGGS ARTIFICIALLY INCUBATED AT VARIOUS SALINITIES (FRESHWATER-SPAWNED, SALINE WATER-HATCHED FRY)

Mean body weights, lengths, and condition factors of seven-day old freshwater-spawned, saline water-hatched fry are presented in Table 2. The single brood successfully hatched at a salinity of 25 ppt had a mean body length and weight which was conspicuously greater than those of broods incubated and hatched in lower salinities. However, there were no significant ($P > 0.05$, t-test) differences between fry hatched in any salinity and those hatched in freshwater with respect to these parameters.

Corresponding ST_{50}, MST, and MLS-96 values of seven-day old fry hatched at various salinities are also presented in Table 2. Since the maximum salinity employed during MLS-96 indexing was 32 ppt, a relative tolerance value was not provided when survival exceeded 50% in all salinities 96 hours following transfer from hatching salinity (MLS-96 > 32 ppt). Therefore, in order to obtain a representative MLS-96 value for fry hatched in a given salinity, mean survival over all trials was computed for each transfer salinity and MLS-96 determined from the resultant generalized survivorship pattern. Fig. 3 illustrates these generalized survivorship patterns for seven-day old freshwater-spawned, saline water-hatched fry, 96 hours following direct transfer to various salinities. As Fig. 3 shows, MLS-96 progressively increased from 19.2 ppt for fry hatched in freshwater to greater than 32 ppt for fry hatched at 20 ppt and above. The survivorship patterns of Fig. 3 reveal that increasing MLS-96 with increasing hatching salinity was related to an elevation of the salinity of incipient mortality. With each 5 ppt increase in hatching salinity, salinity of incipient mortality was elevated by 2.5 to 5 ppt. Incipient mortality for fry hatched at 0, 5, 10, 15, 20 and 25 ppt occurred at approximately 17.5, 20, 22.5, 27.5, 30 and 32 ppt, respectively. Increasing hatching salinity also expanded the range between incipient and final mortality. For example, incipient and final mortalities for the freshwater-hatched broods occurred within a relatively narrow salinity range of 17.5 to 22.5 ppt. In contrast, for broods hatched at 10 ppt, incipient mortality occurred at 22.5 ppt and mortality was still not complete at 32 ppt. Hence, whereas survival declined rapidly as salinity exceeded the level of incipient mortality for fry hatched in freshwater, a progressively more gradual decline in survival was observed as salinity exceeded the level of incipient mortality for fry hatched at elevated salinities.

Since the time period employed for salinity tolerance testing was 96 hours, a relative ST_{50} value was not provided whenever survival did not fall to 50% by 96 hours following direct transfer from hatching salinity to full seawater ($ST_{50} > 5{,}760$ min). Therefore, in order to obtain a representative ST_{50} value for fry hatched in a given salinity, mean survival over all trials was computed at successive time periods following transfer, and ST_{50} derived from the resultant generalized

Table 2. Median survival time (ST_{50}), mean survival time (MST) and median lethal salinity (MLS-96) of seven-day old *O. niloticus* fry hatched from freshwater-spawned eggs artificially incubated at various salinities.

Incubation salinity (ppt)	Body weight (mg)[a]	Body length (mm)[a]	Condition factor[a]	ST_{50} (min)[b]	MST (min)[a]	MLS-96 (ppt)[b]	Assay temperature range (°C)
0	7.8 ± 0.4 (6)	8.9 ± 0.3 (6)	1.14 ± 0.06 (6)	51.0 (6) 42.0 – 71.0	51.0 ± 4.7 (6) 4.20 – 73.4	19.2 (4) 18.5 – 22.0	27.2 – 29.8
5	8.1 ± 0.8 (5)	9.1 ± 0.2 (5)	1.07 ± 0.05 (5)	59.0 (5) 46 – 150	86.2 ± 27.5 (5) 45.1 – 193.3	21.2 (4)	27.4 – 30.5
10	8.2 ± 0.5 (6)	9.0 ± 0.2 (6)	1.14 ± 0.07 (6)	90.0 (6) 64 – >5,760	1,097.0 ± 934.5 (6) 65.3 – 5,760	25.0 (4) 23.8 – >32	27.4 – 29.8
15	8.0 ± 0.9 (5)	9.1 ± 0.2 (5)	1.05 ± 0.06 (5)	4,320 (5) 147 – >5,760	3,411.2 ± 1,073.8 (5) 154.6 – 5,760	30.2 (4) 26.5 – >32	27.3 – 30.5
20	7.7 ± 1.4 (3)	9.0 ± 0.3 (3)	1.03 ± 0.09 (3)	>5,760 (3) 1,402 – >5,760	4,089.5 ± 847.6 (3) 3,005 – 5,760	>32 (2) >32	27.3 – 29.8
25	9.8 (1)	9.8 (1)	1.04 (1)	>5,760 (1)	5,760 (1)	>32 (1)	28.4

[a]Mean ± S.E.M. (no. of determinations). Ranges of values are presented for MST.

[b]Derived from generalized survivorship patterns as described in text. Range is given below median point; no. of determinations in parentheses.

survivorship pattern. Fig. 4 illustrates these generalized survivorship patterns as a function of time for seven-day old freshwater-spawned, saline water-hatched fry following direct transfer from hatching salinity to full seawater (32 ppt). As Fig. 4 shows, ST_{50} increased from 51.0 min for fry hatched in freshwater, to greater than 5,760 min for fry hatched at 20 ppt or above. Mortality occurred primarily during the initial eight hours following transfer from hatching salinity to full seawater (32 ppt), then stabilized thereafter through 96 hours. Increasing hatching salinity resulted in a progressive increase in the percentage of individuals surviving 96 hours following direct transfer from hatching salinity to full seawater (32 ppt). Fry hatched in freshwater or 5 ppt exhibited complete mortality within the initial two or four hours, respectively, following transfer. In contrast, fry hatched in 10, 15, 20 and 25 ppt exhibited mean survival values of 28.7, 51.3, 69.3 and 100%, respectively, 96 hours following transfer.

REPRODUCTIVE PERFORMANCE OF YEARLING *O. NILOTICUS* IN LABORATORY AQUARIA AT VARIOUS SALINITIES

Results of studies on the reproductive performance of yearling *O. niloticus* broodstock in laboratory aquaria at various salinities are summarized in Table 3. The data represent spawnings recorded from 30 May to 18 October 1983. For comparative purposes, Table 3 also presents results of a separate study on reproductive performance of *O. niloticus* in freshwater laboratory aquaria, which employed an older (two to three years) and much larger (mean seasonal body length, weight: 21.5 cm, 203.9 g) broodstock consisting of 16 females. For the older broodstock, data represent spawnings recorded from 26 March to 18 October 1983.

For yearling females, total number of spawnings was greater in the brackish salinities of 5 through 15 ppt than in either full seawater (32 ppt) or freshwater. Low spawning frequency in full seawater can in part be explained by a tendency for the mean interval between spawnings to lengthen in the higher salinities from approximately 18 days at 5 and 10 ppt, to 22.9 days at 15 ppt, and to 31.0 days at 32 ppt. However, as the intervals between successive spawnings varied over a considerable range in any given salinity, these differences were not statistically significant ($P > 0.05$, t-test). The total number of spawnings in each salinity was also influenced by the number of times each female in a salinity group spawned during the period of observation. For example, more spawnings were recorded in 15 ppt than in either 10 ppt or 5 ppt, despite the fact that the mean interval between spawnings was longer in 15 ppt. This was because during the period of observation, each female in the 15 ppt group spawned an average of 6.7 times, whereas each female in the 10 ppt and 5 ppt groups spawned an average of only five times. Only single spawnings from each female were observed in the freshwater control group. In comparison, each female in the older broodstock in freshwater spawned an average of 3.8 times, and the mean interval between spawnings when eggs were removed was 19.7 days: very similar to that observed for yearling females spawning in salinities of 5 to 15 ppt.

