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Original Research Articles

Improved breeding and seed production of climbing perch (*Anabas testudineus*) in controlled tanks and cage systems

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The climbing perch (*Anabas testudineus*) is a valuable fish species with significant potential for aquaculture. However, a low survival rate was observed at its early development stages, requiring an optimized protocol for sustainable aquaculture production. This paper presents an improved breeding and seed production technology for climbing perch. Mature climbing perch were induced to spawn using salmon gonadotropin-releasing hormone analog (sGnRHa) at a dose of 30 µg/kg-1 body weight of the breeder. The female broodfish spawn 9–10 hours after hormone injection. Developmental stages were defined based on the morphological features of the embryos from the first cleavage to the hatching stage. The first cleavage began at approximately 18–20 minutes after fertilization. Newly hatched larvae were observed within 18–20 h at 30°C, having an average larval size of 0.389±0.042 mm (total length). Successful breeding entails a selection of suitable algal species, an optimal aquaculture environment, regular feeding rates, consistent monitoring of larval development, and effective management of water quality. Survival rates from the larval stage to fry were achieved at 84.69% and 77.60%, respectively, while the survival rate to the fingerlings stage was 72.51%. Therefore, by implementing an optimized protocol, aquaculture practitioners can maximize the production potential of climbing perch while ensuring sustainable cultivation practices. The findings from this research contribute to the advancement of climbing perch aquaculture by providing valuable insights for the successful cultivation and increased economic profitability of *A. testudineus* species in hatchery productions.

INTRODUCTION

The climbing perch (*Anabas testudineus*) is a unique and fascinating fish species that has captured the attention of many aquaculture enthusiasts. The climbing perch primarily thrives in freshwater environments like lakes, rivers, and swamps. This species, also known as the walking fish, possesses extraordinary abilities to adapt to diverse aquatic environments and can even navigate on land using specialized adaptation.[1] They can also survive challenging conditions including low-oxygen environments, stagnant water bodies, and even temporary puddles during dry seasons.[1] While climbing perch exhibits unique adaptive characteristics in its body structure and respiratory system, enabling survival in waters with fluctuating oxygen levels, the early life stage of this species has shown challenges, leading to lower survival rates and reduced production output in aquaculture.

Climbing perch has been widely studied to increase production and was considered a candidate fish for species diversification in freshwater aquaculture.[2] Moreover, the climbing perch is produced in many parts of Asia, and research is being conducted to improve its aquaculture potential and production. In countries like India and Bangladesh, climbing perch is already being extensively cultured and is known as a nutritious delicacy.[3,4] This species exhibits superior growth, with two culture cycles annually and achieving a size of 80-100g within 90-120 days. It is well-suited for high-density stocking in ponds and is regionally preferred, commanding a high market price.[5] Climbing perch serves as a cheaper source of essential amino acids, protein, fat and ash required for dietary consumption. It contains considerable vitamin A and cal-

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cium.[6] It is also a rich source of iron and copper, which are essential minerals for hemoglobin synthesis.[7] Its flesh is composed of high amounts of fatty acids like linolenic and docosahexaenoic acids necessary for good heart and proper nerve development.[8] Notably, the climbing perch, with its muscle, eggs, and liver rich in various nutrients, serves as a healthy dietary choice, offering essential nutrients such as potassium and vitamin B12.[9]

In the Philippines, the climbing perch (*A. testudineus*) holds notable significance in aquaculture contributing to the country's fisheries sector and is a significant dietary resource.[10] Aquaculturists often rely on traditional practices, including artificial propagation, involving hormonal induction to synchronize and enhance the breeding process. This species is economically important due to its adaptability to various environments, high market demand and is often sold fresh in local markets.

Despite a good market demand for climbing perch, the current supply relies solely on capture fisheries, which puts more pressure on wild stock.[11] Aquaculture production of climbing perch can address this issue, but constraints on the nonavailability of quality seeds from the natural habitat persist. Furthermore, *A. testudineus* has been labeled as a species with insufficient data by the International Union for Conservation of Nature (IUCN) in the 2020 assessment of global aquatic environments.[12]

Climbing perch farming in the Philippines is uncertain and requires additional refinement.[13] In previous years, the production of this species has been exclusively dependent on catching fish from natural sources. Information from the Philippine Statistics Authority (PSA) indicates a decrease in the capture of climbing perch within Philippine waters. Furthermore, the output of climbing perch derived from inland sources has experienced a decline, falling from 2,072.38 metric tons in 2017 to 1,805.27 mt in 2019.[14] Aya, Gutierrez, and Garcia[15] suggested that further investigations into strategies for rearing larvae were required to enhance the hatchery survival rates of climbing perch.

