Efficacy of 17β-estradiol on survival rate, sex reversal, and growth performance of climbing perch (*Anabas testudineus*) using the immersion method

Tuan Q. Nguyen, Tu C. P. Nguyen, Loi N. Nguyen, Minh V. Tran, Hue N. D. Truyen, & Tam T. Nguyen*

Faculty of Fisheries, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: August 30, 2024

Revised: October 06, 2024

Accepted: October 17, 2024

Keywords

Anabas testudineus
Immersion method
17β-estradiol
Sex reversal

***Corresponding author**

Nguyen Thanh Tam

Email:
nthanhtam@hcmuaf.edu.vn

ABSTRACT

This study aimed to evaluate the efficacy of estradiol on the survival rate, sex reversal ratio, and growth performance of climbing perch (*Anabas testudineus*) using the immersion method. A completely randomized design was applied, involving three estradiol (E2) treatment groups at concentrations of 1.0 mg/L (1E2), 1.5 mg/L (1.5E2), and 2 mg/L (2E2), along with a control group triplications. Seven-day-old fingerlings were exposed to the E2 solution for 2 h before being transferred to nurseries in hapas placed in earthen ponds at a density of 200 fish/m^2 for 60 days. After the hormone treatment and 60 days of rearing, the highest survival rate was observed in the control group (86.7%). The female ratios in the 17β-estradiol (E2) treatments ranged from 72.0% to 90.0%, which were significantly higher than the ratio of the control group (55.6%) (P < 0.05). The 2E2 treatment demonstrated the highest female percentage, which was statistically greater than that observed in the 1E2 and 1.5E2 treatments (P < 0.05). The mean weight and length of fish in the E2 treatments were greater than those in the control treatment, although the differences were not statistically significant (P > 0.05). Additionally, the study revealed a direct proportionality between the average weight of experimental fish and the hormone concentration. Based on these findings, the recommended dose for achieving maximum mono-sex female climbing perch is 2 mg/L of 17β-estradiol.

Cited as: Nguyen, T. Q., Nguyen, T. P. C., Nguyen, L. N., Tran, M. V., Truyen, H. N. D., Nguyen, T. T. (2024). Efficacy of 17β-estradiol on survival rate, sex reversal, and growth performance of climbing perch (*Anabas testudineus*) using the immersion method. *The Journal of Agriculture and Development* 23(Special issue 1), 100-107.

1. Introduction

The study of sex reversal in fish has gained significant attention due to its implications for aquaculture and conservation. Understanding and manipulating sex differentiation can have profound effects on population management and productivity (Pandian & Sheela, 1995). Sex reversal, where an individual fish changes its sex from male to female or vice versa, is a naturally occurring phenomenon in several fish species (Devlin & Nagahama, 2002). This process can be influenced by environmental factors, social cues, and hormonal interventions (Yamamoto, 1969). Globally, species such as groupers, wrasses, and clownfish are well-known for their sex-changing capabilities (Shapiro, 1987). In Vietnam, sex reversal is particularly relevant for species like the Asian sea bass (*Lates calcarifer*) and tilapia species (*Oreochromis* spp.), which are vital to the local aquaculture industry (FAO, 2010). The ability to control sex differentiation through hormonal treatments has opened new avenues for optimizing fish production and maintaining ecological balance (Hunter & Donaldson, 1983).

Climbing perch (*Anabas testudineus*) is an important food fish that is considered to be of high quality, contributing to local people's livelihoods and well-being in the Mekong Delta (Anh et al., 2003; Klemick & Lichtenberg, 2008). In growth-out farming, the use of female climbing perch is particularly important due to their superior growth performance compared to males. Female climbing perch typically exhibit faster growth rates, greater size, and improved feed conversion efficiency, making them more economically viable for large-scale production. By focusing on female fish in farming operations, farmers can optimize yield and enhance overall productivity, contributing to more sustainable and profitable aquaculture practices.