For yearling females, the mean number of eggs released per spawning was roughly equivalent at all salinities, although somewhat lower at 15 ppt. When the data are expressed as mean number of eggs released per g body weight (seasonal average), results at 15 ppt were closely similar to those observed in the other salinities, indicating that lower mean number of eggs released per spawning at 15 ppt was related to the smaller sizes of females in this salinity. Mean number of eggs spawned per g body weight under saline conditions did not differ significantly ($P > 0.05$, t-test) from the mean value for yearling females spawning in freshwater. In comparison, females of the older broodstock in freshwater released a mean of 961.3 eggs per spawn, which greatly exceeded the values obtained for yearling females. However, when these results are expressed as number of eggs released per g body weight (seasonal average), older and larger females released a mean of only 5.2 eggs per g body weight, a value considerably lower than those observed for yearling females, regardless of salinity. The mean number of eggs released per g body weight by the older females was significantly

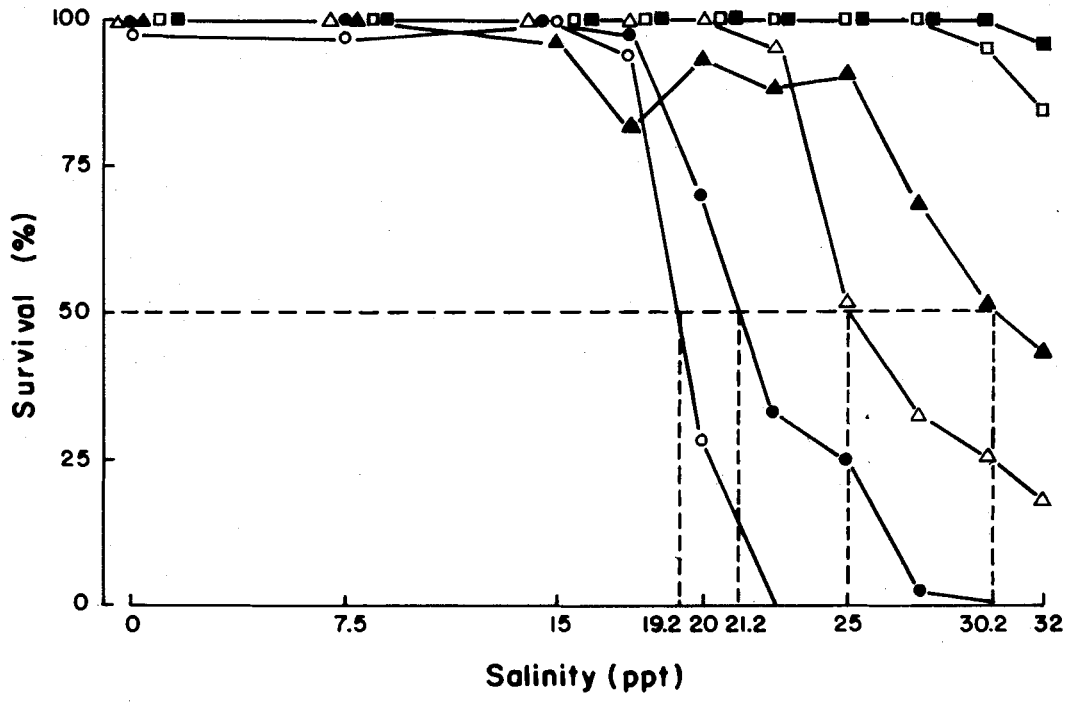

Fig. 3. Survivorship of seven-day old freshwater-spawned, saline water-hatched *O. niloticus* fry, 96 hours following direct transfer from hatching salinity to various salinities. Hatching salinities were: freshwater (○), 5 ppt (●), 10 ppt (△), 15 ppt (▲), 20 ppt (□) and 25 ppt (■). Each plotted point represents the mean survival value for replicate determinations. Number of determinations for each hatching salinity is shown in Table 2. MLS-96 values of Table 2 were derived from these generalized patterns.

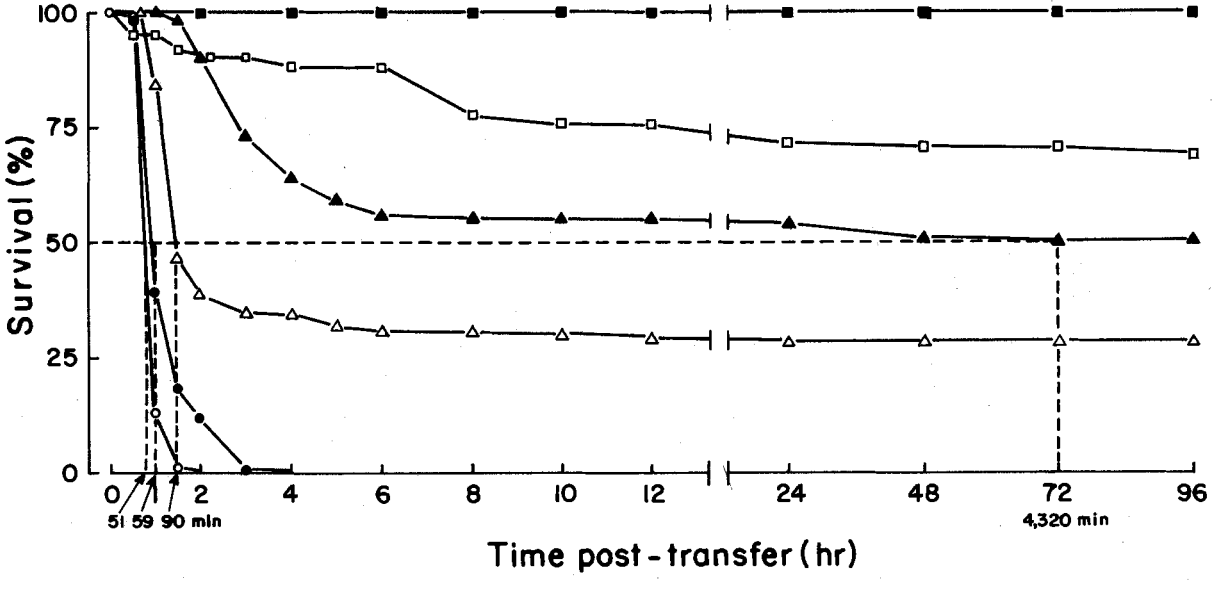

Fig. 4. Survivorship as a function of time for seven-day old freshwater-spawned, saline water-hatched *O. niloticus* fry, following direct transfer from hatching salinity to full seawater (32 ppt). Hatching salinities were: freshwater (○), 5 ppt (●), 10 ppt (△), 15 ppt (▲), 20 ppt (□) and 25 ppt (■). Each plotted point represents the mean survival value for replicate determinations. Number of determinations for each hatching salinity is shown in Table 2. ST_{50} values of Table 2 were derived from these generalized patterns.

Table 3. Reproductive performance of yearling Nile tilapie *(Oreochromis niloticus)* broodstock in laboratory aquaria at various salinities, 30 May-18 October 1983[a], and comparison with older broodstock.

Salinity at spawning (ppt)	No. of spawnings[b]	Mean interval between spawnings (days)[c]	Mean no. of eggs per spawning[c]	Seasonal mean Female body length (cm), body weight (g)[c]	Mean no. of eggs per g body weight[c]	Mean hatching success (%)[d]	Total egg production (per female; per g body wt.)	Total fry production (per female; per g body wt.)
32	7	31.0 ± 8.5 (16 – 49)	260.1 ± 39.2 (136 – 400)	11.9 ± 0.4 34.3 ± 4.1	9.16 ± 1.4 (6.0 – 12.8)	0.7 ± 0.7 (7) (0 – 5)	1,821 (607; 17.7)	13 (abnormal) (0; 0)
15	20	22.9 ± 2.4 (7 – 40)	180.8 ± 21.8 (11 – 367)	10.3 ± 0.6 18.1 ± 3.3	11.3 ± 1.3 (3.8 – 30.1)	36.9 ± 9.5 (14) (0 – 99)	3,616 (1,205; 66.6)	1,334 (444.7; 24.6)
10	15	17.6 ± 2.2 (10 – 26)	295.9 ± 22.5 (189 – 446)	11.4 ± 0.7 25.4 ± 3.7	11.8 ± 0.5 (9.1 – 13.3)	32.7 ± 10.2 (11) (0 – 99)	4,439 (1,479.7; 58.3)	1,452 (484.0; 19.1)
5	15	18.3 ± 1.9 (11 – 30)	226.9 ± 22.9 (90 – 365)	11.5 ± 0.2 26.7 ± 1.4	8.3 ± 0.9 (4.7 – 14.2)	51.6 ± 8.6 (12) (0 – 93.1)	3,404 (1,134.7; 42.5)	1,757 (585.7; 21.9)
0 (yearling)	3	–	273.3 ± 70.4 (150 – 394)	11.4 ± 0.8 24.3 ± 1.5	9.6 ± 2.9 (6.6 – 15.3)	30.9 ± 30.9 (3) (0 – 92.8)	820 (273.3; 11.2)	253 (84.3; 3.5)
0 (2-3 yr)	60	19.7 ± 1.3 (13 – 37)	961.3 ± 61.8 (72 – 1490)	21.5 ± 1.3 203.9 ± 19.7	5.2 ± 0.3 (0.6 – 9.2)	54.2 ± 7.3 (35) (0 – 99.9)	57,678 (3,604.9; 17.7)	31,261 (1,953.8; 9.6)

[a]For yearling broodstock, a group of 3 females and 3 males was maintained in each salinity. For 2-3 yr old broodstock, a group of 16 females was maintained in freshwater aquaria at a ratio of 1-3 females:1 male.

