Re-evaluating euryhaline nature of Nile tilapia, *Oreochromis niloticus*: A hatchery perspective

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**Abstract**

This study re-examined Nile tilapia adaptability for various salinity uses by determining survival rates and growth parameters in 0 ppt to full 40 ppt seawater at 5 ppt treatment increments (Three replicates each) and at a 2.5 ppt daily acclimation rate. Our findings indicate that Nile tilapia could be raised at all salinity levels, including full 40 ppt seawater, but mortality could be expected at 20 ppt and above during acclimation. The Kaplan-Meier log-rank test revealed that the acclimation mortality of the 40 ppt treatment was significantly lower (p<0.05) than the other treatments, whereas there was no significant difference in the acclimation survival rates of the 20-35 ppt treatments. However, the growth parameters of the surviving fish at 40 ppt were significantly higher (p<0.05) than those in the other treatments. We recommend the production of Nile tilapia fingerlings in freshwater and their gradual acclimatization for higher salinity applications.

**Keywords:** Salinity tolerance, acclimation, euryhaline, solution dilution calculator, food security

**1. Introduction**

Tilapia (Family Cichlidae) are naturally freshwater fish but they can tolerate a wide range of salinities [1]. Historical production data (1970–2002) indicate that as a family, it is the third major contributor to aquaculture (Next to carp and salmonids), with Nile tilapia (*Oreochromis niloticus*) constituting more than 80% of the total tilapia production, followed by Mozambique tilapia (*Oreochromis mossambicus*) [1]. As a major contributor to global tilapia aquaculture production, Nile tilapia is by far the most important farmed tilapia species, was ranked 6th among the entire farmed finfish species in 2002 [1]. Recent global aquaculture production data published by the FAO [2] for 2020 shows that aside from now ranking third for inland aquaculture (Three spots higher than two decades ago), it also made a substantial contribution to marine and coastal aquaculture (ranked 13th). Its sturdy characteristics for breeding and culture, coupled with increasing consumer demand, are factors that tilapia (particularly the Genus *Oreochromis*) is recognized as the most significant aquaculture food fish worldwide [3]. Although 87.5 percent of the production still comes from freshwater, tapping its euryhaline nature can further increase global production by utilizing all possible water sources for sustainable aquaculture [4,5].

The declining supply of freshwater sources or lack thereof necessitated the culture of Oreochromis species [4,6], in brackish water and even in seawater, as in the case of Florida Red Tilapia in the Caribbean [7-11]. In addition, recent sustainable aquaculture techniques require their use as integral and complementary fish in brackish water and seawater. Cruz et al. [12], for instance, describe its role in the revival of the shrimp industry in Negros Island, Philippines, after the major collapse of the industry due to luminous bacteria outbreak in the early 1990s. The tilapias cultured in the reservoir or in cages within shrimp ponds act as bio manipulators, improving pond water quality and suppressing harmful cyanobacterial blooms and disease vectors such as luminous bacteria, which subsequently reduce production costs aside from providing additional income to the farmers. On the other hand, in more saline seawater, polyculture of *Penaeus vannamei* with Nile tilapia was proven to increase the shrimp growth rate [13].
Marapornics or marine aquaponics explores growing of salt-tolerant plant species for various purposes such as for pharmaceuticals and biofuels aside from food production. In aquaponics (or marapornics), the inherent traits of tilapia make it the most favored fish for the essential component of the system. Another sustainable technology that makes use of saline tilapia is monoculture or polyculture with shrimp species (tiger prawn *Panaeus monodon* and white leg shrimp *Litopenaeus vannamei*) in almost zero water exchange biofloc technology with the addition of a required carbon source plus probiotics. The use of Nile tilapia or red tilapia was proven to be effective in controlling pathogens causing Acute Hepatopancreatic Necrosis Disease (AHPND) in whiteleg shrimp and could enhance growth in tiger prawns. Finally, the most recent is the integration of aquaponics and biofloc systems, termed Floponics, which replaces the RAS of aquaponics with a biofloc system. Thus, there is a need to supply the increasing demand for saline-tolerant tilapia stock for the purposes of the above-stated sustainable aquaculture technologies.