The manipulation of sex in fish populations is essential for several reasons. In hatchery production, achieving a desired sex ratio can enhance reproductive efficiency and yield (Beardmore et al., 2001). For instance, in species where one sex grows faster or reaches market size more quickly, producing monosex populations can significantly improve economic returns (Mair et al., 1997). In fish farming, controlling the sex ratio can prevent unwanted breeding, thereby reducing competition for resources and promoting uniform growth (Guerrero & Guerrero, 1988). Additionally, in conservation efforts, sex reversal can help restore endangered populations by ensuring a balanced sex ratio crucial for successful breeding programs (Rubin, 1985).

Various methods have been developed to induce sex reversal in fish, including environmental manipulation, genetic techniques, and hormonal treatments (Piferrer, 2001). Hormonal treatments are the most widely used approach due to their effectiveness and relative ease of application (Guerrero, 1975). Common hormones include androgens like methyltestosterone, which induce masculinization, and estrogens such as estradiol, which promote feminization (Yamamoto, 1969). These hormones can be administered through feed, injections, or immersion baths, depending on the species and desired outcome (Pandian & Sheela, 1995). Each method of sex reversal has its advantages and disadvantages. Environmental manipulation, such as temperature changes, is non-invasive and sustainable but can be less precise and effective (Yamamoto, 1969). Genetic techniques offer long-term solutions and are highly specific but involve complex and costly procedures (Beardmore et al., 2001). Hormonal treatments are cost-effective, easy to implement, and provide rapid results (Pandian & Sheela, 1995).

Estradiol, a primary estrogen hormone, is widely used to induce feminization in fish, playing a pivotal role in sex differentiation (Guerrero, 1975). The use of estradiol in fish sex reversal offers several advantages. It is a potent and reliable method for achieving desired sex ratios, particularly in species where female individuals are preferred for their growth rates, market value, or reproductive characteristics (Piferrer, 2001). The dosage and method of administration vary depending on the species and desired sex ratio (Pandian & Sheela, 1995). Typically, estradiol is administered through feed during the early developmental stages when gonadal differentiation is most responsive (Hunter & Donaldson, 1983). The feeding method for administering hormones in fish sex reversal has several disadvantages: (i) individual fish may consume varying amounts of food, leading to inconsistent hormone dosages and affecting sex conversion results; (ii) the processing time is lengthy, typically around 21 days; (iii) the age of the fish must be precise, usually 1-day old after the yolk is fully consumed; and (iv) it incurs high production costs (Guerrero & Guerrero, 1988). In contrast, the immersion bath method helps overcome these disadvantages: (i) it provides rapid and uniform hormone distribution, ensuring consistent sex reversal results; (ii) it is ideal for early developmental stages and hatchery environments where large numbers of fish can be treated simultaneously; and (iii) it has lower production costs (Rubin, 1985). Therefore, the research was carried out to investigate the effects of estradiol on sex reversal, survival, and growth of climbing perch (*Anabas testudineus*) using the immersion bath method (Guerrero, 1975).

2. Materials and Methods

This experimental study was designed to evaluate the effectiveness of producing female monosex climbing perch by immersion method. The experiment was conducted over a period of six months, from January to June 2024.

2.1. Test animals

The experimental fish used in the sex reversal treatment were obtained from five pairs of climbing perch broodstock (female: 13 ± 1.2 cm and 98 ± 0.6 g; male: 11 ± 1.4 cm and 79 ± 0.4 g) through artificial reproduction using hormone induction at the experimental farm of the Faculty of Fisheries, Nong Lam University, Ho Chi Minh City. Fertilized eggs obtained from these broodstocks were pooled and incubated in three separate 50-liter plastic tanks, and maintained with gentle aeration. Post-hatching, the larvae were kept in the same tanks, with the water maintained at a temperature of 29 - 32°C, dissolved oxygen (DO) level at 2 - 4 mg/L, ammonia (NH_3) levels below 0.25 ppm, nitrite levels under 1.0 ppm, and a pH range of 7.0 - 7.5. Starting at 3 - 4 days post-hatch (DPH), the larvae were fed *Moina* four times daily in adequate quantities. Upon reaching 6 days of age, feeding was ceased for one day to facilitate the experiment.