[b]Total spawnings observed per group, not necessarily from the same individual.

[c]Mean ±S.E.M. Values in parentheses denote range.

[d]Data represent hatching success (non-fertile spawns included) during artificial incubation and are expressed as mean values ± S.E.M. Range is given below and no. of determinations in parentheses on the right.

($P < 0.001$, t-test) less than those of yearling females spawning in salinities from 5 to 32 ppt.

During artificial incubation of tilapia eggs, non-fertile spawns, characterized by lack of embryo formation, were occasionally observed. For eggs spawned under saline conditions, it was difficult to differentiate between non-fertile spawns resulting from male inactivity and those caused by salinity effects on egg or sperm quality. Hence, when determining mean hatching success during artificial incubation of eggs spawned in various salinities, non-fertile spawns were included (as 0% hatch) in all calculations. Extremely poor hatching success resulted with eggs spawned in full seawater (32 ppt). In one incubation trial, a few abnormal larvae were hatched, all of which died soon after hatching. Mean hatching successes were similar for eggs spawned by yearling females in freshwater (30.9%), 10 ppt (32.7%) and 15 ppt (36.9%). Mean hatching success was considerably higher for eggs spawned at 5 ppt (51.6%) and compared with that obtained with eggs spawned by the older broodstock in freshwater (54.2%). As hatching success varied from 0% to greater than 90% in any given spawning salinity up to 15 ppt, differences in mean hatching successes between these salinities were not statistically significant ($P > 0.05$, t-test).

Although total egg production was greater at 10 ppt, total fry production was greatest at 5 ppt, due to the improved hatching success at 5 ppt. Seasonal egg production per female was much greater for larger, older breeders in freshwater than for yearling breeders in any salinity. However, seasonal egg production per g body weight was greater for yearling breeders in brackish salinities than for older breeders in freshwater. Seasonal fry production per g body weight was also greater for yearling females in brackish salinities than for older females in freshwater. These results suggest that for a given total weight of fish, smaller, yearling females in brackish salinities of up to 15 ppt will produce a greater number of eggs and fry than larger females in freshwater.

SALINITY TOLERANCE OF FRY SPAWNED AT VARIOUS SALINITIES (SALINE WATER-SPAWNED FRY)

Mean body weights, lengths, and condition factors of six to nine-day old fry spawned at various salinities are presented in Table 4. Since only one successful hatching was recorded for yearling females in freshwater, it was necessary to employ fry produced in freshwater by older females as controls in Table 4. As these results show, mean body weight and length of the freshwater-spawned broods were generally higher than those of broods spawned in brackish salinities. Mean weight and length of the freshwater-spawned broods was significantly ($P < 0.05$, t-test) greater than those of broods spawned at 5 and 10 ppt. In tilapias, egg weight increases with body weight of spawner (Peters 1983). Differences in egg weight are due mainly to differences in yolk content, which should have a corresponding effect on size of fry which develop from these eggs. The relatively large size of broods spawned in freshwater may therefore be related to the much larger sizes of spawners from which they originated. Size differences between broods spawned by yearling females in brackish salinities are difficult to interpret since the ages of these broods varied from six to nine days post-hatching. Condition factors of broods spawned in brackish salinities did not differ significantly ($P > 0.05$, t-test) from those of broods spawned in freshwater.

Corresponding ST_{50}, MST and MLS-96 values for these six to nine-day old fry spawned at various salinities are also presented in Table 4. ST_{50} and MLS-96 values were derived from generalized survivorship patterns as described earlier. Fig. 5 illustrates generalized survivorship patterns for six to nine-day old saline water-spawned fry 96 hours following direct transfer from the spawning salinity to various salinities. MLS-96 increased from 19.2 ppt for broods spawned in freshwater to greater than 32 ppt for broods spawned in 15 ppt. The patterns of these changes are very similar to those described earlier for freshwater-spawned, saline water-hatched fry (Fig. 3). As Fig. 5 shows, increasing MLS-96 with increasing spawning salinity is also related to an elevation of the salinity of incipient mortality and an increase in the salinity range between incipient and final mortality. In general, an increase in spawning salinity of 5 ppt elevated salinity of incipient mortality by 2.5 to 7.5 ppt.

Teble 4. Median survival time (ST_{50}), mean survival time (MST) and median lethal salinity (MLS-96) of 6-9 day old *O. niloticus* fry spawned at various salinities.

Spawning salinity (ppt)	Body weight (mg)[a]	Body length (mm)[a]	Condition factor[a]	ST_{50} (min)[b]	MST (min)[a]	MLS-96 (ppt)[b]	Assay temperature range (°C)
0	7.8 ± 0.4 (8)	8.8 ± 0.2 (8)	1.13 ± 0.05 (8)	51.0 (8) 29.0 – 72.0	51.1 ± 5.3 (8) 29.9 – 73.4	19.2 (6) 18.5 – 22.0	25.5 – 29.8
5	5.2 ± 0.3 (6)	8.1 ± 0.1 (6)	0.99 ± 0.05 (6)	600.0 (6) 131 – 780	970.2 ± 270.4 (6) 145.6 – 1,768.4	28.1 (4) 27 – 30.4	27.4 – 30.2
10	5.6 ± 0.3 (7)	8.1 ± 0.1 (7)	1.07 ± 0.08 (7)	360.0 (7) 59.0 – >5,760	2,218.2 ± 639.4 (7) 85.0 – 5,048.5	⩾32 (4)	27.0 – 29.5
15	6.9 ± 1.3 (6)	8.8 ± 0.3 (6)	0.93 ± 0.08 (6)	>5,760 (6)	5,326.4 ± 199.6 (6) 4,702.6 – 5,760	>32 (1)	27.0 – 30.1

[a]Mean ± S.E.M. (no. of determinations). Ranges of values are presented for MST.
[b]Derived from generalized survivorship patterns as described in text. Range of values is given below median values; no. of determinations in parentheses.

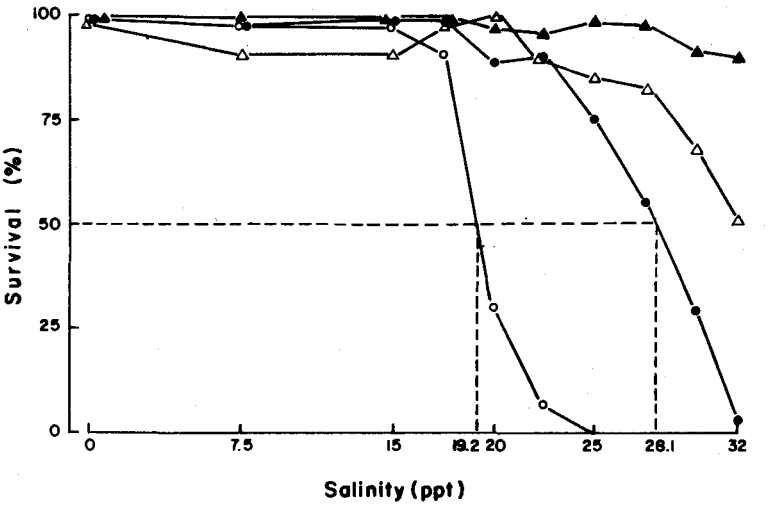

Fig. 5. Survivorship of six to nine-day old saline water-spawned *O. niloticus* fry, 96 hours following direct transfer from spawning salinity to various salinities. Spawning salinities were: freshwater (○), 5 ppt (●), 10 ppt (△), 15 ppt (▲). Each plotted point represents the mean survival value for replicate determinations. Number of determinations for each spawning salinity is shown in Table 4. MLS-96 values of Table 4 were derived from these generalized patterns.

Generalized survivorship patterns for broods spawned or hatched at identical salinities are compared in Figs. 6a, 6b and 6c for salinities of 5, 10 and 15 ppt, respectively. As these results show, at identical salinity of spawning or hatching, saline water-spawned fry exhibited higher MLS-96 values than freshwater-spawned, saline water-hatched fry. These patterns also suggest that the range between incipient and final mortality may be expanded to a relatively greater degree by increasing spawning salinity than by increasing hatching salinity. From the statistical variability of data illustrated in Figs. 6a, 6b and 6c, it is evident that saline water-spawned progeny generally exhibited more consistent survival values between broods than did freshwater-spawned, saline water-hatched progeny.

Fig. 7 illustrates generalized survivorship patterns as a function of time for six to nine-day old saline water-spawned fry following direct transfer from spawning salinity to full seawater (32 ppt). As Fig. 7 shows, ST_{50} increased from 50.9 min for broods spawned in freshwater to greater than 5,760 min for broods spawned at 15 ppt. Unexpectedly, broods spawned at 5 ppt had a higher ST_{50} than those spawned at 10 ppt. However, mean survival at 96 hours was higher in broods spawned at 10 ppt.

