SEX REVERSAL OF NILE TILAPIA, *Oreochromis niloticus* L. BY EGG IMMERSION TECHNIQUE: THE EFFECT OF HORMONE CONCENTRATION AND IMMERSION TIME

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Abstract

The study evaluated sex reversal of Nile tilapia *O. niloticus* by immersing the eggs in different concentrations of 17-α methyl testosterone (0, 200, 400, 600, 800 μg l^{-1}) exposed for different times (24, 48, 72, 96 hrs). The main effects of hormone concentration (HC), immersion time (IT) and their interaction effect (HC x IT) on hatching percentage, survival and percentages of male and female were determined.

The main effects of HC and IT and their interaction significantly influenced hatching percentage, sex and survival of *O. niloticus* ($P<0.001$). Hatching percentage decreased with increased IT. Similarly, survival of treated fry in net enclosures was affected by the increasing IT. Increased per cent male was observed with increasing HC and IT. The main effect of HC showed that the highest per cent male of about 84% was obtained at 800 μg l^{-1}, followed by about 79% at 600 μg l^{-1}, 75% at 400 μg l^{-1}, about 67% at 200 μg l^{-1}, and lowest in the control (59%) ($P<0.05$). The main effect of IT that gave the highest per cent male of about 79% was at the longest duration of 96-hours. The interaction effect of HC x IT showed that the HC greater than 400 ug.l^{-1} appeared to be better in effecting higher percentages of males at longer ITs. Highest per cent male of 91% was attained at 800 ug l^{-1} HC at 96-hour IT comparable with the 88-89% in 400-600 μg l^{-1} HC at the same IT. Hence, from this study the HC and IT of eggs that gave the highest per cent male was identified. The study has proven that it is possible to induce sex reversal of *O. niloticus* by egg immersion, an alternative technique from the traditional sex reversal method of feeding the fry with hormone-treated feeds.

Introduction

Early sexual maturity in tilapia culture is a well recognized problem. There are a number of ways to control reproduction in mixed sex population. One of these is the culture of all-male tilapia. Sex reversal by oral administration of feed incorporated with methyl testosterone (MT) is probably the most effective and practical method for the production of all male tilapia. This is the most common method of sex reversal in the Philippines. However, the technique has some limitations such as the uniform age of fish that should be used at the first feeding stage to ensure high reversal rate; and less control of reversal

efficiency especially when done in the natural environment where natural food is present. Moreover, widespread use of large quantities of sex reversal hormone in hatcheries may pose a health risk to workers (Mair, 1997) and there is little information on the fate of the hormone in the effluent and ground water.

Studies on sex reversal by immersion in hormone solution of methyl testosterone (MT) are few and focused mainly on fry instead of eggs such as in Coho salmon *Onchorynhus kisutch* (Baker *et al*., 1988; Piferre *et al*., 1994); *Oreochromis aureus* (Torrans *et al*., 1988; Eckstein and Spira, 1965 and Al-Duham, 1970 as cited in Phelps and Popma, 2000); *O. mossambicus* (Varadaraj and Pandian, 1987); *O. niloticus* (Gale *et al*., 1995 and 1999; Green *et al*., 1995; Contreras-Sanchez *et al*., 1997). A successful study by Gale *et al*. (1995) showed that 3-hr exposure of *O. niloticus* fry at 10 and 13 days post fertilization in mestanolone at 500 mg l^{-1} produced greater than 93% male. There is a lack of information on sex reversal of *O. niloticus* by egg immersion technique except that reported in Thailand (Anonymous, 2002) where the immersion of 2-day old eggs in 500 μg l^{-1} of MT at 24 hours resulted in 88% male (Srisakultiew, pers. com.). The mechanism of action of the immersion technique is that the hormone is absorbed through passive diffusion across the lipid membrane of the eggs. During the embryonic development, gonadal differentiation can be affected by the administration of steroid sex hormone (Jobling, 1995) in the holding water. Straussman and Nakamura (2003), however, pointed out that the mechanism of action of exogenous steroids during sex differentiation is not sufficiently clear.

Sex reversal by egg immersion may lessen the duration of treatment and lower the cost of hormone used relative to the traditional technique of sex reversal by oral administration. This alternative technique of administering the sex reversal hormone may be of great help in hatcheries employing artificial incubation because of greater control of sex reversal and lower risk to health of workers. A study to explore this alternative technique of sex reversal is relevant. Hence, this investigation aimed to determine the effect of hormone concentration and immersion time on the hatching percentage, per cent survival and percentages of male and female of Nile tilapia *O. niloticus*.