2.2. Preparation of hormone treated solutions

The 17β-estradiol hormone utilized in this study was purchased from Sigma Aldrich Ltd., Germany. A stock solution was prepared by dissolving 400 mg of the hormone in 1 L of 96% ethanol, yielding a nominal concentration of 0.4 mg/mL. To achieve the treatment concentrations, appropriate amounts of the stock solution were dissolved in a 20 L glass flask containing 10 L of water, followed by gently aeration to facilitate ethanol evaporation.

2.3. Experimental design

The experiment was carried out in 20 L glass tanks using completely randomized design.

The experiment involved three 17β-estradiol (E2) concentration treatments: 1 mg/L (1E2), 1.5 mg/L (1.5E2), and 2 mg/L (2E2), along with a control treatment (C). Each treatment was replicated three times. 7-day-old fry were randomly selected from three nursery tanks and placed into experimental glass tanks at a density of 500 fish/L for 2 h. Post hormone treatment, the fry were transferred to separate nursery hapas (1 m x 1 m x 0.5 m) at a density of 200 fish/m^2 for a duration of 60 days. During this period, the fish were fed commercial floating pellets with varying crude protein levels: 40% from days 1 to 15, at a feeding rate of 10% of body weight, administered four times daily; 35% from days 16 to 30, at a feeding rate of 7% of body weight, administered three times daily; and 30% from days 31 to 60, at a feeding rate of 5% of body weight, administered twice daily.

2.4. Sampling and data collection

a) Sex reversal

At the end of experiment, 30 fish were randomly collected in each experimental replicate to identify the sex of the fishes (total 90 fish/treatment). Morphology of the gonads were examined and recorded. Sexing determination was done by standard aceto-carmine gonad squashing technique (Guerrero & Shelton, 1974).

b) Growth performance

Every 30 days, 10 fish were randomly collected in each experimental replicate to measure the mean growth (average weight and length), using digital calipers and an electronic balance, respectively, then released the fish back into experimental hapas. Survival rates were recorded at two hours after immersion of fish in E2 solutions and at the end of the growth experiment (day 60).

c) Statistical analysis

The data were statistically analyzed by statistical package SPSS version 16.0 in which data were subjected to one-way ANOVA and Duncan's multiple range test (DMRT) was used to determine the significant differences between the means at 5% level of significance.

3. Results

The water quality parameters maintained during the experiment are summarized in Table 1. The temperature ranged from 29 to 32°C, DO levels varied between 2.0 and 4.0 mg/L, pH levels ranged from 7.0 to 7.5, and NH_3 levels were kept below 0.25 mg/L. These parameters were within the optimal range for the growth and survival of climbing perch (*Anabas testudineus*), ensuring a stable environment throughout the study (Boyd, 1998).

Table 1. Water quality parameters during experiment

Temperature (°C)	Dissolved oxygen (mg/L)	pH	NH_3 (mg/L)
29.0 - 32.0	2.0 - 4.0	7.0 - 7.5	< 0.25

The survival rates and mean percent females for each estradiol-17β treatment group and the control group are shown in Table 2. All groups exhibited a 100% survival rate after 2 h of treatment, indicating that the initial exposure to 17β-Estradiol did not negatively impact the immediate survival of the fish. However, the survival rate at day 60 showed slight variations among these groups. The control group had a survival rate of 86.7%, whereas the treated groups showed lower survival rates: 82% for 1E2, 81.3% for 1.5E2, and 80.7% for 2E2.

Histological analysis of gonadal tissues revealed no intersex individuals across all estradiol treatments and control groups (Table 2; Figure 1). The effectiveness of 17β-Estradiol in sex reversal was evident in the increase in the percentage of females in the treated groups compared to the control. The control group exhibited a normal sex ratio of 1 female to 0.80 male. In contrast, the female ratios in the E2 treatments varied from 72% to 90%, which were significantly higher than the 55.6% observed in the control group (P < 0.05). The experimental results demonstrated that the highest feminization rate of 90% occurred in the 2E2 treatment (2 mg/L E2 concentration), a statistically significant difference compared to other E2 treatments (P > 0.05). This indicates that a concentration of 2 mg/L E2 is an effective dose for feminizing climbing perch via immersion. Consequently, the 2E2 treatment (2 mg/L E2) is identified as the most suitable concentration for sex reversal in climbing perch fry using the immersion method.