SALINITY TOLERANCE OF FRESHWATER-SPAWNED AND HATCHED FRY ACCLIMATIZED TO VARIOUS SALINITIES (FRESHWATER-SPAWNED, FRESHWATER-HATCHED, SALINE WATER-ACCLIMATIZED FRY)

Mean body weights, lengths and condition factors of 11 to 18-day old *O. niloticus* fry spawned and hatched in freshwater, but acclimatized for seven to eight days to various salinities, are presented in Table 5. Broods maintained in freshwater were all 15 days old while those acclimatized to salinities of 5 through 15 ppt ranged from 11 to 18 days old. Since these fry were older, they were larger than the freshwater-spawned, saline water-hatched fry (seven days old) or saline water-spawned fry (six to nine days old) whose salinity tolerances were described earlier. Corresponding ST_{50} and MST values for these 11 to 18-day old fry, spawned and hatched in freshwater, but acclimatized to

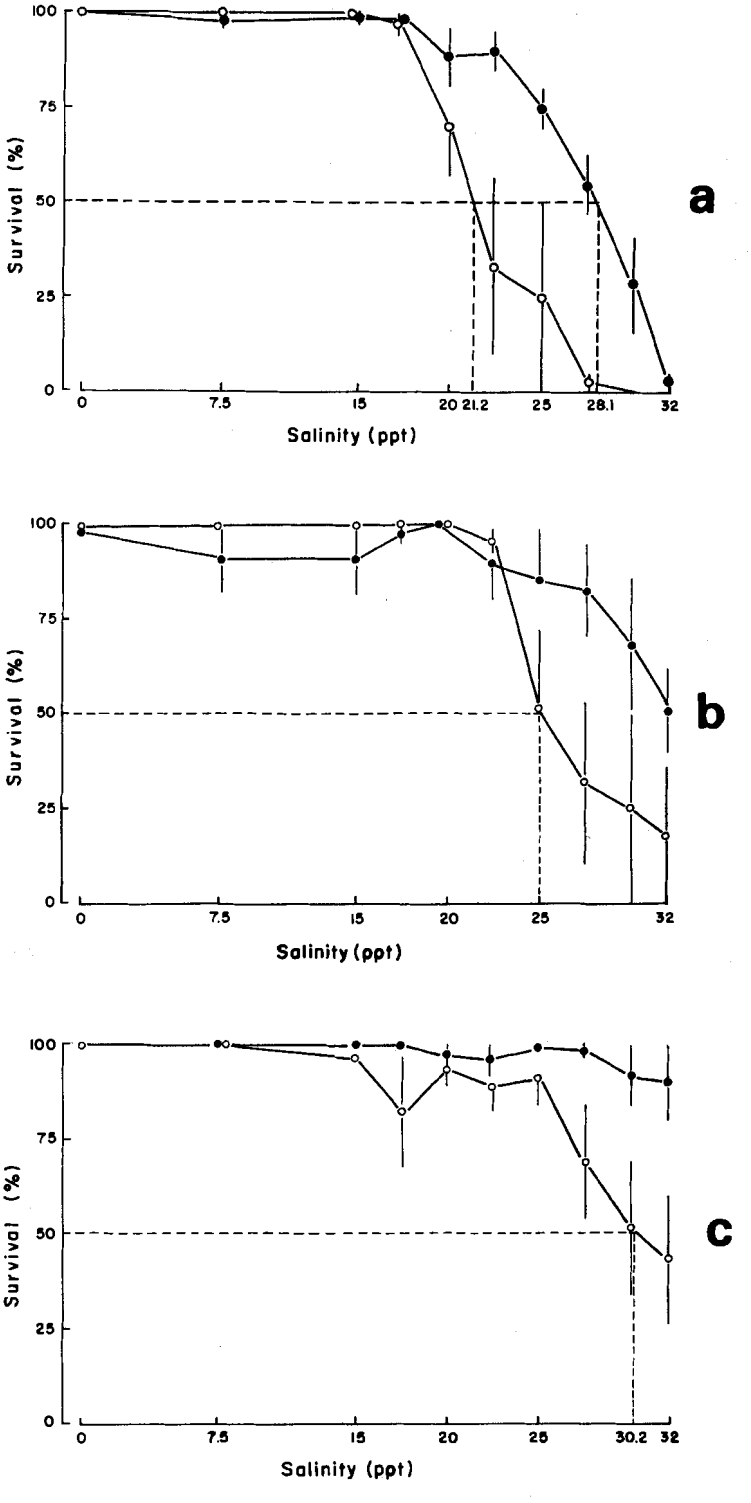

Fig. 6. Comparison of survivorship between freshwater-spawned, saline water-hatched (○) and saline water-spawned (●) *O. niloticus* fry, 96 hours following direct transfer from identical hatching or spawning salinity, respectively, to various salinities. Survivorship patterns are compared for hatching and spawning salinities of 5 ppt in Fig. 6a, 10 ppt in Fig. 6b and 15 ppt in Fig. 6c. Each plotted point represents the mean survival value for three to four determinations. Vertical bars represent ± S.E.M. Absence of vertical bars indicates that the S.E.M. lies within the area of the plotted point.

various salinities, are also presented in Table 5. ST_{50} values were derived from generalized survivorship patterns as described earlier. Fig. 8 illustrates generalized survivorship patterns as a function of time for 11 to 18-day old freshwater-spawned, freshwater-hatched, saline water-acclimatized fry following direct transfer from acclimation salinity to full seawater (32 ppt). ST_{50} progressively increased from 29.0 min for broods retained in freshwater, to 270 min for broods acclimatized to 15 ppt.

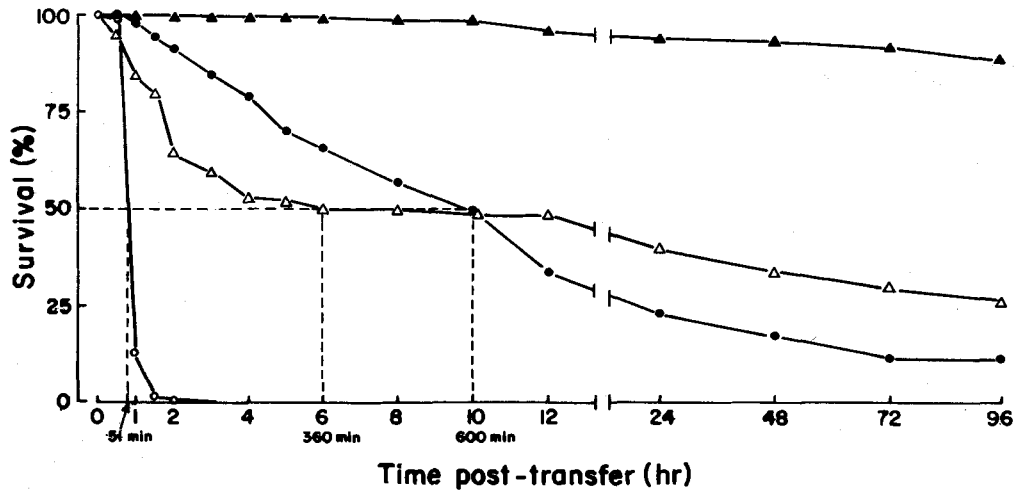

Fig. 7. Survivorship as a function of time for six to nine-day old saline water-spawned *O. niloticus* fry, following direct transfer from spawning salinity to full seawater (32 ppt). Spawning salinities were: freshwater (○), 5 ppt (●), 10 ppt (△), and 15 ppt (▲). Each plotted point represents the mean survival value for replicate determinations. Number of determinations for each spawning salinity is shown in Table 4. ST_{50} values of Table 4 were derived from these generalized patterns.

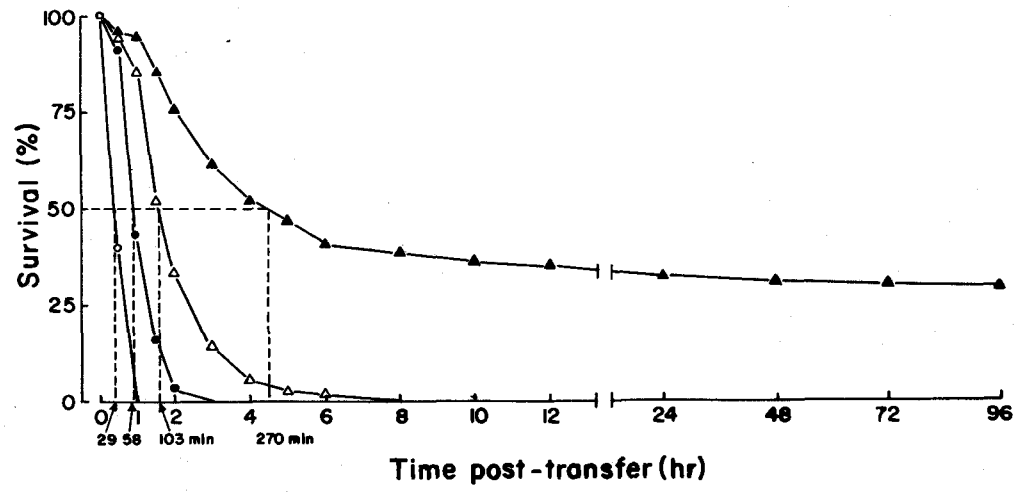

Fig. 8. Survivorship as a function of time for 11 to 18-day old freshwater-spawned, freshwater-hatched, saline water-acclimatized *O. niloticus* fry, following direct transfer from acclimetization salinity to full seawater (32 ppt). Acclimatization salinities were: freshwater (○), 5 ppt (●), 10 ppt (△), and 15 ppt (▲). Each plotted point represents the mean survival value for replicate determinations. Number of determinations for each acclimetization salinity is shown in Table 5. ST_{50} values of Table 5 were derived from these generalized patterns.

