SEX REVERSAL OF NILE TILAPIA *OREOCHROMIS NILOTICUS* L. BY EGG IMMERSION TECHNIQUE: THE EFFECT OF HORMONE CONCENTRATION AND IMMERSION TIME

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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, oral administration of the hormone, 17 α-methyl testosterone (MT), is the most common method for the production of all male tilapia. It is probably the most effective and practical method currently available in the Philippines.

Limitations of traditional sex reversal method

- uniform age of fish that should be used at the first feeding stage to ensure high reversal rate
- less control of reversal efficiency especially when done in the natural environment where natural food is present
- widespread use of large quantities of sex reversal hormone in hatcheries may pose a health risk to workers
- little information on the fate of the hormone in the effluent and ground water

Why sex reversal by immersion of eggs in MT?

- **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.**

- **May lessen environmental risk**

- **Androgen when disposed of may be filtered, thus, water that goes to the aquifer may be safe of this steroid.**

What have been done along the line of SRT by immersion?

- **Studies on sex reversal by immersion in hormone solution of methyl testosterone (MT) are few and focused mainly on fry**

- 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 ug.l-1 of MT at 24 hours resulted in 88% male (Srisakultiew, pers. com.)**

Objectives

- The study generally aimed to masculinize Nile tilapia *O. niloticus* by egg immersion.

- Specifically, this study aimed to determine the **effect of hormone concentrations and immersion times on the hatching percentage of *O. niloticus* eggs, per cent survival and percent of male.**

Methodology

- **FAC Selected Strain (FaST) *O. niloticus* breeders (100-150 g.) were conditioned in net enclosures (each with dimension of 3 m x 5 m x 1.5 m) and paired at a sex ratio of 1male:3female equivalent to 5 males:15females 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.**

Methodology

Treatment	Immersion time	Hormone rate (*u*g.l^{-1})
1	0	0
2	24	200, 400, 600 and 800
3	48	200, 400, 600 and 800
4	72	200, 400, 600 and 800
5	96	200, 400, 600 and 800

Each of the treatments was replicated three times.

- One hundred eggs were immersed in different 17-α methyl testosterone (MT) hormone concentrations (HC) at varying immersion times (IT).
- 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.

Methodology

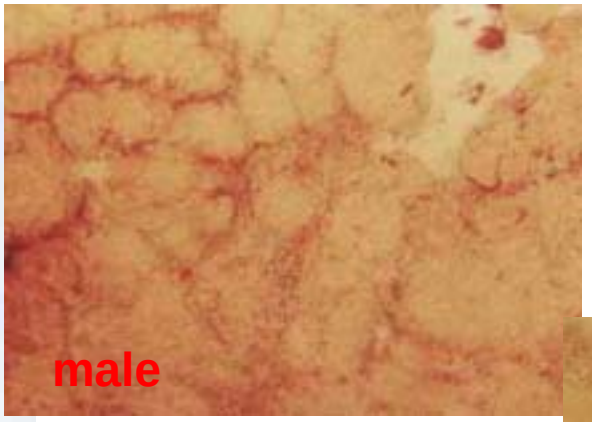
- After immersion, treated eggs were transferred in down-welling egg incubators where they were placed for hatching.
- Fry were placed in the incubators for period of 5-7 days after which they were reared in net enclosures.
- Fry whose yolk sac have been absorbed were fed with fry booster feed.
- The 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.

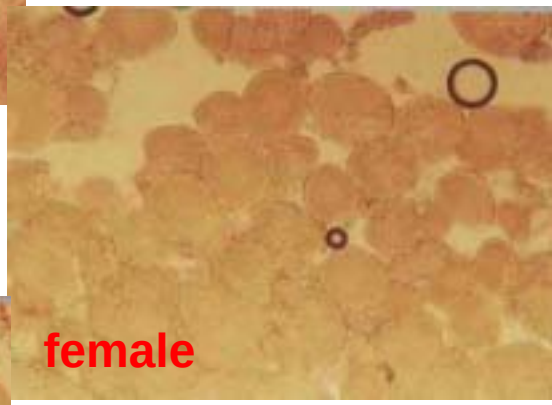
Methodology

- 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.
- Experimental layout = randomized complete block design.
- Per cent survival - survival was expressed as a proportion of the treated fry stocked in the net enclosure.
- Water quality parameters (temperature, pH and dissolved oxygen (DO)) were measured weekly up to the end of the experiment.
- All surviving fish were placed in iced box and immediately dissected for sex differentiation.