Table 2. Effects of 17β-Estradiol on survival, sex reversal, and growth in climbing perch (*Anabas testudineus*)

Treatment	Number of analyzed fish	Survival rate after 2 h (%)	Survival rate at day 60 (%)	Female (%)	Male (%)	Sex ratio Female:Male
Control	90	100	86.67 ± 1.15	55.56 ± 5.09^{a}	44.4	01:00.8
1E2	90	100	82.00 ± 2.00	72.00 ± 4.00^{b}	28.0	01:00.4
1.5E2	90	100	81.33 ± 3.06	72.92 ± 3.61^{b}	27.1	01:00.4
2E2	90	100	80.67 ± 3.06	90.0 ± 3.33^{c}	10.0	01:00.1

Values (mean ± standard deviation of data for triplicate groups) with different superscripts in the same column are significantly different (one-way ANOVA and Tukey test, P < 0.05).

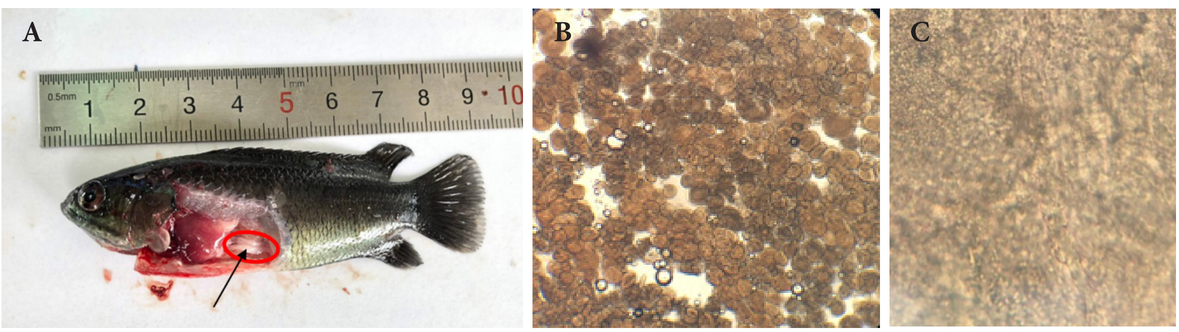

Figure 1. The observed gonad of male and female climbing perch after 60 days of 17β-Estradiol treatment. (A) Gonad removal (B) Eggs, and (C) Testis under light microscope.

The growth performance of the experimental fish over 60 days is detailed in Table 3. The control group showed a final weight of 8.112 ± 0.203 g and a specific growth rate (SGR) of 14.564 ± 0.042 %/day. The group treated with 2 mg/L estradiol-17β had the highest final weight (8.494 ± 0.214 g) and SGR (14.641 ± 0.042 %/day), indicating that higher doses of estradiol-17β may positively influence growth performance (Pandian & Sheela, 1995).

Table 3. Growth performance of experimental fish

Treatment	Initial Weight (g)	Initial Length (cm)	Final Weight (g)	Final Length (cm)	WG (g)	SGR (%/day)	DWG (g/day)
Control	0.001	0.48	8.112 ± 0.203	7.530 ± 0.129	8.111 ± 0.203	14.564 ± 0.042	0.135 ± 0.003
1E2	0.001	0.48	7.027 ± 0.436	7.173 ± 0.319	7.026 ± 0.436	14.323 ± 0.102	0.117 ± 0.007
1.5E2	0.001	0.48	7.203 ± 0.485	7.208 ± 0.239	7.202 ± 0.485	14.364 ± 0.110	0.120 ± 0.008
2E2	0.001	0.48	8.494 ± 0.214	7.643 ± 0.083	8.493 ± 0.214	14.641 ± 0.042	0.142 ± 0.004

Values (mean ± standard deviation of data for triplicate groups). WG: weight gain; SGR: specific growth rate; DWG: daily weight gain.