Table 5. Median survival time (ST_{50}) and mean survival time (MST) of 11-18 day old freshwater-spawned and freshwater-hatched *O. niloticus* fry following acclimatization to various salinities.

Acclimatization salinity (ppt)	Body weight (mg)[a]	Body length (mm)[a]	Condition factor[a]	ST_{50} (min)[b]	MST (min)[a]	Assay temperature range (°C)
0	12.2 ± 1.2 (4)	10.5 ± 0.2 (4)	1.03 ± 0.04 (4)	29.0 (4) 24.0 – 32.0	29.8 ± 1.9 (4) 26.2 – 34.0	25.8 – 30.2
5	11.5 ± 1.3 (6)	9.1 ± 0.3 (6)	1.51 ± 0.06 (6)	58.0 (6) 41.0 – 81.0	62.1 ± 15.3 (6) 41.6 – 83.6	27.0 – 28.0
10	11.4 ± 0.8 (6)	9.3 ± 0.1 (6)	1.42 ± 0.05 (6)	103.0 (6) 64.0 – 165.0	116.0 ± 47.6 (6) 72.2 – 185.4	27.5 – 28.0
15	12.0 ± 0.7 (6)	9.7 ± 0.2 (6)	1.32 ± 0.02 (6)	270.0 (6) 155.0 – >5,760	1,966.4 ± 1,608.0 (6) 430.2 – 4,319.3	27.2 – 28.0

[a]Mean ± S.E.M. (no. of determinations). Ranges of MST values are presented.
[b]Derived from generalized survivorship patterns. Range of values is given below median values; no. of determinations in parentheses.

Generalized survivorship patterns for broods spawned, hatched or acclimatized at identical salinities are compared in Figs. 9a, 9b and 9c, for salinities of 5, 10 and 15 ppt, respectively. As Fig. 9a shows, survivorship was similar for broods acclimatized or hatched at 5 ppt. In both groups, direct seawater transfer resulted in complete mortality within four hours. The survivorship for broods spawned at 5 ppt was distinctly different with more gradual mortality and a mean survival of 12.3% at 96 hours following transfer. At a salinity of 10 ppt, differences in survivorship between groups became more distinct (Fig. 9b). Whereas direct transfer of saline water-acclimatized broods to full seawater resulted in complete mortality within eight hours, a mean survival of approximately 28% was recorded in both the saline water-hatched and saline water-spawned broods 96 hours following transfer. At a salinity of 15 ppt, survival was considerably enhanced in all groups (Fig. 9c). Mean survival values of 30.5, 51.3 and 89.9% were recorded for saline water-acclimatized, saline water-hatched and saline water-spawned broods, respectively, 96 hours following direct transfer to full seawater.

Fig. 10 compares the relationships between salinity tolerance (MST) and spawning salinity, hatching salinity and acclimatization salinity for saline water-spawned, freshwater-spawned, saline water-hatched, and freshwater-spawned, freshwater-hatched, saline water-acclimatized fry, respectively. MST rose non-linearly with increasing spawning, hatching or acclimatization salinity. The relationship between MST and spawning salinity is very similar to that between MST and hatching salinity. Rate of increase in MST with acclimatization salinity was comparatively lower. It is evident from these relationships that at equivalent salinity, early exposure (spawning) produced progeny of comparatively higher salinity tolerance than those spawned in freshwater and hatched at elevated salinity. Similarly, at equivalent salinity, progeny spawned in freshwater but hatched at elevated salinity exhibited higher salinity tolerance than those spawned and hatched in freshwater, then acclimatized to an elevated salinity. Saline water-spawned progeny generally exhibited more consistent MST values between broods than did the saline water-hatched progeny. Mean MST values for the saline water-spawned broods were significantly ($P < 0.05$, t-test) higher than those of the saline water-hatched broods at 5 ppt, and than those of the saline water-acclimatized broods at 5, 10 and 15 ppt. Mean MST values for saline water-hatched and saline water-acclimatized broods were not significantly different at any salinity.

General Discussion and Conclusions

The ontogeny of salinity tolerance in freshwater-spawned and reared *O. niloticus* from 7 to 395 days post-hatching, was described in an earlier report (Watanabe et al. 1984). The salinity tolerance of this species during the early embryonic period of development was determined in the present study. From the generalized survivorship pattern of freshwater-spawned eggs artificially incubated at various salinities, an MLS-96 value of 18.9 ppt was derived, a value equivalent to that found earlier to characterize broods from 7 to 120 days post-hatching. Therefore, characteristic salt tolerance in *O. niloticus* is evident during the initial stages of its ontogeny.

The ability of fertilized eggs of certain teleosts to develop over a wide range of salinities has been described previously. In the plaice, *Pleuronectes platessa,* this ability was attributed to the osmoregulatory activity of the vitelline membrane which is capable of regulating the osmotic concentration of the yolk from the time of fertilization (Holliday and Jones 1967). In the herring, *Clupea harengus,* this ability was present only after completion of gastrulation, and was attributed to osmoregulatory activity of embryonic ectodermal cells rather than the vitelline membrane (Holliday and Jones 1965).

From the generalized survivorship pattern as a function of time for freshwater-spawned *O. niloticus* eggs transferred directly to full seawater (32 ppt), a median survival time (ST_{50}) of 978 min was derived, a value far greater than those found to characterize 7 to 395-day old *O. niloticus* fry and fingerlings, which ranged from 28.8 to 179 min. The relatively high ST_{50} value exhibited by