Thus, rearing protocols were executed by this study to increase survival and focus on the efforts established by hatchery techniques in captive conditions. Detailed procedures that were employed from larval rearing up to the nursery stage have been implemented for the mass production trials of climbing perch. Furthermore, a cost-return analysis has been incorporated to examine the overall profitability of the entire rearing process.

MATERIALS AND METHODS

BROODSTOCK COLLECTION AND TRANSPORT PROTOCOL

Fish were collected from the wild and conditioned for three months in preparation for this study. 123 samples of climbing perch broodstock were collected in May 2022 from a private fish farm in Barangay Tiwi, Barotac Nuevo, Iloilo, Philippines (10.9216° N, 122.7407° E). Fish were transported to the hatchery facility of the Bureau of Fisheries and Aquatic Resources–National Freshwater Technology Center (BFAR–NFTC), packed in polyethylene plastic bags with water and oxygenated air, and sealed with rubber bands due to the thick and spiny dorsal fins of climbing perch, which have the potential to tear regular packaging materials, a double packaging method consisting of sturdy cardboard and plastic bags was used. The fish were anesthetized using tricaine methanesulfonate during transport.

BROODSTOCK ACCLIMATIZATION AND STOCKING IN HAPA NET CAGES

Upon arrival at the hatchery facility, the fish samples were acclimatized in tanks with water for a period of 7–14 days for quarantine and monitoring. Selected fish were healthy with no lesions or ulcerations. They were free from parasites and had proportional body parts without deformities. Nevertheless, fish that exhibited certain ulcerations and were found to have parasites within a 2-week acclimatization time frame were retained in tanks for an extended period of 1 month for further observation and administration of necessary treatments. This included undergoing salt baths designed to target and eradicate the ectoparasites affecting the fish. Simultaneously, regular water changes and proper sanitation protocols were implemented to maintain a clean environment. After an acclimatization period, the collected fish samples were stocked in 1 m x 1 m x 1 m hapa nets in an earthen pond with a size of 165 m^2 for conditioning. Each cage was populated with a group of 10–15 fish and were labeled with codes that corresponded to the specific collection location of the fish.

Before stocking in conditioning hapa net cages, fish were examined to make sure no parasites were attached to their bodies. They were disinfected in a 30-part-per-thousand (ppt) salt bath for 10 seconds prior to stocking in the pond.

BROODSTOCK MANAGEMENT

Climbing perch breeders were fed 5% of their body weight with commercial tilapia starter feed pellets containing 31.22% crude protein, 8.31% ash, 0.47% moisture and 12.21% fat. They were fed once or twice a day, morning (09:00h) and afternoon (16:00h), and their hapa net cages were changed once a month.

Sampling was conducted to determine the condition of the broodstock. Sexually mature females have round bodies with bulged bellies and reddish swollen genital openings. Compared to the females, the male breeders were slender with oozing milt. Both female and male breeders were selected for induced spawning. This experimental study was implemented at the Fisheries Biotechnology Center hatchery in the Science City of Muñoz, Nueva Ecija.

PREPARATION AND ADMINISTRATION OF HORMONES

Nine climbing perch breeders out of the 123 collected samples were used in the study: 3 females and 6 males. The sample size of 3 females and 6 males was selected to ensure diversity and representativeness while addressing resource constraints such as space and equipment limitations. This approach is aligned with common research practices, considering the focus on specific traits and practical considerations for handling and maintaining breeding popula-

tions. The sample size of 3 females and 6 males was chosen to ensure diversity and representativeness, considering resource constraints and focusing on specific traits in line with research objectives and practical considerations for handling breeding populations. The female breeders weigh from 24–29 g, with body lengths spanning from 115–130 cm. The male breeders, on the other hand, weigh from 19–27 g, with body lengths spanning from 105–125 cm. Breeders were assigned at a 1:2 female-to-male ratio.

A ready-to-use, commercially available sGnRHa hormone in a liquid formulation was used at 30 µg of hormone /g of fish body weight. The sGnRHa was injected intramuscularly into the base of the pectoral fin of male and female climbing perch fish using a sterile 1-cc syringe. The fish were then placed inside 20-liter plastic tubs without aerators. The plastic tubs were covered with nets to prevent the fish from escaping and to provide them with a dark environment for spawning.