The salinity tolerance of three *Oreochromis* tilapia species (Nile, Mozambique, and Florida Red Tilapia and their varieties) for aquaculture has been extensively studied to include brood stock and hatchery management in salinities above freshwater to full seawater. Among the three, information on seed production, growth, and survival of red tilapia at different salinities is the most established in the literature and has been extensively cultured in seawater. Mozambique tilapia is recognized as the most tolerant species, but it is also the least preferred commercially because of its slower growth rate. Although Nile Tilapia has the fastest growth rate and the most accessible, it is identified as the least tolerant that most authors further emphasize that a culture above 20 ppt is not possible. For this reason, experimental studies that explore tilapia conditions in the recent aquaculture technologies mentioned above in high-salinity water typically utilize red tilapia as samples.

Efforts to improve the survival of Nile tilapia at higher salinities have been made through selective breeding and genetic modifications. El-Zaeeem *et al.* introduced seabream and artemia DNAs into the gonads of fish samples. Rigorous genetic selection among Nile tilapia strains and associated species has been conducted in the Philippines and Vietnam to improve salinity tolerance. However, the role of acclimation in growing above 20 ppt has seldom been emphasized or recognized in recent studies, as the results often limit its tolerance to brackish water only (less than 20 ppt). The Marine Environment Research Department (MERD) of the Ministry of Climate Change and Environment (MOCCAE) in Umm Al Quwain, United Arab Emirates, operates a Nile tilapia hatchery for local freshwater growers. The adaptability of Nile tilapia to seawater culture was first tested when the International Center for Biosaline Agriculture (ICBA) in Dubai requested saline tilapia fingerlings and juveniles for research purposes. Although there were no formal studies at that time, MERD was able to establish that this was possible through acclimation (without specific standards), and the Nile Tilapia provided and some left at MERD tanks were able to grow and survive in seawater. Consequently, this study was initiated to assess the mortality of Nile tilapia small fingerlings (0.5 g) during acclimation and to assess growth parameters and survival during one month grow-out at various salinities in 5-ppt treatment increments from fresh to seawater. We hope that this study will provide information and hatchery protocols for preparing seeds for growing at elevated salinities, particularly in hatcheries with both freshwater and seawater sources.

2. Materials and Methods

2.1 Experimental set-up

This study was conducted at the freshwater tilapia hatchery of MERD, with both freshwater and seawater reservoirs, from 16 March to 11 May 2022. Nine experimental setups were used with each replicate, with a salinity increment of 5 ppt from 0 ppt (pure freshwater) to 40 ppt (pure seawater), were prepared indoors at room temperature. The upper limit of treatment was set at 40 ppt since refractometer readings of the seawater source averaged 40 ppt. Twenty-seven 30-litre circular plastic tanks were used in the experiment, each filled with 25 L according to the salinity requirement with one aerator each. Although a light was installed in each tank, only the room fluorescent light was turned on to mimic a common indoor hatchery setup.

Nile tilapia fingerlings (270 pieces) produced by the same set of breeders at the MERD approximately two months ago were used. Initially with freshwater, each tank was stocked with ten Nile tilapia fingerlings with average weights intended to be as homogenous as possible on 16 March 2022. The fish were allowed to settle for 5 days to recover from weighing stress prior to salinity adjustments, and any mortality observed would be replaced from the reserved tank.