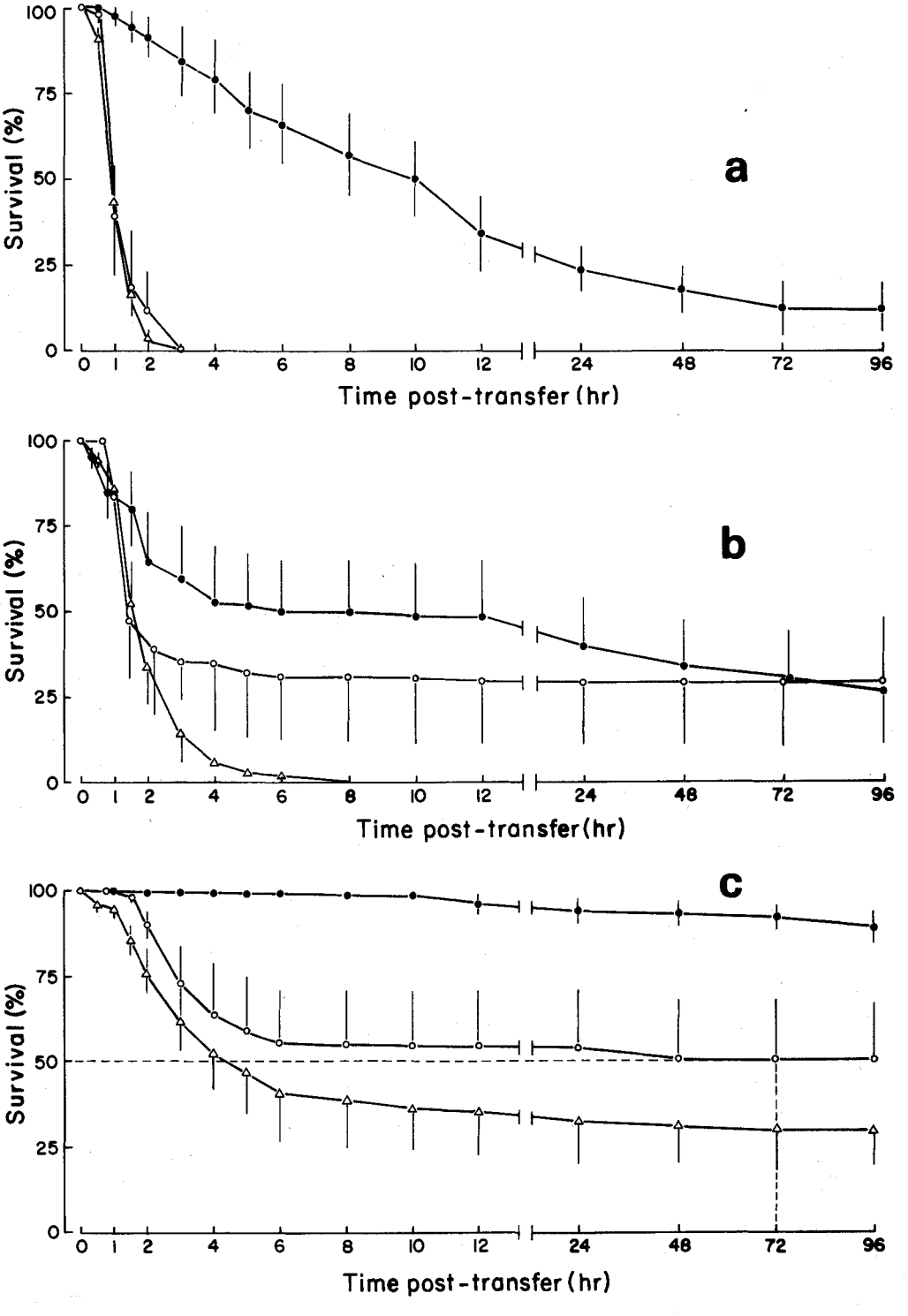

Fig. 9. Comparison of survivorship as a function of time between saline water-spawned (●), freshwater-spawned, saline water-hatched (○), and freshwater-spawned, freshwater-hatched, saline water-acclimatized (△) *O. niloticus* fry, following direct transfer from identical spawning, hatching or acclimatization salinity, respectively, to full seawater (32 ppt). Survivorship patterns ere compared for hatching, spawning, or acclimatization salinities of 5 ppt in Fig. 9a, 10 ppt in Fig. 9b, and 15 ppt in Fig. 9c. Each plotted point represents the mean value for five to seven determinations. Vertical bars represent ± S.E.M. Absance of vertical bars indicates that the S.E.M. lies within the area of the plotted point.

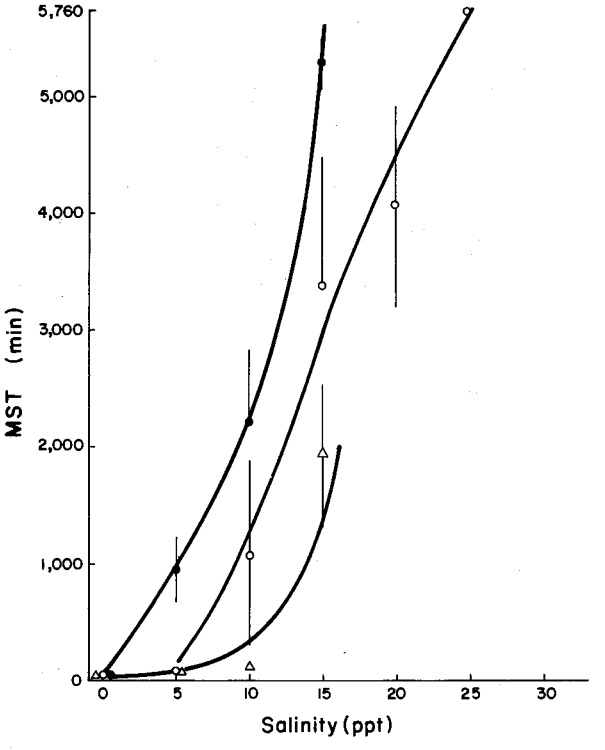

Fig. 10. Salinity tolerance (MST) of *O. niloticus* fry as a function of the spawning salinity (saline water-spawned fry) (●), hatching salinity (freshwater-spawned, saline water-hatched fry) (○), or the acclimatization salinity (freshwater-spawned, freshwater-hatched, saline water-acclimatized fry) (△). Plotted points represent mean values of Table 4, 2 and 5, respectively. Vertical bars represent ± S.E.M. Absence of vertical bars indicates that the S.E.M. lies within the area of the plotted point. Regression analysis produced relationships as follows: saline water-spawned fry, $Y = 51.10 + 253.02X - 24.05X^2 + 2.04X^3$; $R^2 = 1.00$; freshwater-spawned, saline water-hatched fry; $Y = 3.90 - 98.90X + 29.15X^2 - 0.65X^3$; $R^2 = 0.98$; freshwater-spawned, freshwater-hatched, saline water-acclimatized fry, $\ln Y = 2.68 + 0.31X$; $R^2 = 0.86$.

embryos reflects their ability to survive direct seawater transfer for longer periods of time than fry or fingerlings, although complete mortality was observed by 96 hours post-transfer for both embryos and fry or fingerlings. Weisbart (1968) found that the presence of the chorion imparted increased salinity resistance on embryos of Pacific salmon (*Oncorhynchus* spp.). He could not attribute this increased resistance to impermeability of the chorion since the perivitelline fluid of eggs immersed for four or more hours in 31.8 ppt seawater was slightly hyperosmotic to the external medium. Dechorionated embryos, nevertheless, exhibited decreased survival times in seawater. The high ST_{50} values exhibited by embryos of *O. niloticus* in the present study may similarly be related to the presence of the chorion.

The vitelline membrane can also function to protect the embryo against osmotic changes through either impermeability or osmoregulatory activity (Hempel 1979). Differential mortality during incubation in various salinities prior to hatching, however, discounts the possibility of vitelline membrane impermeability. The results are more consistent with the possibility that the vitelline membrane provides protection to the embryo through osmoregulatory activity, which becomes ineffective at high salinities. That ST_{50} values are substantially lowered following hatching further suggests that high salinity tolerance exhibited by embryos is related to the presence of the egg membranes rather than to osmoregulatory activity by embryonic ectodermal cells as postulated for herring (Holliday and Jones 1965).

The large difference in ST_{50} values exhibited by embryos and fry or fingerlings should be interpreted with some reservation, however, as embryos surviving extended periods in full seawater exhibited structural abnormalities, generally characterized by an under-development of organs. Descriptions of developmental abnormalities resulting from the effects of salinity during egg incubation have been summarized by Holliday (1969). Nevertheless, it is remarkable that eggs spawned in freshwater may be incubated and successfully hatched at salinities as high as 25 ppt, whereas 7 to 395-day old fry and fingerlings are unable to survive direct transfer to this salinity. These results may reflect a relatively greater degree of adaptability of early embryos to high environmental salinity than fry or fingerlings. Kinne (1962) proposed that in the desert pupfish *(Cyprinodon macularius),* the capacity and intensity of non-genetic adaptations to environmental salinity may be maximal during early ontogenic development. In his review of the effects of salinity on the eggs and larvae of teleosts, Holliday (1969) similarly expressed surprise at reports (Oliphan 1940, 1941) of successful hatching of several freshwater spawning species in salinities as high as 20 ppt.

No consistent relationship was observed between incubation salinity and time to hatching. It is well known, however, that the effects of salinity on development rate of teleost eggs can be profoundly modified by many factors including temperature, dissolved oxygen and genotype of parental fish (Holliday 1969). The interaction of such factors may have obscured any salinity-related effects on hatching time in the present study.

Mean body weights, lengths and condition factors of seven-day old freshwater-spawned fry hatched at various salinities were not significantly different from those of fry hatched in freshwater. Forrester and Alderice (1966) suggested that in Pacific cod *(Gadus macrocephalus),* maximum larval size was associated with those salinity and temperature conditions producing maximum survival to hatching. At 5 to 7°C, maximum survival and larval size were achieved at salinities associated with least osmotic stress. It was inferred that environmental conditions allowing maximum distribution of energy to growth, while satisfying requirements for maintenance and physical activity, should maximize survival and size of larvae in a minimum period of incubation (Alderice and Forrester 1968). Lack of significant size differences of larvae hatched in various salinities despite differential mortality in these salinities is difficult to explain on this basis.

In the present study, the salinity tolerance of fry subjected to various kinds of early salinity exposure was determined. A progressive increase in salinity tolerance with increasing exposure salinity was observed. Rao (1975) reported that the salinity tolerance of newly-hatched larvae of California killifish *(Fundulus parvipinnis)* was influenced by incubation salinity; larvae hatched in lower incubation salinities exhibited greater freshwater tolerance than those hatched at higher salinities. Conversely, tolerance of newly-hatched larvae to 70 ppt increased with salinity of incubation. Pfeiler (1981) similarly observed in bonefish (*Albula* sp.) juveniles that increasing adaptation salinity increased the upper incipient lethal salinity (defined as the salinity at which theoretically 50% of the population can survive indefinitely). That exposure to low salinities may not necessarily result in greater salinity tolerance is suggested by the observation that in Pacific salmon (*Oncorhynchus* spp.) alevins, exposure to 10 ppt for two days followed by 20 ppt for a subsequent two days, produced median survival times following seawater transfer that were the same as those for alevins transferred directly from freshwater to 31.8 ppt seawater (Weisbart 1968).