The computation of hormone concentration was accomplished using the following equation:

Hormone concentration = (hormone dose x average fish body weight)/(injection volume)

EGG COLLECTION AND INCUBATION

Eggs were collected using scoop nets (100µm mesh size) 2–3 h after spawning to ensure their fertilization. Prior to transferring the larvae, water in the hatching tank was prepared with *Chlorella vulgaris* water a day before stocking to support successful incubation. The collected eggs were stocked in a concrete tank containing 1500 L of green water with a cell density of 1–3 x 10^5 cells/mL, where they remained until hatching. To initiate the green water culture, a suitable water medium is enriched with a balanced mix of nutrients, often including nitrogen and phosphorus compounds, to stimulate microalgae growth. Proper light conditions, temperature, and aeration support optimal algal growth. Regular monitoring of water quality parameters ensures a stable and nutritious environment, fostering the development of robust and healthy aquatic organisms within the system. Proper handling and management were taken to prevent any damage to the eggs during the transfer process. After egg collection, breeders were taken out of the breeding tubs and placed back into their individual hapa nets. The hatching took place 20–21 h after fertilization, with a temperature range of 28–30°C.

LATENCY PERIOD AND SPAWNING PERFORMANCE

The latency period is the duration between hormone injection and the first appearance of spawned eggs. After complete spawning, all eggs were collected and transferred to separate incubation tanks (replicated groups) for larval rearing. The calculation of total fecundity and relative fecundity can be expressed in equations as follows:

Total Fecundity (TF):

$$TF = \text{Number of eggs per gram} \times \text{Total volume of released eggs}$$

In this equation, the "Number of eggs per gram" refers to the count of eggs found in 1 gram of female fish.

Relative Fecundity (RF):

$$RF = \frac{Total\ amount\ of\ eggs}{weigh\ of\ female\ fish\ (g)}$$

Here, the "Total amount of eggs" is the overall count of eggs released, and the "Weight of female fish" is the weight of the female fish in grams.

After the completion of spawning, representative samples of eggs were collected from the incubation tanks. The eggs were then carefully examined under a microscope to distinguish fertilized from unfertilized ones. The number of fertilized eggs was enumerated, providing the basis for calculating the fertilization rate. This rate was expressed as a percentage, calculated using the formula:

$$Fertilization\ rate\ (FR) = \frac{Number\ of\ fertilized\ eggs}{Total\ number\ of\ eggs} \times 100$$

Following this, the hatching rate was determined by monitoring the emergence of larvae from the fertilized eggs over a specified period. The number of hatched larvae was then enumerated, and the hatching rate was calculated as a percentage:

$$Hatching\ rate\ (FR) = \frac{Number\ of\ hatched\ larvae}{Total\ number\ of\ fertilized\ eggs} \times 100$$

OBSERVATION OF EMBRYONIC TO LARVAL STAGES OF CLIMBING PERCH UNDER A MICROSCOPE

To study embryonic development, 30 eggs were sampled and examined under a compound microscope. Photographs were captured using a digital camera attached to the microscope whenever there were noticeable differences in embryonic development stages as shown in **Figure 1**. The time taken for the first newly hatched larvae to appear was recorded. For the larval stages, a collection of 10 larvae were preserved in vials with 10% buffered formalin. They were viewed using Olympus CX23, a light microscope. The vials containing the larvae were labeled accordingly by the time they were taken. Additionally, ToupView software was used to measure the size of embryos and larvae.

LARVAL REARING

The tanks were thoroughly cleaned the day before stocking. Disinfection was carried out by introducing chlorinated water into the tanks and allowing the water to stand overnight. Typically, a chlorine concentration of 10 ppt was employed. The initial fish stocking density comprised 100 larvae within each square meter, which was equivalent to 1,500 larvae within tanks measuring 1 m x 5 m x 1 m in length (3 replicates) with a water volume of 200 liters. The estimated quantity of larvae required to be moved from the hatching box to the larval rearing tanks needed to be calculated. Prior to the egg transfer, the water in the rearing tank was conditioned with green water (*Chlorella vulgaris water)*. A weekly water exchange of 50% was conducted. From the third to the fifth day, food for climbing perch larvae consisted of *Brachionus* sp. at a concentration of 5 rotifers/mL. The feeding rate was incrementally raised to 10 rotifers/mL from the sixth to the tenth day. Subsequently, the rate was further elevated to 20 rotifers/mL mixed with *Moina* sp. (5–7 individuals/mL) from the 11^{th} to the 14^{th} day of the rearing period as shown in **Table 1**. Each larva could consume the food within 24 h. During the entire period of