Methodology

FAC Selected Strain (FaST) *O. niloticus* breeders (100-150 g) were conditioned in hapas or net enclosures (each with dimension of 3 m x 5 m x 1.5 m) and paired at a sex ratio of 1 male:3 female equivalent to 5 males:15 females per hapa. Two days after pairing, daily checking of eggs in the mouth of the female was done. Once eggs were observed, it was recorded as the first day of mouthbrooding. Eyed-eggs (3-4 day old) were collected from the mouth between 1600-1700 hrs and were transferred to the laboratory.

One hundred eggs were immersed in different concentrations of 17-α methyl testosterone (MT) hormone concentrations (HC) of 0, 200, 400, 600 and 800 μg l^{-1} for varying immersion times (IT) of 0 (control), 24, 48, 72 and 96 hours. The different HCs were prepared from a stock solution. Immersion was done using plastic containers (1.5 liter capacity each) which were suspended in aquarium measuring 24 cm x 50 cm x 30 cm.

Aerators were provided in each container to facilitate the continuous movement of eggs in the water column.

After immersion, treated eggs were transferred in down-welling egg incubators where they were placed for hatching. Fry were placed in the incubators for period of 5-7 days after which they were reared in net enclosures. Fry whose yolk sac have been absorbed were fed with fry booster feed. The hatching percentage was evaluated by counting the number of hatched fry divided the number of eggs stocked multiplied by 100. The experiment was arranged in completely randomized design.

Hatched fry were transferred in net enclosures placed in a single earthen pond and they were reared for two months to allow for gonad development. The experimental layout was in randomized complete block design. After this period, per cent survival was evaluated. Survival was expressed as a proportion of the treated fry stocked in the net enclosure. All surviving fish were placed in iced box and immediately dissected for sex differentiation. Sexes of fingerlings (male female, intersex) were identified by gonad squash technique (Guerrero and Shelton, 1974).

During the rearing period, water quality parameters (temperature, pH and dissolved oxygen (DO)) in the net enclosures were measured weekly up to the end of the experiment. Temperature and DO concentration were measured by YSI DO meter Model 55 while pH by a pen-type HANNA pH meter.

Data were analyzed using ANOVA in 5 x 4 factorial with three replications. Comparison of means was done using Duncan's Multiple Range Test (DMRT). Pearson correlation analysis was used to determine the relationship of HC, IT and hatchability. The general linear model in the Statistical Package for Social Sciences (SPSS) version 10 was used.

Results

Analysis of variance showed strong significant effects of hormone concentration (HC) and immersion time (IT) and their interaction (HC x IT) on the differences in hatching percentage, per cent survival and per cent male ($P<0.001$).

Hatching percentage

The main effect of HC showed that 200 $\mu g\ l^{-1}$ gave the highest hatching percentage (95.67%) followed by 800 $ug\ l^{-1}$ (92.58%) with these highest rates of sex reversal being not significantly different ($P>0.05$) (Figure 1). The hatching percentage of 90.17% in the control (0 $ug\ l^{-1}$) was comparable with the hatching percentages in 400 $\mu g\ l^{-1}$ (89.67%) and 800 $\mu g\ l^{-1}$ (92.58%) ($P>0.05$). The lowest hatching percentage of 86.75% occurred at the concentration of 600 $\mu g\ l^{-1}$ which was not significantly different from the hatching percentage in the control (0 $\mu g\ l^{-1}$) and at 400 $\mu g\ l^{-1}$ ($P>0.05$). Although the main effect of HC gave significant variations in the hatchability percentages in the different treatments, there seemed to be no