Exposure to dilute seawater may minimize the osmotic variations associated with direct transfer to full seawater. For example, Iwata et al. (1982) found that pre-acclimatization of chum salmon *(O. keta)* fry to a salinity of 12 ppt for 12 hours resulted in a gradual increase in plasma sodium to the seawater acclimatized levels. Subsequent exposure to full seawater (36 ppt) did not cause a significant change in plasma sodium level. Boeuf and Harache (1982) similarly observed that preadaptation of coho salmon *(Oncorhynchus kisutch)* to 25 ppt for three weeks suppressed the large fluctuation in internal body fluids observed during direct transfer to full seawater (36 ppt).

According to Bashamohideen and Parvatheswararao (1972), prior acclimatization of *O. mossambicus* to 75% seawater facilitated acclimatization to 100% seawater so that there was less osmotic

work and therefore less energy expended in 100% seawater. This was evidenced by a lower rate of glucose utilization.

Information on reproduction in tilapias in relation to environmental salinity is scanty. General ranges of salinities over which various species are known to reproduce have been summarized by Wohlfarth and Hulata (1983). The Nile tilapia *(O. niloticus)* was reported to reproduce, along with *T. zillii* and *S. galilaeus* at salinities of 13.5 to 29 ppt in the Great Bitter Lakes of Egypt (El Saby 1951, in Kirk 1972). However, in Lake Qarun, a former freshwater lake which became progressively more saline, only *T. zillii* continued to persist at 29 ppt after other species including *O. niloticus* and *S. galilaeus* had disappeared (El Zarka 1956, in Kirk 1972).

Chervinski (1961) observed spawning of *O. niloticus* in 50% seawater (19 ppt) during growth experiments in concrete tanks. From the small number of young produced, he inferred that relatively fewer young are produced in brackishwater than in freshwater. Similar results had been previously reported for *O. mossambicus* which produced considerably less spawns at 36.2 ppt than in freshwater (Zaneveld 1958, in Chervinski 1961). Chervinski and Yashouv (1971) noted that during growth experiments of *O. aureus* in seawater ponds, there was no reproduction, no nest building and a drop in gonadosomatic index, which they suggested to be due to a resorption of eggs.

Experimental evidence on reproductive performance of tilapias at various salinities has been lacking. In the present study, reproductive performance of yearling *O. niloticus* was monitored under laboratory conditions at various salinities. An inhibitory effect of high salinity on reproduction was also observed. In general, there was a tendency for the intervals between spawns to lengthen in higher salinities, resulting in considerably fewer spawns in full seawater than in brackishwater. These results should be interpreted with some caution, however, as resorption of ripe spawns is a common phenomenon in tilapias and the number of completed spawns may not necessarily indicate the number of spawns actually elaborated by a given fish (Peters 1983). An apparently anomalous result was that total spawns were lowest among yearling females in freshwater. In tilapias, early maturity at small sizes is thought to be a common response to unstable or stressful environmental conditions (Payne 1983). Therefore, greater spawning activity in brackish and seawater may have been related to the salinity exposure history of these individuals. Alternatively, infrequent spawning in freshwater may have resulted from greater resorption of ripe spawns for reasons which are presently unclear. No firm conclusions can be drawn on the basis of available data.

Hatching successes were comparable for yearling females at 5 ppt and older females in freshwater. However, the inhibitory effect of high salinity on reproduction was evidenced by considerably lowered hatching successes at 10 and 15 ppt. Successful hatching was not achieved in full seawater. Therefore, no fry were produced in full seawater despite the fact that eggs continued to be produced and spawned at this salinity. Rearing at high salinities has been suggested as a way to prevent overpopulation in fishponds without the need for sex separation (Chervinski and Yashouv 1971). Present results show that although *O. niloticus* failed to reproduce in full seawater, energy was nevertheless channelled into egg production in females of small sizes. Therefore, all-male rearing through sex separation, sex reversal or hybridization is still an appropriate technique for maximizing growth rates at high salinities.

In a detailed study of egg development in tilapias, Peters (1983) observed the number of eggs released per spawn to increase with body weight in the substrate spawner *T. tholloni,* as well as in the mouthbrooding species *O. mossambicus, S. melanotheron* and *S. galilaeus.* His results revealed, however, that the curves relating an increase in number of eggs spawned with increasing body weight tended to become flat when body weights reached high values, indicating that large females released relatively fewer eggs per unit weight at each spawn. Payne and Collinson (1983) similarly reported that in both *O. aureus* and *O. niloticus,* smaller females produce more eggs per unit body weight. Since adverse environmental conditions stimulate early maturity at small sizes in tilapias, relatively higher fecundity in smaller fish further enhances the chances of survival under such conditions (Payne and Collinson 1983). Present results clearly show that small, yearling *O. niloticus*

females release fewer eggs at each spawn than do older, larger females. However, in agreement with results of previous studies, larger females released fewer eggs per unit weight than did yearling females, regardless of spawning salinity.

Since smaller individuals are more productive per unit weight than larger individuals, it is important for the culturist attempting to maximize broodstock productivity to determine maximum size above which egg production per unit weight begins to decline. Higher egg productivity per unit weight has little practical value, however, if spawnings are less frequent or hatching successes relatively poorer. Results of the present study indicate that seasonal egg and fry production per unit weight was greater among yearling females spawning in brackish salinities of 5 to 15 ppt than in larger females spawning in freshwater. Therefore, seasonal fry production would be expected to be greater for smaller females for a given total weight of fish, even under brackishwater conditions.

Kinne (1962), in his experiments with the euryhaline teleost, *Cyprinodon macularius,* demonstrated that fish hatched from eggs remaining in the spawning salinity exhibited higher food conversion efficiencies than those transferred between three and six hours after fertilization into another salinity within its ecological tolerance range. For example, at a rearing temperature of 30° C, fish spawned and reared in seawater (35 ppt) showed better conversion efficiencies than those of the same brood hatched and reared in freshwater. Likewise, those spawned and reared in freshwater showed better conversion efficiencies than those of the same brood hatched and reared in seawater. His results suggest, however, that if rearing is to be performed at a salinity outside the optimal growth range for the species, then maximal growth and food conversion efficiencies may be achieved by spawning at the same salinity. Kinne concluded that the osmotic environment to which the eggs are exposed within three to six hours after spawning induces adjustments which persist throughout the lives of the fish hatched from these eggs and that these adjustments are non-genetic adaptations of the organism to environmental salinity which are not transmitted to the next generation. He further proposed that the effects of spawning salinity were based on the passage of the external medium through the egg chorion in forming the perivitelline fluid, thereby modifying the environment in which the embryo develops.

It was determined in the present study that at equivalent salinity, early exposure (spawning) produces Nile tilapia progeny of comparatively greater salinity tolerance than those spawned in freshwater but hatched at elevated salinity. These results are consistent with the idea that significant adjustments to environmental salinity are made within a short period of time following spawning. In tilapia eggs, formation of the perivitelline space due to water absorption is completed two to three hours after fertilization (Peters 1983). It may be similarly hypothesized that the osmotic environment to which eggs are exposed within two to three hours after spawning induces adjustments which affect the salinity tolerance of fry hatched from these eggs.

The relatively more consistent results obtained with saline water-spawned progeny compared to saline water-hatched progeny may be related to the fact that whereas fry spawned and hatched at a given salinity were exposed to a constant saline environment throughout development, those removed from freshwater for incubation at elevated salinities were removed at different stages of development. This resulted from the fact that since the exact time of spawning was sometimes unknown, time of removal may have ranged from 12 to 36 hours post-spawning. Hence, each brood was exposed to the freshwater spawning medium for non-identical periods of time. Broods transferred to elevated salinities at an earlier stage of development would be expected to exhibit greater salinity tolerance.

It was also determined in the present study that at equivalent salinity, Nile tilapia progeny spawned in freshwater but hatched at elevated salinity exhibit comparatively higher salinity tolerance than those spawned and hatched in freshwater, then subsequently acclimatized to an elevated salinity. These results suggest that adaptability to a given environmental salinity during early development diminishes as development proceeds. The more closely similar salinity tolerance values exhibited by saline water-hatched and saline water-acclimatized fry compared to saline water-spawned fry

also suggest that the most profound adjustments to environmental salinity are indeed made during the period of early development following fertilization.