4. Discussion

The climbing perch (*Anabas testudineus*) is an exceptionally resilient species, capable of thriving in challenging environmental conditions, including low dissolved oxygen levels and poor water quality. This resilience was further evidenced by the high survival rates (> 80%) observed across all treatments in this study's experimental conditions.

The results of this study demonstrate that 17β-Estradiol significantly influences the sex reversal process in climbing perch. Fish treated with the hormone exhibited a higher proportion of females compared to the control group, suggesting that 17β-Estradiol effectively directs gonadal differentiation toward feminization. This finding aligns with previous research on other fish species, where 17β-Estradiol has been shown to promote female sex differentiation when administered during the critical period of sexual development (Tayamen & Shelton, 1978; Pandian & Sheela, 1995). Similarly, studies by Piferrer (2001) have shown the effectiveness of estradiol-17β in inducing feminization across different species, confirming the hormone's broad applicability. The observed sex ratios are consistent with previous studies on the effects of estrogenic compounds in fish. The ability to manipulate sex ratios through hormonal treatments can be beneficial for aquaculture practices, particularly in species where one sex is more desirable for production purposes (Hunter & Donaldson, 1983). Moreover, the immersion method appears to be an effective means of hormone delivery, ensuring consistent and adequate exposure to 17β-Estradiol.

Survival rates varied slightly among the treatment groups, with the highest survival observed in the control group (86.7%) and the lowest in the group treated with 2 mg/L estradiol-17β (80.7%). These results suggest that while higher doses of estradiol-17β can increase the feminization rate, they may also have a marginal impact on survival (Johnstone, 1985). The slight reduction in survival rates at higher doses could be due to the physiological stress induced by the hormone, as noted by Devlin & Nagahama (2002) in their comprehensive review of sex determination and differentiation in fish.

Growth performance, as indicated by final weight and specific growth rate (SGR), was also positively influenced by higher doses of estradiol-17β. The group treated with 2 mg/L exhibited the highest final weight (8.494 ± 0.214 g) and SGR

(14.641 ± 0.042 %/day). These findings align with the observations of (Pandian & Sheela, 1995), who noted enhanced growth in hormone-treated fish. Furthermore, Gale et al. (1999) observed that estradiol-17β not only influences sex differentiation but also positively affects growth rates by promoting anabolic processes in fish.

Comparative studies on the use of estradiol-17β in other fish species, such as Nile tilapia (*Oreochromis niloticus*), have shown similar patterns of feminization and growth enhancement. For instance, Mair et al. (1997) reported that higher doses of estradiol-17β resulted in a higher proportion of females and improved growth performance. This cross-species consistency underscores the potential of estradiol-17β as a universal agent for sex reversal and growth enhancement in aquaculture.

The implications of these findings for aquaculture are significant. Furthermore, the findings align with the theory that estrogen plays a crucial role in sex differentiation in teleost fish. The high efficacy of 17β-Estradiol in inducing female sex differentiation suggests its potential application in the aquaculture industry to control sex ratios and improve production efficiency (Li & Wang, 2019). By optimizing the dose of estradiol-17β, it is possible to achieve high rates of feminization, which is desirable in species where females grow faster or have better market value. The optimal dose identified in this study (2 mg/L) balances high feminization rates with acceptable growth performance and survival, making it a practical choice for commercial applications. Future studies should aim to further optimize these parameters and investigate the long-term effects of hormone treatment on fish health and reproductive capabilities, as indicated by Hunter & Donaldson (1983), who highlighted the importance of evaluating the long-term sustainability of hormone treatments in aquaculture.

5. Conclusions

The use of estradiol-17β at a dose of 2 mg/L is effective for inducing sex reversal in climbing perch (*Anabas testudineus*) through the immersion method. This dose achieves a high feminization rate, enhances growth performance, and maintains acceptable survival rates. These findings support the use of estradiol-17β as a valuable tool in aquaculture for optimizing the production of female fish. Further research is recommended to refine these parameters and assess the long-term impacts on fish health and reproductive success.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This study was made with financial support from Nong Lam University (Ref. no. CS-SV23-TS-05)

References

Anh, V. T., Chiem, N. N., Dung, D. T., & Sultana, P. (2003). *Understanding livelihoods dependent on inland fisheries in Bangladesh and South East Asia (DFID/FMSP Project R8118): Vietnam country statsus report*. Retrieved April 20, 2023, from https://mrag.co.uk/sites/default/files/fmspdocs/R8118_VietStatus.pdf.