Table 1. Feeding rate of *Chlorella vulgaris* and *Brachionus* sp. given as food for *A. testudineus* from larval to fry stage

Days	*Microalgal cell concentration (cells individual/day)*	*Rotifers individual (cells and-^{12} day-^{12})*	*Moina individual (cells and-^{12} day-^{12})*
0	1 x 10^5/day	-	-
3–5	1 x 10^5/day	5 individuals/mL	-
6–10	2 x 10^5/day	10 individuals/mL	-
11–14	3 x 10^5/day	20 individuals/mL	5–7 individuals/mL

tank-based culture, the prevailing temperature remained at 28-30°C. A consistent and modest aeration process was initiated once the larvae reached the eighth day.

After 2 weeks of being reared in tanks, the climbing perch fry were placed into hapa nets within the pond with an average length of 17.12 mm. The juvenile climbing perch was transferred to hapa net cages suspended in the pond following a 2-week duration of tank rearing. A consistent water level of 15–20 cm was monitored in every enclosure. The ponds were introduced to different zooplankton and fertilized with chicken manure. After a preparation period of 1 week, the climbing perch fry were transferred in a fine-mesh net cage of 1 m x 5 m in size. Supplementary feeding consisted of fry mash feed and natural plankton in the pond. They were provided with natural planktonic food present in ponds and a commercial tilapia fry mash diet (Crude Protein:42%; Crude fat:5%; Crude fiber:5%; Crude Ash:16%; Moisture:12%). They were fed twice daily, 09:00h in the morning and 16:00h in the afternoon, to facilitate their growth and development. Regular water quality monitoring was performed to maintain optimal rearing conditions. Consistent monitoring of water parameters, including temperature, pH, dissolved oxygen, and ammonia levels, was conducted. Partial water changes were implemented to reduce pollutants and restore essential minerals, while routine removal of debris, sediment, and accumulated sludge was carried out to prevent the accumulation of organic matter that could compromise water quality.

FISH SAMPLING

Precise length and weight measurements of the wet fish samples were recorded during the initial phase of the study. The growth and survival rate data for climbing perch were carried out through a series of samplings of length (mm) and weight (mg) at specific time points: 0, 5, 10, 15, 20, 25, 30, 35, 40, and 45 days after hatching (DAH). Samples were collected 2 h after feeding, and their length was measured with a stereo microscope during the early stage and with a vernier caliper as they developed. The weight measurements were taken in wet samples using a semi-microanalytical balance. Fish counts were conducted to determine the survival rate.

On the 15th, 35th, and 45th days, a comprehensive counting method, referred to as total counting, was employed. This likely involved visually counting every fish in the designated cages during hapa change. The purpose of these counts was to track changes in the number of fish over time and ultimately calculate the survival rate, providing insights into the overall success of the fish population during the experiment. Enumeration on these specific days allowed for calculating the percentage of fish that persisted throughout the experimental period. Total length (mm) and weight (mg) growth were measured from 30 larval samples in cages per replicate. The larvae grew to 23.61±0.77 mm and weighed 970 ± 1.563472 mg during the 20-day culture period. For 35 DAH, the nurseries attained a size of 28.089±1.4955mm with a weight of 1600 ± 2.3945 mg, which accounts for a survival rate of 77.60%. Moreover, on day 45, the climbing perch grown in ponds obtained a survival rate of 72.51%, measuring between 50.07±1.6779mm in length and between 3700 ± 1.763834 mg in weight. Enumeration on these specific days allowed for the calculation of the percentage of fish that persisted throughout the experimental period. Growth in terms of total length (mm) and weight (mg) was measured from 30 larval samples per replicate **(Table 4).**

ECONOMIC PARAMETERS

The variable expenses, depreciation costs, and gross profit were considered in the cost return analysis of the experimental study. This assessment was based on a production cost of PhP 2.00 per piece (selling price after a 45-day culture period) and an electricity cost of PhP 299.86 per cycle. The study was carried out in an earthen pond with an area of 165 m^2. The pond used was equipped with eleven hapa net cages measuring 1m x 5m x 1m. The stocking density was set at 100 pieces per square meter with a total of 1500 per net cage, and the production duration was calculated for the period of 45 days, equivalent to one cycle.