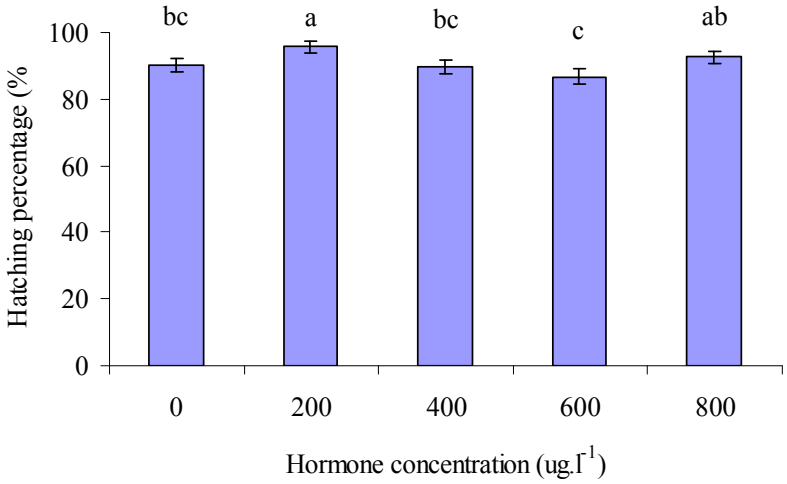

Figure 1. Main effect of HC on hatching percentage across all ITs.

trend in relation to the increasing hormone concentration. Pearson correlation coefficient between HC and hatchability was not significant (r=-0.081; P>0.05).

The main effect of IT showed that the highest hatching percentage of 97.87 was obtained at 24-hr IT, followed by 92.53% at 48-hr, and lowest at 72-hr and 96-hr ITs (87.13% and 86.33%, respectively) (P<0.001) (Figure 2). Results showed a decreasing trend in hatching percentage as immersion time increased (r = -0.626; P = 0.01).

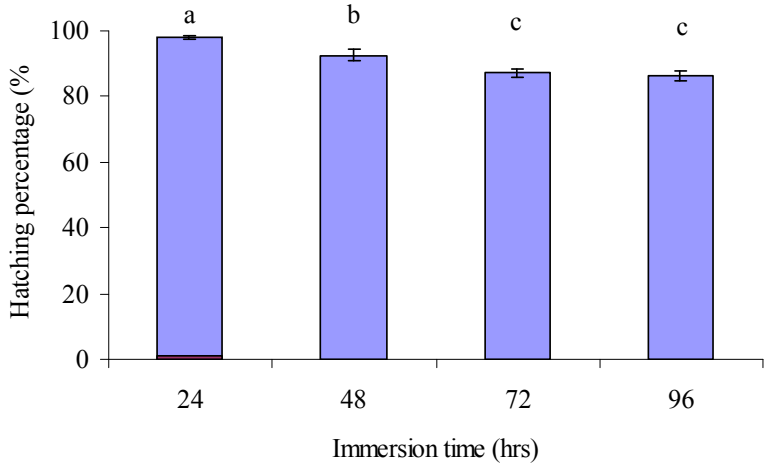

Figure 2. Main effect of IT on hatching percentage across all HCs.

The interaction effect of HC x IT showed that the highest hatching percentages generally occurred at 24-hr IT in HCs of 0, 200,400, 600 and 800 ug l^{-1} HC (96.67- 99.33%) and at 48-hr IT in HCs 0, 200 and 800 µg l^{-1} (93-99.67%) (Table 1). Lowest hatching percentage of 79.67% occurred at 96-hr IT in 600 µg l^{-1} HC.

Table 1. Interaction effect of HC x IT on hatching percentage.

HC (µg l^{-1})	Hatching (%) at different IT (hrs)			
	24	48	72	96
0	97.67 a (0.88)	93.00 abc (5.51)	86.00 cde (1.53)	84.00 de (2.65)
200	98.33 a (0.88)	99.67 a (0.33)	95.67 ab (1.33)	89.00 bcd (4.58)
400	96.67 ab (1.76)	86.00 cde (3.06)	84.33 de (2.85)	91.67 abcd (3.53)
600	97.33 a (1.33)	86.67 cde (1.20)	83.33 de (1.76)	79.67 e (4.10)
800	99.33 a (0.67)	97.33 a (1.45)	86.33 cde (1.45)	87.33 cde (0.33)

Note: Means with similar letters are not significantly different at P >0.05. Values in parentheses are standard error of the mean (SEM).

Survival in net enclosures

The control with HC 0 ug l^{-1} gave the highest post-treatment survival of 97.33% followed by 200 µg l^{-1} with 95.48% survival with these being not significantly different (P>0.05) (Figure 3). The lowest survival percentages were observed at 400, 600 and 800 µg l^{-1} HC (86.46%, 85.92%, and 87.95%, respectively) with these being significantly lower than the lower doses.