Methodology

- Sexes of fingerlings (male, female, intersex) were identified by **gonad squash technique** (Guerrero and Shelton, 1974).





•The stained gonad was examined under the compound microscope. It was male if gonad was densely packed with spermatocytes; female if densely pack with oocytes; intersex if gonad had very few and scattered oocytes.

Methodology

- 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 and Discussion

- **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 percentages of male and female (P<0.001).**

Hatching percentage: Effect of HC

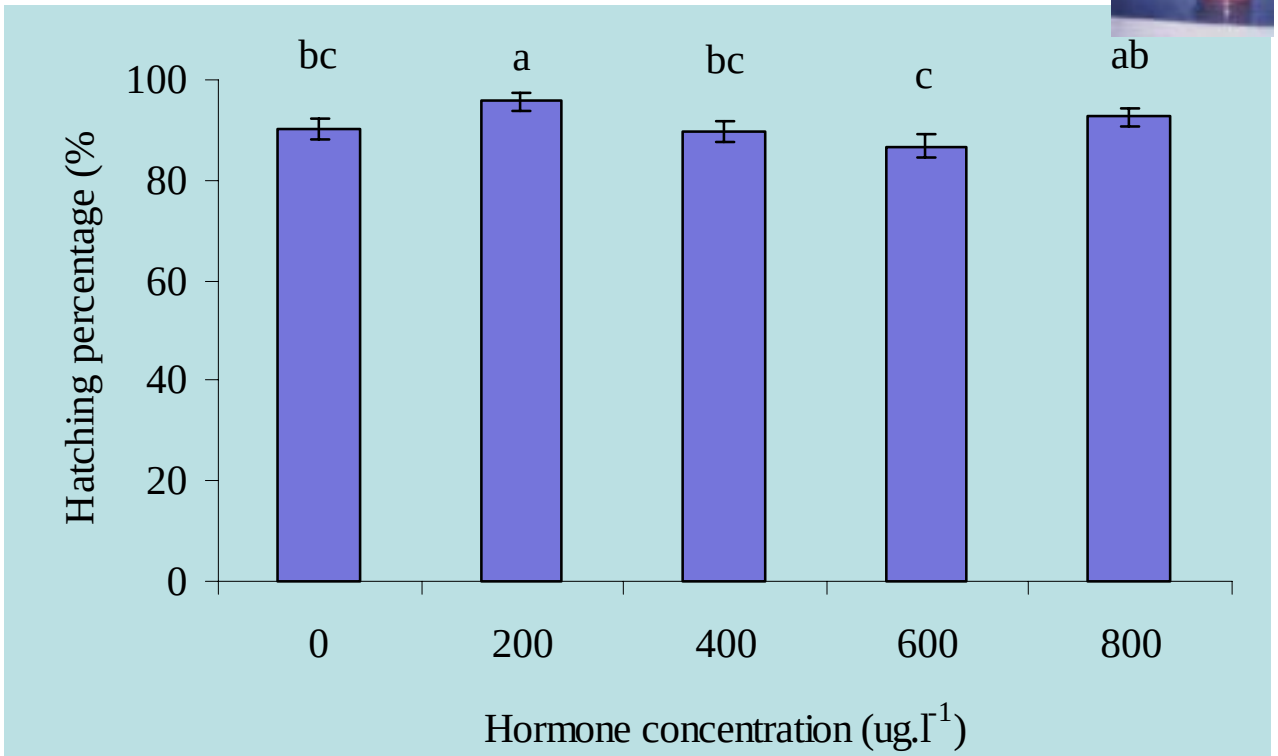
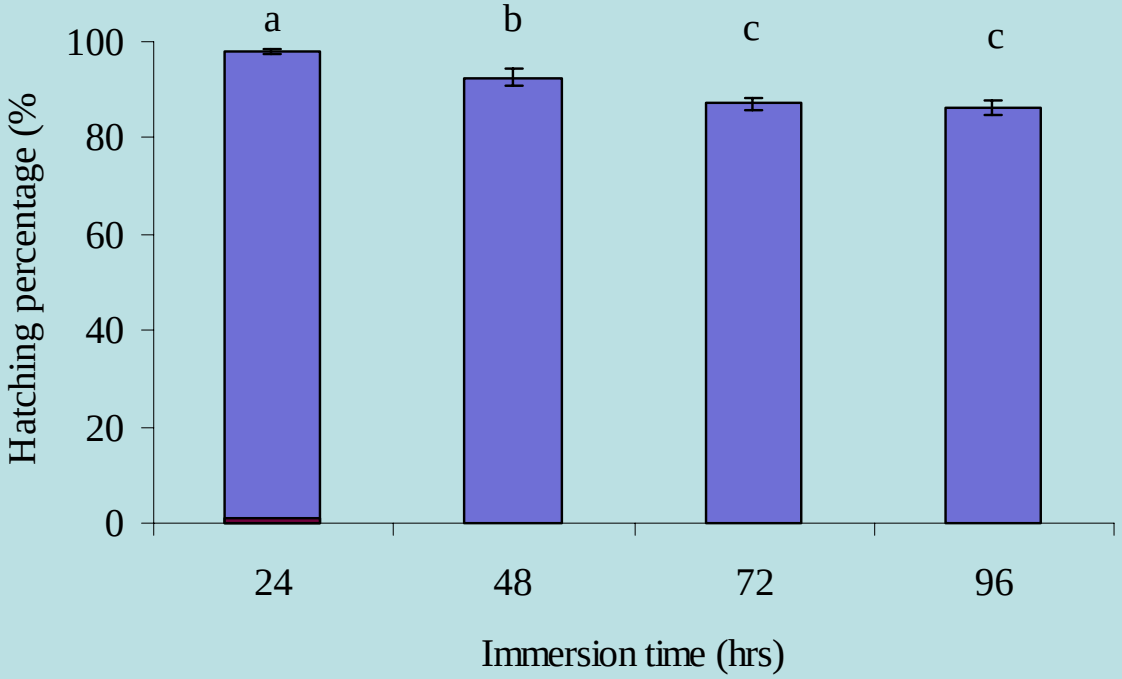