Beardmore, J. A., Mair, G. C., & Lewis, R. I. (2001). Monosex male production in finfish as exemplified by tilapia: applications, problems, and prospects. *Aquaculture* 197 (1-4), 283-301. https://doi.org/10.1016/B978-0-444-50913-0.50015-1.

Devlin, R. H., & Nagahama, Y. (2002). Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture* 208 (3-4), 191-364. https://doi.org/10.1016/S0044-8486(02)00057-1.

FAO (Food and Agriculture Organization of the United Nations). (2010). Cultured aquatic species information programme. Retrievied August 10, 2024 from, https://www.fao.org/4/i1820e/i1820e.pdf.

Gale, W. L., Fitzpatrick, M. S., Lucero, M., Contreras-Sanchez, W., & Schreck, C. B. (1999). Masculinization of Nile tilapia (*Oreochromis niloticus*) by immersion in 17α-methyltestosterone at different temperatures. *Aquaculture* 178 (3-4), 349-357. https://doi.org/10.1016/S0044-8486(99)00136-2.

Guerrero, R. D. (1975). Use of androgens for the production of all-male Tilapia aurea (Steindachner). *Transactions of The American Fisheries Society* 104(2), 342-348.

Guerrero, R. D., & Guerrero, L. A. (1988). Feasibility of commercial production of sex-reversed Tilapia in the Philippines. *SEAFDEC Asian Aquaculture* 1(2), 4-6.

Hunter, G. A., & Donaldson, E. M. (1983). Hormonal sex control and its application to fish culture. *Fish Physiology* 9B, 223-303. https://doi.org/10.1016/S1546-5098(08)60305-2.

Johnstone, R. (1985). Hormonal sex reversal in fish. *Aquaculture* 57 (1-4), 211-220.

Klemick, H., & Lichtenberg, E. (2008). Pesticide use and fish harvests in Vietnamese rice agroecosystems. *American Journal of Agricultural Economics* 90(1), 1-14. https://doi.org/10.1111/j.1467-8276.2007.01059.x.

Li, M., Sun, L., & Wang, D. (2019). Roles of estrogens in fish sexual plasticity and sex differentiation. *General and Comparative Endocrinology* 277, 9-16. https://doi.org/10.1016/j.ygcen.2018.11.015.

Mair, G. C., Abucay, J. S., Skibinski, D. O. F., Abella, T. A., & Beardmore, J. A. (1997). Genetic manipulation of sex ratio for the large-scale production of all-male tilapia *Oreochromis niloticus* L. *Canadian Journal of Fisheries and Aquatic Sciences* 54(2), 396-404.

Pandian, T. J., & Sheela, S. G. (1995). Hormonal induction of sex reversal in fish. *Aquaculture* 138(1-4), 1-22. https://doi.org/10.1016/0044-8486(95)01075-0.

Piferrer, F. (2001). Endocrine sex control strategies for the feminization of teleost fish. *Aquaculture* 197(1-4), 229-281. https://doi.org/10.1016/S0044-8486(01)00589-0.

Rubin, D. A. (1985). Effects of pH and temperature on sex ratio in cichlids and a poeciliid (*Acanthophacelus*). *Copeia* 1985(1), 233-235.

Shapiro, D. Y. (1987). Differentiation and evolution of sex change in fishes. *BioScience* 37(7), 490-497. https://doi.org/10.2307/1310421.

Tayamen, M. M., & Shelton, W. L. (1978). Inducement of sex reversal in *Sarotherodon niloticus* (Linnaeus). *Aquaculture* 14(4), 349-354. https://doi.org/10.1016/0044-8486(78)90017-0.

Yamamoto, T. (1969). Sex differentiation. In Hoar, W. S., & Randall, D. J. (Eds.). *Fish Physiology, Volume 3: Reproduction and growth bioluminescence, pigments, and poisons* (117-175). New York, USA: Academic Press.