2.2 Acclimation

Daily changes in salinity for treatments 5 ppt to 40 ppt started on 21 March 2022, at 2.5 ppt per day (except on Saturdays), which lasted for 22 days until 10 April 2022, when full seawater (40 ppt) was reached for the highest salinity treatment (Figure 1). From the newly placed freshwater on Day 0, 1.5 L of each tank's water was replaced with 1.5 L of seawater to increase the salinity by 2.5 ppt, following the solution dilution calculator (*C1V1=C2V2*). To raise the salinity to 5 ppt the following day, the same procedure was performed, replacing 1.5 of the current volume with 1.5 L seawater for the scheduled 2.5 ppt increase (but higher volume of seawater was needed to add to further elevate the water salinity beyond 12.5 ppt). This was performed until the desired salinity was reached for each treatment. After addition of seawater, the derived salinity was checked using a refractometer. Each week, 80% of the water (20 L) was replaced with the prepared mixture of freshwater and seawater for the desired salinity, as presented in Table 1, targeting and siphoning of the accumulated waste at the tank bottom (Days 7, 16, and 22). The number of mortalities in each tank was recorded daily to determine the acclimation survival rate.
Fig 1: The 22-day acclimation procedure from 21 March 21 to 11 April 2022, to reach the respective salinities of each treatment at 2.5 ppt per increase (all tanks started at 0 ppt). The blue vertical line indicates a rest day (Saturday) for water salinity adjustment. Adjustments on days 15 and 19 were postponed due to the number of mortalities observed in the specified tanks. Circles indicate the schedule for 80% water tank replacement.

Table 1: An exact mixture of seawater (40 ppt refract meter reading) and freshwater (0 ppt) was used to derive 25 L of water at each desired salinity or treatment, particularly during weekly 80% water tank replacement. The seawater source salinity and the salinity of the mixtures must be validated using a refract meter.

<table>
<thead>
<tr>
<th>Salinity (ppt)</th>
<th>Seawater (L)</th>
<th>Freshwater (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00</td>
<td>25.00</td>
</tr>
<tr>
<td>5</td>
<td>3.13</td>
<td>21.88</td>
</tr>
<tr>
<td>10</td>
<td>6.25</td>
<td>18.75</td>
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<tr>
<td>15</td>
<td>9.38</td>
<td>15.63</td>
</tr>
<tr>
<td>20</td>
<td>12.50</td>
<td>12.50</td>
</tr>
<tr>
<td>25</td>
<td>15.63</td>
<td>9.38</td>
</tr>
<tr>
<td>30</td>
<td>18.75</td>
<td>6.25</td>
</tr>
<tr>
<td>35</td>
<td>21.88</td>
<td>3.13</td>
</tr>
<tr>
<td>40</td>
<td>25.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

2.3 One-Month Grow-out
Those who survived during acclimation were further raised for a month to test the survival of fish for a prolonged period and the growth parameters for each salinity treatment from 11 April to 11 May 2022. Water exchange was also performed every week (For 80% of the tank volume). Mortalities were also recorded during this period to determine the grow-out survival rate, with the number surviving during acclimation used as the denominator. At the end of one month, the individual weights of the fish that survived the challenge were recorded with a strainer to drain extra water prior to weighing. The total weight gain, specific growth rate, average daily gain, and feed conversion ratio (FCR) were computed based on the final (live) and initial stocking weights [25].

2.4 Feeding and Water Quality
The feed consisted of 52% crude protein and 10% crude fat at 0.3–0.9 mm size. From the start of stocking until the end of the acclimation period, fish were fed at 10 percent of the initial biomass (Set at 5 grams), or 0.5 g each day given twice daily. The amount of feed in each tank was reduced proportionally based on the recorded mortalities. During one-month grow out period, the amount of feed was increased 0.1 g per week or 0.6 on the first week, 0.7 on the second week, 0.8 on the third week and 0.9 g on the final week (Specific for tanks with still 10 pieces). For tanks with mortalities, the exact amount of feed was computed by multiplying this number with the cumulative survival rate (The number of surviving fish divided by the initial stock of 10).

Water temperature and pH were the only water parameters tested during the experiment and ranged from 26-28 to °C and 8.1-8.2 respectively.

2.5 Re-acclimation
After the final weighing, the fish were combined into one tank for each treatment and reacclimatized at 5 ppt per day. When the tank reached 0 ppt, the fish were placed in a larger tank to record mortality. All fish were stocked in one freshwater tank after 8 days.

2.6 Statistical Analysis
Descriptive statistics and Analysis of Variance (ANOVA: single factor) were performed using the Data Analysis feature of Microsoft Excel for growth performance parameters. The Kaplan-Meier log-rank test was used to evaluate the survival statistical differences among treatments during acclimation in Excel [32].