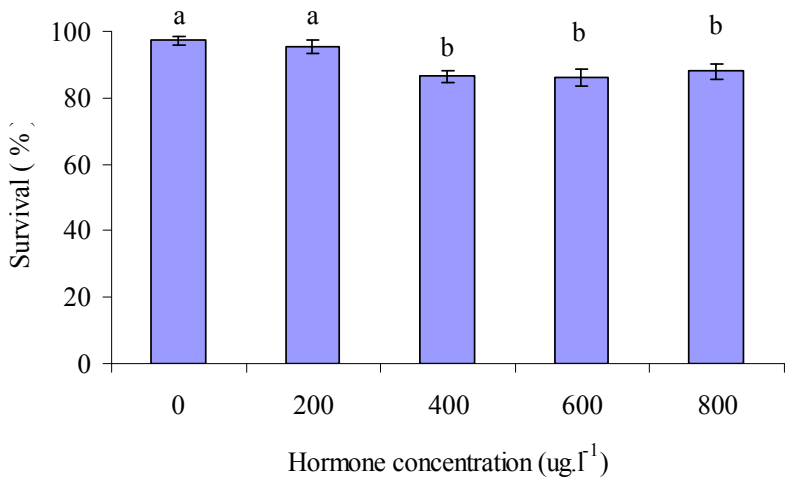

Figure 3. Effect of hormone concentration on percent survival across all ITs.

Survival was significantly higher in 24-hr IT (95.32%) than for the longer durations (88.26 – 90.98%) (Figure 4). Results showed that increasing HC and IT resulted in decreasing per cent survival (r = -0.444; P=0.01).

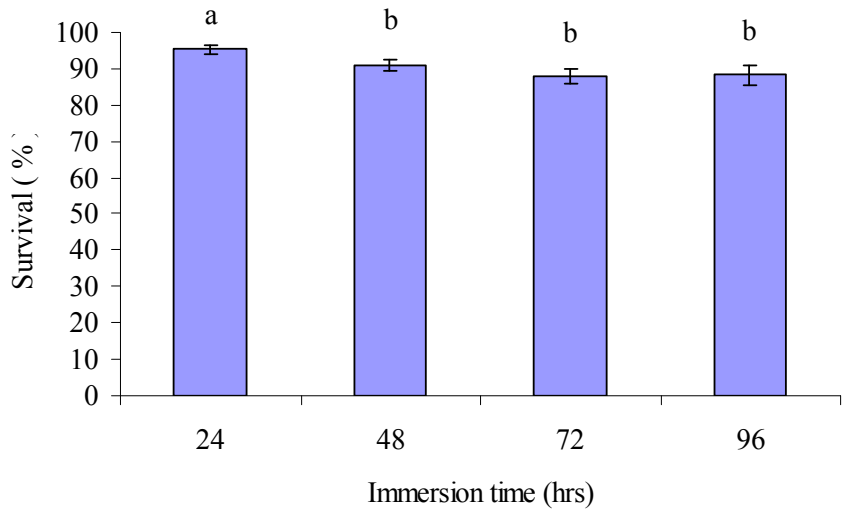

Figure 4. Effect of immersion time on per cent survival across all HCs.

The highest per cent survival of greater than 90% was observed at 24-hr, 48-hr, 72-hr, and 96-hr in 0 µg l^{-1} and 200 µg l^{-1} (Table 2). Survival greater than 90% was also observed at 600 µg.l^{-1} and 800 µg.l^{-1} for 24-hr IT. Lowest survival percentages (76.80 and 80.94%) were found in the longest ITs 96-hr at 600 µg l^{-1} and 800 µg l^{-1} HC, respectively.

Table 2. Survival as affected by the interaction effect of HC x IT.

HC (µg l^{-1})	Survival (%) at different IT (hrs)			
	24	48	72	96
0	97.93 abc (1.21)	96.27 abc (2.79)	100.00 a (0.00)	95.13 abc (4.87)
200	96.57 abc (2.09)	98.32 abc (1.68)	90.49 abcde (4.37)	96.55 abc (6.74)
400	87.53 cde (1.75)	83.99 def (1.89)	82.46 def (2.58)	91.88 abc (4.66)
600	96.88 abc (3.12)	88.07 bcde (1.03)	81.93 def (2.67)	76.80 f (2.95)
800	97.67 abc (1.20)	88.27 bcde (2.85)	84.90 def (1.39)	80.94 ef (3.56)

Note: Means with similar letters are not significantly different at P >0.05. Values in parentheses are standard error of the mean (SEM).