It was determined in a previous study (Watanabe et al. 1984) that hybrid progeny of *O. mossambicus* (♀) and *O. niloticus* (♂) exhibit higher salinity tolerance than either *O. aureus* or *O. niloticus*. As size-related changes in salinity tolerance were observed in these species, it was suggested that a combination of hybridization (to increase salinity tolerance levels) and maximization of early freshwater growth to size of maximum tolerance (to minimize freshwater requirements) may optimize conditions for economic culture of tilapias in seawater. Results of the present study have demonstrated that early salinity exposure, through spawning and incubation at elevated salinities, can effectively enhance salinity tolerance levels in young tilapia fry. The feasibility for spawning under saline conditions was demonstrated by successful fry production in salinities as high as 15 ppt. Furthermore, seasonal fry production per unit weight by females in brackish salinities of 5 through 15 ppt exceeded that observed for larger females in freshwater. In addition to enhancing salinity tolerance levels of fry, early salinity exposure provides the added benefit of reducing freshwater requirements associated with broodstock holding and early rearing.

Maximization of early freshwater growth to size of maximum salinity tolerance and early salinity exposure through spawning and hatching at elevated salinities appear to be conflicting approaches. However, if increased tolerance with size is related to body surface:volume relationships (Parry 1960), to development of the hypoosmoregulatory system (Wedemeyer et al. 1980) or to ontogenic changes in hemoglobin (Perez and Maclean 1976), then it seems reasonable to assume that progeny spawned and hatched at elevated salinities would similarly exhibit ontogenic changes in salinity tolerance. In salmonids, the rate of development of the hypoosmoregulatory system is influenced by prior acclimatization to low salinities and by growth rate (Wagner et al. 1969). It follows that optimum time for seawater transfer of fry spawned and hatched in elevated salinities is when size of maximum salinity tolerance is attained. Therefore, hybridization, early salinity exposure and maximization of early growth to size of maximum salinity tolerance are all compatible techniques for saltwater tilapia culture. Temperature control, application of growth promoters or all-male rearing are all potentially useful techniques for attaining size of maximum salinity tolerance in the shortest period of time. Further experiments are required to determine the ontogeny of salinity tolerance in progeny spawned and hatched at elevated salinities.

It is not possible to equate high salinity tolerance with high growth rates in seawater on the basis of available data. However, as results to date have demonstrated that salinity tolerance varies considerably with age, size, and salinity exposure history of the individual, it is important that these factors be defined and standardized when designing experiments for evaluating the growth performance of tilapias in relation to environmental salinity.

The genetic approach to developing strains or hybrids which exhibit good growth and survival in seawater remains an important research priority for saltwater tilapia culture. In addition, non-genetic techniques such as early salinity exposure and selection of optimal transfer times may help to maximize the potential of moderately salt-tolerant species, such as *O. niloticus,* for survival and growth during culture at higher than optimal salinities.

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References

Alderice, D.F. and C.R. Forrester. 1968. Some effects of salinity and temperature on early development and survival of the English sole *(Parophrys vetulus).* J. Fish. Res. Board Can. 25(3): 495-521.

Bashamohideen, M. and V. Parvatheswararao. 1972. Adaptations to osmotic stress in the freshwater euryhaline teleost *Tilapia mossambica.* IV. Changes in blood glucose, liver glycogen and muscle glycogen levels. Mar. Biol. 16: 68-74.

Boeuf, G. and Y. Harache. 1982. Criteria for adaptation of salmonids to high salinity seawater in France. Aquaculture 28: 163-176.

Chervinski, J. 1961. On the spawning of *T. nilotica* in brackishwater during experiments in concrete tanks. Bamidgeh 13(1): 30.

Chervinski, J. and A. Yashouv. 1971. Preliminary experiments on the growth of *Tilapia aurea* (Steindachner) (Pisces, Cichlidae) in sea water ponds. Bamidgeh 23(4): 125-129.

Chervinski, J. 1982. Environmental physiology of tilapias, p. 119-128. *In* R.S.V. Pullin and R.H. Lowe-McConnell (eds.) The biology and culture of tilapias. ICLARM Conference Proceedings 7, 432 p. International Center for Living Aquatic Resources Management, Manila, Philippines.

Forrester, C.R. and D.F. Alderice. 1966. Effects of salinity and temperature on embryonic development of the Pacific cod *(Gadus macrocephalus).* J. Fish. Res. Board Can. 23(3): 319-340.

Hempel, G. 1979. Early life history of marine fish. University of Washington Press, Seattle.

Holliday, F.G.T. 1969. The effects of salinity on the eggs and larvae of teleosts, p. 293-311. *In* W.S. Hoar and D.J. Randall (eds.) Fish physiology. Vol. 1, Academic Press, New York.

Holliday, F.G.T. and M.P. Jones. 1965. Osmotic regulation in the embryo of the herring *(Clupea harengus).* J. Mar. Biol. Ass. U.K. 45: 305-311.

Holliday, F.G.T. and M.P. Jones. 1967. Some effects of salinity on the developing eggs and larvae of the plaice *(Pleuronectes platessa).* J. Mar. Biol. Ass. U.K. 47: 39-48.

Iwata, M., T. Hirano and S. Hasegawa. 1982. Behavior and plasma sodium regulation of chum salmon fry during transition into seawater. Aquaculture 28: 133-142.

Kinne, O. 1962. Irreversible non-genetic adaptation. Comp. Biochem. Physiol. 5: 265-282.

Kirk, R.G. 1972. A review of recent developments in Tilapia culture, with special reference to fish farming in the heated effluents of power stations. Aquaculture 1: 45-60.

Landless, P.J. and A.J. Jackson. 1976. Acclimatising young salmon to sea water. Fish Farming Int. 3(2): 15-17.

Lee, J.C. 1979. Reproduction and hybridization of three cichlid fishes, *Tilapia aurea* (Steindachner), *T. hornorum* (Trewavas) and *T. nilotica* (Linnaeus) in aquaria and in plastic pools. Auburn University, Auburn, Alabama. 84 p. Ph.D. dissertation.

Oliphan, V.I. 1940. Contributions to the physiological ecology of the eggs and larvae of fishes. I. The effect of salinity on early developmental stages of *Abramis brama* L., *Lucioperca lucioperca* L. and *Caspialosa volgensis* Berg. Zool. Zh. 19: 73-98.

Oliphan, V.I. 1941. Effect of salinity on the eggs and larvae of carp, vobla and bream. Vses. Nauchn.-Issled. Inst. Morsk. Nybnogo. Khoz. i Okeanogr. Tr. 16: 159-172.

Parry, G. 1960. The development of salinity tolerance in the salmon, *Salmo salar* (L.) and some related species. J. Exp. Biol. 37: 425-434.

Payne, A.I. 1983. Estuarine and salt tolerant tilapias, p. 534-543. *In* L. Fishelson and Z. Yaron (compilers). International Symposium on Tilapia in Aquaculture, Nazareth, Israel, 8-13 May 1983. Tel Aviv University, Israel.

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.

Perez, J.E. and N. Maclean. 1976. The haemoglobins of the fish *Sarotherodon mossambicus* (Peters): functional significance and ontogenetic changes. J. Fish Biol. 9(5): 447-455.

Peters, H.M. 1983. Fecundity, egg weight and oocyte development in tilapias (Cichlidae, Teleostei). ICLARM Translations 2, 28 p. International Center for Living Aquatic Resources Management, Manila, Philippines.

Pfeiler, E. 1981. Salinity tolerance of leptocephalus larvae and juveniles of the bonefish (Albulidae : Albula) from the Gulf of California. J. Exp. Mar. Biol. Ecol. 52: 37-45.

Rao, T.R. 1975. Salinity tolerance of laboratory-reared larvae of the California killifish, *Fundulus parvipinnis* Girard. J. Fish Biol. 7(6): 783-790.

Trewavas, E. 1983. Tilapiine fishes of the genera *Sarotherodon, Oreochromis* and *Danakilia.* British Museum (Natural History), London.

Wagner, H.H., F.P. Conte and J.L. Fessler. 1969. Development of osmotic and ionic regulation in two races of chinook salmon *Oncorhynchus tshawytscha.* Comp. Biochem. Physiol. 29: 325-341.

Watanabe, W.O., C-M. Kuo and M-C. Huang. 1984. Salinity tolerance of the tilapias *Oreochromis aureus* (Steindachner), *O. niloticus* (L.), and *O. mossambicus* (Peters) x *O. niloticus* hybrid. ICLARM Technical Reports 16. (In press)

Wedemeyer, G.A., R.L. Saunders and W.C. Clarke. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Mar. Fish. Rev. 42(6): 1-14.

Weisbart, M. 1968. Osmotic and ionic regulation in embryos, alevins, and fry of five species of Pacific salmon. Can. J. Zool. 46: 385-397.

Wohlfarth, G.W. and G. Hulata. 1983. Applied genetics of tilapias. ICLARM Studies and Reviews 6, 26 p. Second edition. International Center for Living Aquatic Resources Management, Manila, Philippines.