Figure 1. Main effect of HC on hatching percentage across all ITs.

Although the main effect of HC gave significant variations in the hatchability %s in the different treatments, there seemed to be no trend in relation to the increasing hormone concentration.

Pearson correlation coefficient between HC and hatchability was not significant (r=-0.081; P>0.05).

Hatching percentage: Effect of IT



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

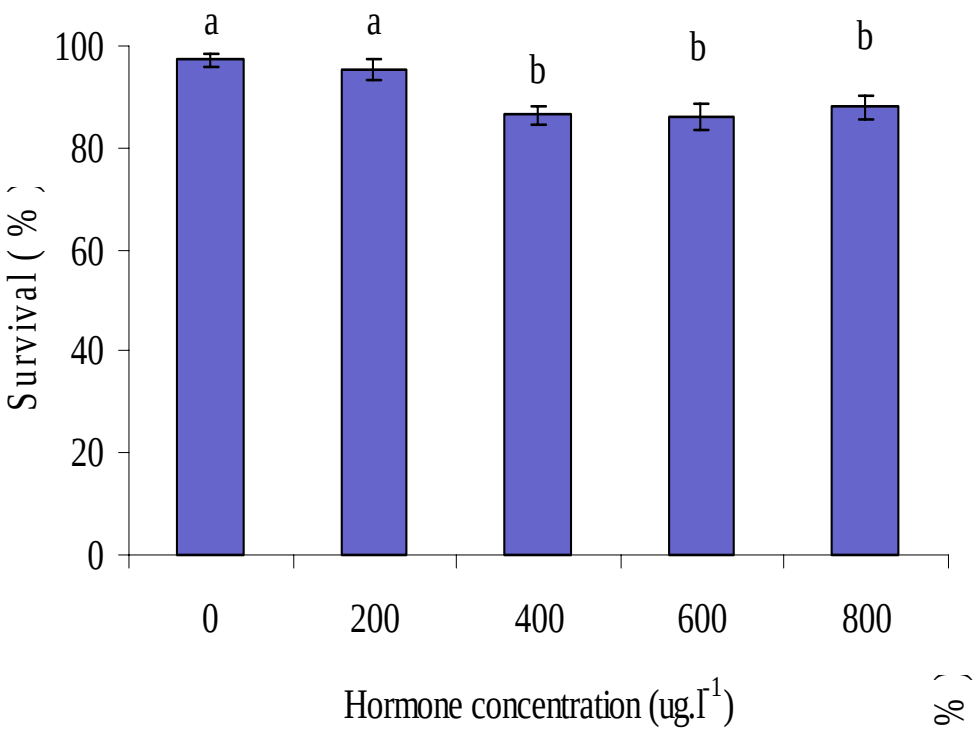
Results showed a decreasing trend in hatching percentage as immersion time increased (r = -0.626; P = 0.01).