Formula:

$$\text{Gross profit} = \text{Total production per cycle } X \text{ cost per fingerling}$$

$$\text{Payback period} = \frac{\text{Total cost}}{\text{Net Profit}}$$

$$\text{Net profit} = \text{Gross Profit} - \text{Total cost}$$

$$\text{Return on Investment (ROI)} = \text{Net income/total cost X 100}$$

$$\text{Depreciation Cost} = \frac{(\text{Asset Value}) - 0\,(\text{Market Value})}{\text{Useful life in Years}\,/8\text{ cycles (per year)}}$$

RESULTS

The breeding performance of female climbing perch breeders, with weights of 24.2g, 28.5g, and 29.4g in replicates 1, 2, and 3, was observed and measured according to these five parameters: latency period, fecundity, fertilization rate, incubation period, and hatching rate. Data on these parame-

Table 2. Effect of 30 µg/kg sGnRHa hormone on latency, fecundity, fertilization rate, incubation time, and hatching rate of climbing perch at 28-30°C.

Code	*Latency (h)*	*Fecundity per g/ BW*	*Fertilization rate (%)*	*Incubation time (h)*	*Hatching rate (%)*	*Remarks*
Replicate 1	9 h:30 min	14,900.46	99.08	20	96.8	Complete spawning
Replicate 2	9 h:33 min	8,778.87	98.74	21	97.1	Complete spawning
Replicate 3	10 h:10 min	14,634.69	99.56	20	98.7	Complete spawning

Note: BW= body weight; g=grams; h=hours

ters are shown in **Table 2**. Results showed that the latency and incubation periods ranged from 9–21 h. The 30 µg/g sGnRHa have acquired an average fecundity of 2,253.48 eggs/g of body weight. At the end of the experiment, fertilization and hatching rates reached their average at 99.12± 0.137 and 97.5±0.342 at 28-30°C, respectively. No adverse effects were observed in spawners injected with sGnRHa. Mueanwhile, the embryonic development of climbing perch, which was timed hours after spawning (HAS), was presented in **Table 2**.

In the present study, the initial observation on the first day after fertilization revealed that the newly hatched larvae were tiny, translucent larvae with basic characteristics, and had relatively larger yolk sacs. 0.032–0.046 mm in length and weighed 0.0069 mg. By the third day, there was a noticeable increase in color pigmentation, making them slightly less transparent, and they started to exhibit a subtle curvature in their body shape. The larvae measure about 7.699 mm in length and 0.911 mg in weight. On the fifth day, they showed improved mobility, as they developed distinct fins, allowing them to move more efficiently in the water. Their average length was 8.84mm, and they weighed 1.906 mg. By the tenth day, the larvae's fins became more pronounced, and their body structure became more defined, with an average length of 14.12 mm, and an average weight of 44 mg. By the 15th day, a remarkable transformation had occurred, with the larvae now displaying well-formed fins, a clearly articulated body structure, and distinct color patterns. Their length measured 17.12 mm, and they weighed 80 mg. On the 45th day, the climbing perch exhibited an increase in growth length, reaching approximately 50.07 mm. Simultaneously, a weight increase of around 3700 mg was observed on the same day, as shown in **Table 4**.

Survival rates were remarkably outstanding, with 84.69% of larvae successfully transitioning to the fry stage and 77.60% of fry progressing to the fingerling stage. However, as the climbing perch advanced to the fingerling stage, the survival rate slightly decreased to 72.51%, as shown in **Table 5**.

COST RETURN ANALYSIS

The cost return analysis of the climbing perch production demonstrates a favorable outcome in this study. The depreciation cost, amounting to PhP 1,920.00, covers essential expenses like equipment (breeding tanks, fine mesh nets and GI pipes) that shall remain constant regardless of production levels. It accounts for the decline in the assets worth and allocates this expense over its estimated useful period. The variable cost at PhP 17,951.86, which fluctuates with production, includes inputs like feed, labor, electricity, other agricultural supplies etc. Each cycle production stands at 11,418 fish, with an annual output of 91,344 fish. The cost per fingerling for 45 days of rearing is PhP 2.00. When combining depreciation and variable costs, the total expenditure amounts to PhP 18,497.36 as presented in **Table 6**. The ROI resulted at 23.455 with a payback period of 4.26 cycles, indicating the profitability of the venture as shown in **Table 7**.