Sex ratio

Significant increase (P<0.001) in per cent male was observed with increasing HC being significantly different from each other (Figure 5). The HC 800 µg l^{-1} gave 83.97% male; 600 µg l^{-1} gave 79%; 400 µg l^{-1} contributed about 75% male; 200 µg l^{-1} about 67% and lowest was the control (0 µg l^{-1}) which gave only 58.62% male.

Highest per cent male (78.69%) occurred at 96-hr IT, followed by about 72% in 48-hr and 72-hr, and lowest (68.60%) in 24-hr. Results showed that the effective immersion time to achieve higher percentage of male is 96 hours (Figure 6).

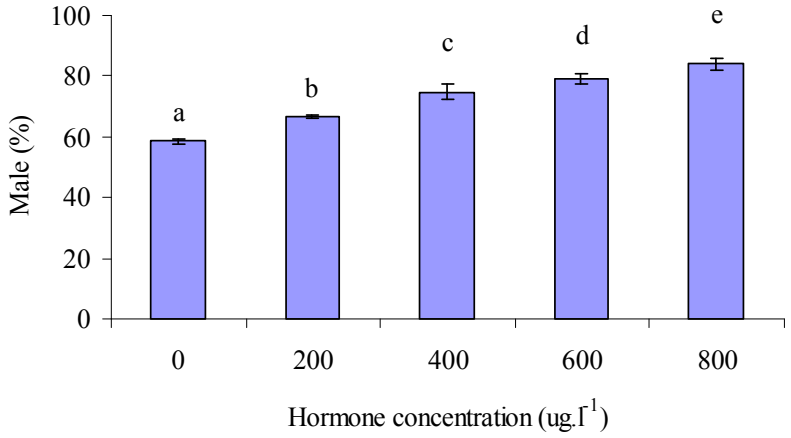

Figure 5. Main effect of hormone concentration on per cent male across all ITs.

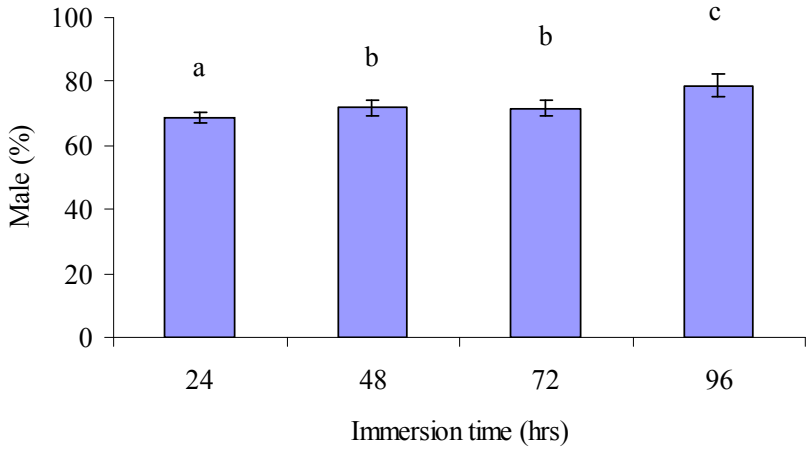

Figure 6. Main effect of immersion time on per cent male across all HCs.

The interaction effect of IT x HC was highly significant ($P<0.001$). Results showed that higher HC greater than 400 µg l^{-1} seemed to be better in effecting higher percentages of males at longer ITs (Table 3). The longer IT and the higher the HC, the higher the percentage male can be achieved. Highest per cent male of 91.09% was attained in 800 µg l^{-1} at 96-hr IT. However, this per cent male was comparable with the 88.88% and 87.65% male found in 400 µg l^{-1} and 600 µg l^{-1} both at 96-hr IT.

Table 3. Interaction effect of HC x IT on per cent male.