Interaction effect of HC x IT

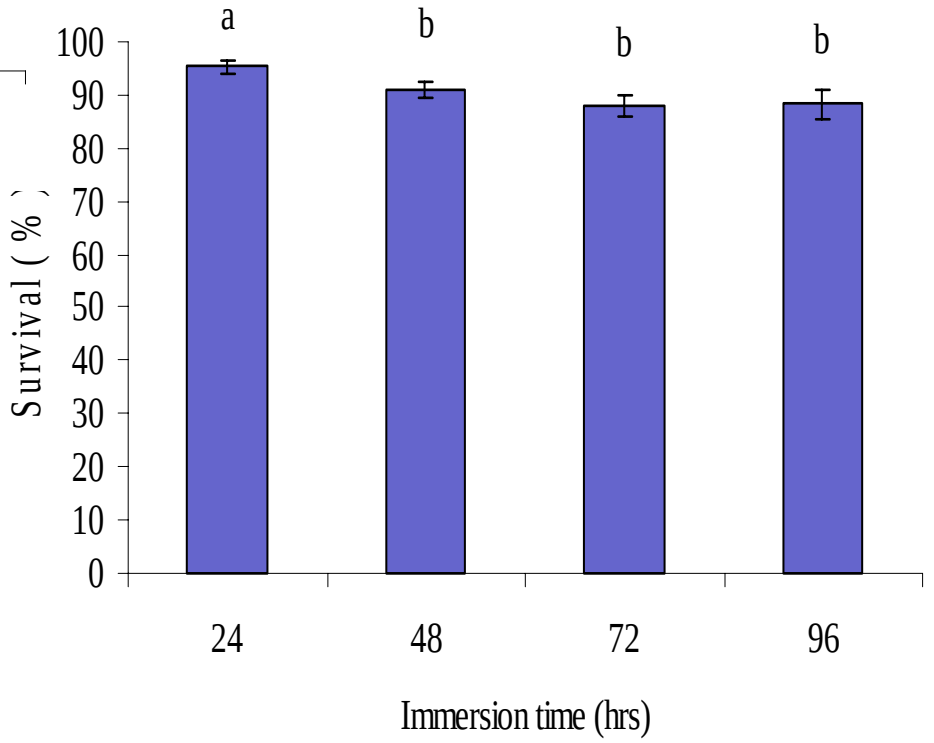
HC (ug.l^{-1})	Hatching (%) at different IT (hrs)			
	24	48	72	96
0	97.67 a (*0.88*)	93.00 abc (*5.51*)	86.00 cde (*1.53*)	84.00 de (2.65)
200	98.33 a (*0.88*)	99.67 a (*0.33*)	95.67 ab (*1.33*)	89.00 bcd (*4.58*)
400	96.67 ab (1.76)	86.00 cde (*3.06*)	84.33 de (*2.85*)	91.67 abcd (*3.53*)
600	97.33 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



Main effects of HC and IT



Results showed that increasing HC and IT resulted in decreasing per cent survival (r = -0.444; P=0.01).