DISCUSSION

Breeding and early rearing are important practices for the sustainable production of climbing perch in captivity, and a common technique to ensure a stable supply of larvae is the use of synthetic hormones. Induced spawning is a common technique used in hatcheries to increase fish ovulation through the application of hormones necessary to stimulate internal reproduction factors. This technique enables aquaculture farmers to breed fish species that do not reproduce naturally in captivity and to manipulate the timing of the egg production cycle.[16] Gonadal development in fish is primarily regulated by two major hormones, the luteinizing hormone and the follicle-stimulating hormone.[17] The sGnRHa, a synthetic gonadotropin-releasing hormone, resembles these naturally occurring GnRHs produced from the hypothalamus of fish, which then triggers hormone secretion from the pituitary gland. Breeding of climbing perch necessitates an optimal hormone dose to encourage more gonadotropin activity for successful ovulation.[18] Various fish species have been effectively induced to spawn with sGnRHa, either alone or in combination with dopamine receptor antagonists.[19]

The results of this study provided evidence that the use of 30 µg/kg sGnRHa was effective for inducing spawning in *A. testudineus*. The fish spawned 9–10 h after hormone administration, where an average spawning fecundity of 12,771.34 per female was obtained. Fertilization and hatching rates were also remarkable at 99.13% and 97.50%, re-

Table 3. Time of different embryonic stages in climbing perch at 28-30°C.

Development stages	*Development time*	*Descriptions*
Fertilized egg	0 HAS	The fertilized eggs are small with diameter ranging between 0.90–0.97 mm. The fertilized eggs of *A. testudineus* were noted to be nearly spherical or oval in shape, exhibiting a clear, pearl-like appearance and floating freely on the water surface.
2-cell stage	1.0 HAS	Two-cell stages of cleavage showed blastodiscs that separated into two divisions.
4-cell stage	1.25 HAS	Second cleavage occurred perpendicularly to the first cleavage.
8-cell stage	1.5 HAS	Third cleavage began to develop parallel to the first cleavage plane.
16-cell stage	2.0 HAS	Cells were found and a layer of 16 cells was formed.
32-cell stage	2.25 HAS	Fifth cleavage divided blastoderm into 32 cells.
64-cell stage	2.5 HAS	Six cleavages developed and a 64-cell layer was formed.
128-cell stage	3.0 HAS	Multiple cells with almost regular shape were formed, having been through numerous rounds of cell division.
Morula stage	3.5 HAS	Blastodiscs consists of blastomers.
Early blastula stage	5.0 HAS	Blastodic showed 2 layers of cells indicating the start of the blastula stage.
Blastula stage	6.0 HAS	Epibolic cells have developed and germ ring appeared
Pre-early gastrula stage	7.0 HAS	Blastoderm became flattened down onto the yolk sphere.
Early gastrula	7.5 HAS	Blastodic showed a thick region that extended toward the yolk.
Pre-mid gastrula stage	8.0 HAS	Differentiation of early notochord was clearly seen. Blastoderm had almost covered the entire yolk sac. Ectoderm and endoderm were visible.
Mid gastrula stage	9.0 HAS	Poles can be on both ends and the gastrula. Neural plates formed.
Early neurula stage	10.0 HAS	Neural crests formed from neural plates.
Late neurula stage	10.5 HAS	Optic bud, brain and spinal cord were formed.
4-somite stage	11.0 HAS	Four-somite cells were found adjacent to the notochord. The optic cup was visible.
6-somite stage	12.0 HAS	Six-somite cells were visible beside the notochord. Optic lens have started to develop.
9-somite stage	13.0 HAS	Nine-somite cells were found adjacent to the mid-posterior part of the notochord.
12-somite stage	14.5 HAS	Chromatophores were completely developed.
16-somite stage	16.5 HAS	The first heartbeat was observed.
Newly hatched larva	20.0 HAS	Hatching was complete. The newly hatched larva has a transparent slender body with a round yolk sac.
1-day old larva	24 HAS	Total length of first day old larvae ranged from 0.48-0.65 mm. The pigmentation extended to the yolk sac both dorsally and ventrally. Typical star-shaped melanophores clusters appear around the final tract of the intestine

spectively. Moreover, the incubation period for the eggs lasted for up to 20–21 h at a temperature range of 28–30°C. The sGnRHa is chosen as an inducing agent due to its effectiveness, species compatibility, advancement in reproduc-