HC ($\mu g\ l^{-1}$)	Male (%) at different IT (hrs)			
	24	48	72	96
0	59.23 i (1.64)	59.29 i (0.60)	58.93 i (0.57)	57.01 i (1.07)
200	66.72 gh (1.12)	67.03 gh (1.63)	64.29 h (1.91)	68.79 fgh (0.28)
400	68.56 fgh (1.48)	69.92 fg (0.18)	72.06 f (2.07)	88.88 ab (0.89)
600	71.83 f (2.35)	79.91 de (3.09)	77.47 e (0.32)	87.65 ab (0.96)
800	76.66 e (1.32)	83.10 cd (1.71)	85.02 bc (2.18)	91.09 a (1.38)

Note: Means with similar letters are not significantly different at P >0.05. Values in parentheses are standard error of the mean (SEM).

Water quality

Mean pH ranged from 7.5 – 8.8 were within the suitable values for fish culture (Boyd, 1982). Dissolved oxygen (DO) concentrations ranged from 4.73 – 12.5 $mg.l^{-1}$ in the morning and 5.03 – 11.2 mg l^{-1} in the afternoon. Temperature was 26.9 – 28.1^{o}C (morning) and 27.9 – 29.4^{o}C (afternoon). According to Phelps and Popma (2000), DO concentrations should remain above 4 mg l^{-1} and the optimum temperature between 26- 28^{o}C for ideal fish culture.

Discussion

The study revealed significant information on the effect of hormone concentration (HC) and immersion time (IT) and their interaction (HC x IT) on hatching percentage, per cent survival and masculinization percentage. Although the main effect of HC had significant variations on the hatchability percentages in the different treatments, there seemed to be no trend on the hatching percentage in relation to the increasing hormone concentration. However, as immersion time increased, the hatchability percentage decreased. It may be possible that the tolerance level of the eggs may have been compromised in higher HC at longer IT that contributed to lower hatching percentage. A decreasing trend in the survival (%) of fish was observed as hormone concentration and immersion time increased.

Based from the results, masculinization of Nile tilapia was achieved by egg immersion. The per cent of males produced tended to increase as HC and IT increased. Similarly, there was significant HC x IT interaction for sex ratio. The highest average proportion of males (91%) was obtained at 800 $\mu g\ l^{-1}$ comparable with the 88- 89% male obtained at 400 $\mu g\ l^{-1}$ and 600 $\mu g\ l^{-1}$ both at 96-hr IT. These values of per cent males were lower compared to the reported average masculinization rate employing the traditional sex reversal using hormone-treated feed. A HC of 40 $mg.kg^{-1}$ administered to first feeding fry for approximately 25 days fed 4 times a day, can give an excess of 90% male, even averaging 95% male although 100% populations are seldom achieved (Mair, 1997). In Thailand,

immersion of 30-40 *O. niloticus* eggs (2-day old) in 500 μg l^{-1} of MT at 24 hours resulted in 88% male (Srisakultiew, pers. comm.).

The average per cent male of 91% obtained in our experiment compared to that reported by Mair (1997) using the traditional sex reversal may be attributed to lower hormone concentrations (i.e. 400, 600 and 800 μg l^{-1}). These HCs are equivalent to 0.4, 0.6 and 0.8 mg kg^{-1}, respectively, based from the assumption that 1 liter of water is equal to 1 kg. These concentrations may still be suitable because eggs were used instead of fry. Hence, it may also be possible that the lower per cent male obtained in this study compared to that reported by Mair (1997) was due to the management employed during the immersion and incubation. Therefore, this may be worthwhile to look at in future investigations.

Conclusion and recommendations

The study showed that it is possible to induce sex reversal by immersing eggs in MT hormone. The study was able to identify the hormone concentration and immersion time that gave the highest per cent masculinization. Generally, the highest per cent male was obtained at 96-hr IT starting at 400 μg l^{-1} to 800 μg l^{-1} HC. A 91% masculinization was attained in 800 μg l^{-1} at 96-hr IT which was similar with about 88-89% masculinization in 400 and 600 μg l^{-1} at 96-hr IT.

It is suggested that future studies should consider using higher rates of hormone to see if there is an increase in the masculinization percentage and determine the limit of hormone dosage that can be used. The management practices during immersion and incubation and the density of eggs to be immersed should be looked into in an attempt to achieve consistently higher rates of masculinization of fry. It may also be interesting to determine the effectivity of immersion technique using fry instead of eggs. Assuming that consistently high rates of sex reversal could be achieved with variations on this immersion method, it would be important to look at the practical logistics, safety and economics of using this technique for commercial scale application.

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