Survival in net enclosures

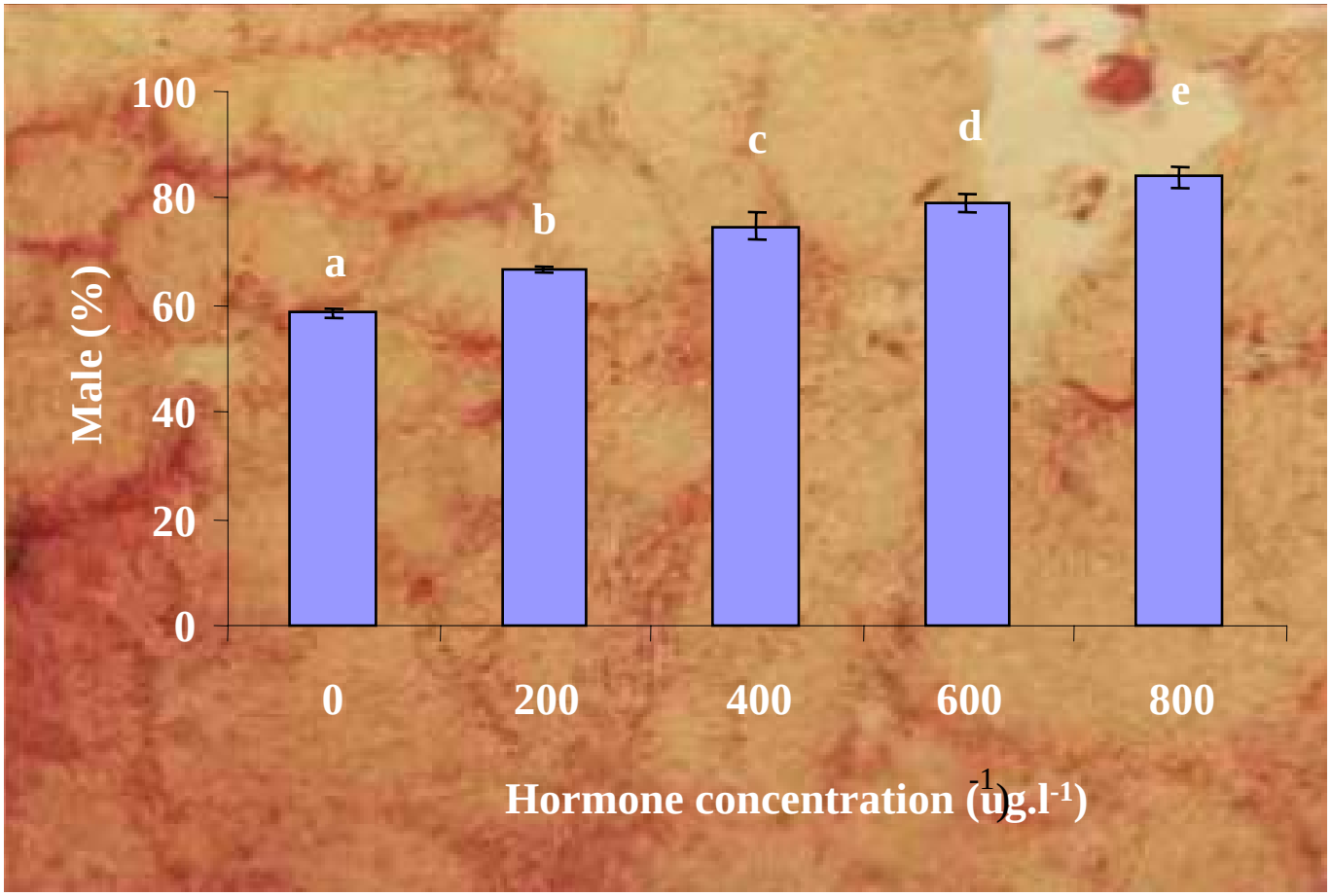
Interaction effect of HC x IT

HC ($ug.l^{-1}$)	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).