3. Results
3.1 Survival Rates
No mortality was observed until Day 12 of the acclimation process, when two pieces died at Tank 3 of the 40 ppt treatment, the day after the salinity was increased to 27.5 ppt
Comparing the three treatments that had 30 ppt salinity on Day 15, only the 40 ppt treatment resulted in a substantial reduction with a survival rate of less than 50%. Mortalities were further observed in treatments of 35 and 40 ppt when salinity was increased to 32.5 and 35 ppt on Day 17 and 18, respectively (survival rates of 83% and 70% for 35 ppt; 27% and 20% for 40 ppt). Treatments 20, 25, and 30 had 100% survival rates when the desired salinity was reached, but some mortality was observed thereafter. Figure 2 indicates the final mortalities of the five treatments after acclimation for treatments of 0–15 ppt achieved a 100% survival rate.

![Survival of Nile Tilapia for each treatment during the respective salinity adjustments (acclimation) over time. Treatments of 0, 5, 10, and 15 ppt were not included because no mortalities were observed until the last day of the experiment. The final survival rates of the five treatments after the 22-day acclimation period are shown in the graph.](image)

![Kaplan-Meier survival curves for all treatments during the 22-day acclimation period. The results of the log rank test (n=30, DF=1, α = 0.05) classified the survival results of the treatments into three groups: 1. The 40 ppt treatment obviously differed significantly from the other treatments; 2. The survival curves of treatments 20, 25, 30, and 35 were statistically similar.; 3. The 100% survival rate of Treatments 0,5,10, and 15 was significantly higher than of the other treatments with mortalities](image)
The observed daily mortality for each treatment during acclimation is further reflected in Figure 3 or the Kaplan-Meier survival curves, which were subsequently compared using log rank test (n=30, DF=1). The results revealed three distinct survival patterns. Treatments 20, 25, 30, and 35 were statistically similar (p>0.05), although the survival distribution between 20 and 35 ppt had a P-value of 0.089 or very close to 0.05. Second, the low survival rate of 40 ppt was significantly lower than that of treatment 35, with a P-value of 0.0000002, which indicates that it is even more significantly lower than the rest of the treatment. Finally, the 100 percent mortality with replicate 2 having the lowest final mean weight of 2.5 g (Figure 4). In other replicates, with replicate 2 having the lowest final mean weight of 2.5 g (Figure 4).

Table 2: Growth and survival performance (Mean ± Standard Deviation) of Nile Tilapia grown at different salinities (3 replicates each) for 52 days (22-day acclimation plus 30-day grow-out) in reference to mean initial weights.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Salinity Treatments</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0 PPT</td>
</tr>
<tr>
<td>Mean initial Weight</td>
<td>0.57±0.03</td>
</tr>
<tr>
<td>Mean Final Weight</td>
<td>3.92±0.16</td>
</tr>
<tr>
<td>Total Weight Gained</td>
<td>3.35±0.14</td>
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<tr>
<td>Specific Growth Rate</td>
<td>3.58±0.07</td>
</tr>
<tr>
<td>Average Daily Gain</td>
<td>0.06±0.06</td>
</tr>
<tr>
<td>FCR</td>
<td>1.07±0.05</td>
</tr>
<tr>
<td>Acclimation Survival Rate (%)</td>
<td>100±0</td>
</tr>
<tr>
<td>Initial N (Start of 30-Day Grow-out)</td>
<td>10±0</td>
</tr>
<tr>
<td>Final Number</td>
<td>10±0</td>
</tr>
<tr>
<td>30-day Grow-out Survival Rate (%)</td>
<td>100±0</td>
</tr>
<tr>
<td>Overall Survival Rate (%)</td>
<td>100±0</td>
</tr>
</tbody>
</table>