Per cent male

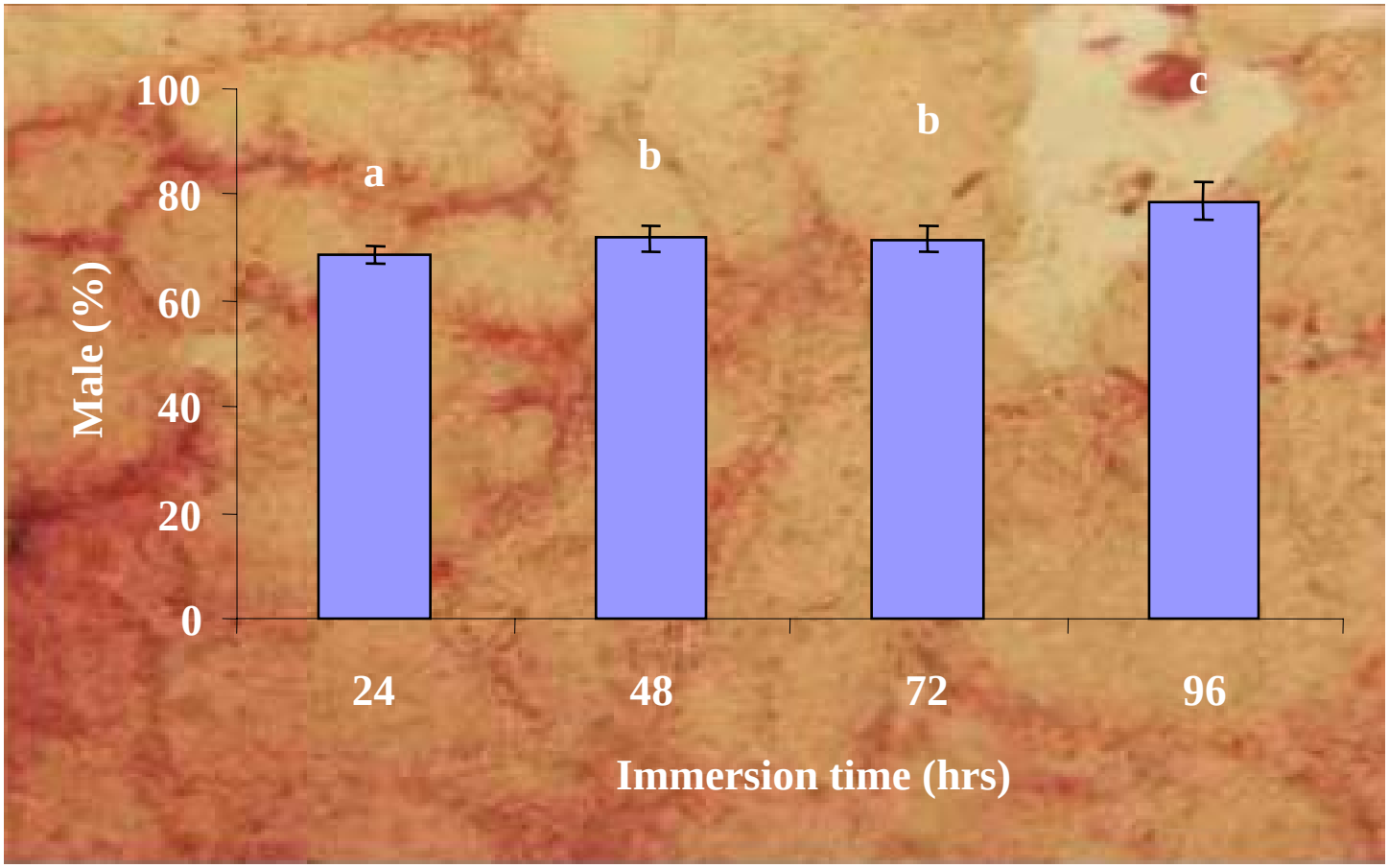
- **Main effect of HC**



Significant increase in per cent male was observed with increasing HC (P<0.001) .

Per cent male

- **Main effect of IT**



Results showed that the most effective immersion time to achieve higher percentage of male is 96 hours

Per cent male Interaction effect of HC x IT

HC ($ug.l^{-1}$)	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*)

1. Results showed that higher HC starting at 400 $ug.l^{-1}$ seemed to be better in effecting higher percentages of males at longer ITs.
2. The longer IT and the higher the HC, the higher the percentage male can be achieved.

Water Quality

- Mean pH ranged from 7.5 – 8.8 were within the suitable values for fish culture.
- 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°C (morning) and 27.9 – 29.4°C (afternoon).

Conclusions

- 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 ug.l^{-1} to 800 ug.l^{-1} HC.
- A 91% masculinization was attained in 800 ug. l^{-1} at 96-hr IT which was similar with about 88-89% masculinization in 400 and 600 ug.l^{-1} at 96-hr IT.

Recommendations

- 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**.


Thank you !!!
Maraming salamat po!!!