3.2 Growth Parameters
The Single Factor Analysis of Variance (ANOVA) of the replicate mean final weight for each treatment (n=3) showed that only 10 ppt had a significant difference among the three replicates, with replicate 2 having the lowest final mean weight of 2.5 g (Figure 4). In other words, each replicate mean of other treatments did not differ significantly. However, the treatment means (mean of the replicates), excluding 40 ppt, correspondingly showed no significant difference. Although the final mean weight, total weight gained, specific growth rate, and daily weight gained of Treatments 10 and 15 ppt did not differ significantly with the rest of the treatments (excluding 40 ppt), the derived values were slightly lower when compared to the rest of the treatments excluding 40 ppt (Fig. 5). Consequently, their Feed Conversion Ratios are slightly higher than the rest of the treatments (Fig. 6). Furthermore, the growth parameters of 20, 25, 30, and 35 ppt treatments are almost equal to those of their low salinity counterparts (0 and 5 ppt). The results of 40 ppt treatment is significantly different with rest of the treatments (Fig. 6). ANOVA of the growth parameters of Treatments 0–35 found no significant difference.

Fig 4: Weights of the surviving fish at the end of the 52-day experiment showing replicates of each treatment (except for 40 ppt with only one surviving fish per replicate). ANOVA of the replicate means of each treatment shows no significant difference (p> 0.05) except for the 10 ppt treatment.
Fig 5: Mean Weight Gained (mean weight gained of each replicate as samples, n=3) in reference to the mean initial weight of the mean of each replicate.

Fig 6: Mean Feed Conversion Ratio (FCR) of each treatment.

Table 3: ANOVA of the growth rate parameters of the treatment means showed no significant difference (P > 0.05) among all treatments when the 40 PPT treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>ANOVA (Single Factor) All Treatments</th>
<th>ANOVA (Single Factor) Except 40 PPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α = 0.05, DF (Total)= 26, F Crit= 2.510</td>
<td>α = 0.05, DF (Total)= 23, F Crit= 2.657</td>
</tr>
<tr>
<td></td>
<td>SS (Total)</td>
<td>F</td>
</tr>
<tr>
<td>Mean initial Weight</td>
<td>0.015</td>
<td>1.014</td>
</tr>
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<td>Mean Final Weight</td>
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<td>Total Weight Gained</td>
<td>22.539</td>
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<td>Specific Growth Rate</td>
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<tr>
<td>Average Daily Gain</td>
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<tr>
<td>FCR</td>
<td>1.427</td>
<td>1.040</td>
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</table>

4. Discussion
The results of this study indicate that Nile tilapia seeds produced in freshwater can be grown through acclimation even at water salinities higher than the commonly established limit of 18-20 ppt. We recommend that this is the best option to meet the demand for saline Nile tilapia fingerlings (e.g., for polyculture with shrimp in a biofloc system at 30 ppt), particularly in areas where freshwater for brood stock is not an issue. It is also less labor-intensive for hatchery operators with both freshwater and seawater sources, which could add a premium to their business by supplying fingerlings for any desired salinity. Watanabe, et al. [33] highlighted this as a general approach to saltwater tilapia culture, but it has not been adequately emphasized in recent literature.

Although brackish water production is technically feasible in areas with a limited freshwater supply [34], the results of previous studies and our findings indicate that freshwater fingerling production is the most favorable and only to
acclimate fish to any desired salinity when needed. Watanabe and Kuo [35], for instance, emphasized that although Nile tilapia can spawn in seawater, the hatching rate is extremely poor. Fridman, et al. [23] had a similar result, adding that yolk sac larvae exposed to 15, 20, and 25 ppt had higher mortality rates, shorter lengths, and lower viability. Furthermore, El Sayed, et al. [21] reported that broodstock fed with equal protein content in freshwater had better growth, higher fecundity, higher spawning frequency, and produced eggs with higher hatchability and fry with larger weights than those reared at 7 and 14 ppt. In conclusion, the reproductive potential of Nile tilapia, including red tilapia, is significantly reduced at higher salinities but is still feasible in brackish water [36, 37, 38].

The daily acclimation increment we followed was 2.5 ppt based on earlier conservative recommendations for tilapia acclimation [39], while 5 ppt is commonly used [35, 36, 40, 41]. The first mortality was observed at 27.5 ppt, which may indicate that the fish were able to stabilize until 25 ppt, which is relatively similar to the results of Lemarie et al. [24]. They applied seven daily salinity increments from 2 to 14 ppt to test Nile tilapia resistance until 100% sample mortality. The first mortalities were observed at 30 ppt for 4–8 ppt daily salinity increments, while higher salinity daily increments of 10–14 ppt showed significant mortalities on the second day. These findings led to the conclusion that the optimal daily increase in salinity is 8 ppt per day.

Our results indicate a 100% survival rate for 0 to 15 ppt treatments, signifying that Nile tilapia fingerlings can cope well up to 15 ppt [39]. Tolerance is high because it is lower than the mean lethal salinity-96 h (MLS-96) of 18.9 ppt (critical point) established by Watanabe et al. [33], or salinity wherein the mortality is 50% after direct exposure within 96 h. Other studies further emphasized that Nile tilapia can withstand direct transfer up to 20 ppt at optimal water temperature [36, 42] but gradual acclimation is a must beyond this point to improve survival rates [43]. Comparing the mortality of the three highest treatments, the fish in the 40 ppt tanks were reduced to less than 40 percent when the salinity level reached 30 ppt on day 15 (Figs. 1, 2), which eventually caused the survival curve to be significantly lower (p < 0.05) than the rest of the treatments (Figure 3). As this did not occur in the 30 and 35 ppt tanks, the cause of mortality may be attributed to other factors aside from the salinity adjustment. In addition, the mortality encountered at treatments 20–40 indicates that deaths should be expected to occur during the acclimation process, which may not vary significantly based on the result of Kaplan-Meier log rank test (Figure 3). Furthermore, using fingerlings as small as 0.5 grams would not cause substantial loss to hatchery operators when they intend to supply saline Nile tilapia.

The high survival rate during the one-month growth window implies that the fish adjusted well to various salinity treatments after acclimation. Treatments 30 and 35 had 100% survival during this period, which was higher than treatments 20 and 25, with one mortality each. Therefore, we concluded that after acclimation, the fish could now be transported with the same or close to the salinity of their intended purpose in preparation for stocking.

Growth parameter patterns were similar to the outcome of the research of Larumbe-Moran et al. [25] to evaluate the effects of different feed protein contents (20, 30, 40, and 50%) on fry raised at four salinities (0, 15, 20, and 25 ppt). Their results indicated that the FCR for fish at 0 and 25 ppt (the highest fed 50% crude protein (as we had in this experiment) had no significant difference but was significantly lower than the FCR of the two mid-salinity treatments (15 and 20 ppt). Different FCR results were established for fish fed with 30 and 40 percent protein content; salinity treatments of 20 and 25 ppt (no significant difference) had lower FCR than those of the two lower salinity treatments, with 15 ppt having the highest. Comparably, our results also showed that treatments with 10 and 15 ppt had the two highest FCRs. The results of the two related studies indicate that feeding with 50% protein content is not optimal for the growth of Nile tilapia cultured in saline water. It is recommended feeding the stock a lower protein content of 30–40%.

The high mortality rate of the 40 ppt treatment was compensated by the higher weights of the three surviving fish, which significantly differed from the mean final weights of the other treatments (p < 0.05). Better growth performance at higher salinity (brackish water and seawater) was first documented in red tilapia which was associated with reduced aggressive behavior [5, 11, 44, 48]. Finally, the tolerance of Nile tilapia to salinity fluctuations after long-term exposure to high salinity becomes more apparent, such as when there is a need to re-acclimate them back to freshwater for breeding purposes, as shown by the zero mortality of samples, including the three fish that survived 40 ppt.

5. Conclusion

The recent developments in aquaculture, particularly in the past two decades (including green water technology, maraponics/aquaponics, marine biotechnology, and floponics) and the dwindling supply of freshwater worldwide, more specifically in arid regions, require the use of salt-tolerant tilapia. Nile tilapia is one of the best choices among tilapia species because it has the highest growth rate and the highest global production, however, it is also renowned for having the lowest salinity tolerance. With proper acclimation to the desired salinity by appropriate mixing of freshwater and seawater, we can tap its euryhaline nature for culture in brackish water and even in full-strength seawater.

6. Acknowledgement

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7. Conflict of Interest

The authors declare no conflicts of interest.

8. References


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