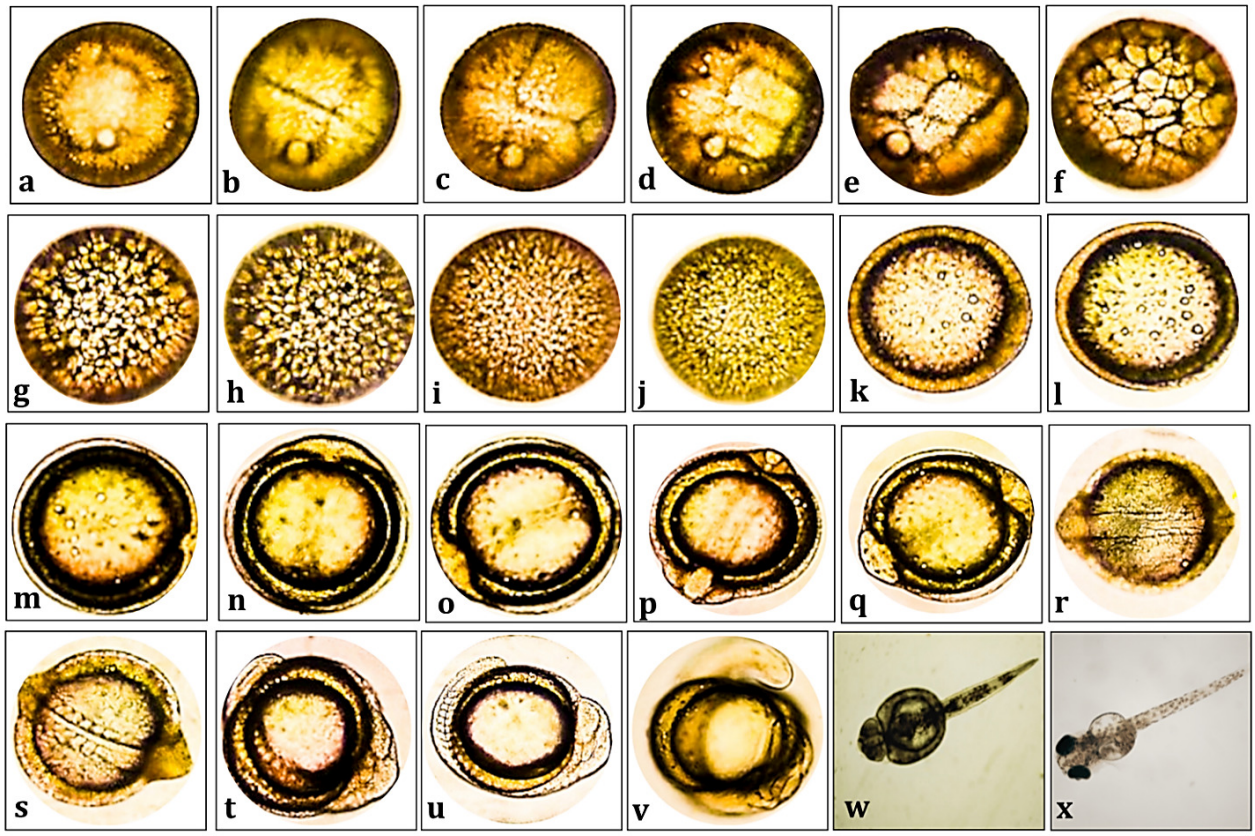

Figure 1. Embryonic development of Climbing perch, *Anabas testudineus*

(a) fertilized egg, (b) 2- cell stage, (c) 4- cell stage, (d) 8- cell stage, (e) 16-cell stage, (f) 32-cell stage, (g) 64-cell stage, (h) 128-cell stage, (i) morula stage, (j) early blastula stage, (k) blastula stage, (l) pre-early gastrula, (m) early gastrula, (n) pre- mid gastrula, (o) mid-gastrula, (p) early neurula, (q)late neurula, (r) 4- somite stage, (s) 6-somite stage, (t) 9- somite stage (u) 12- somite stage, (v) 16- somite stage, (w) newly hatched larva (x) 1 day old larva.

Table 4. Climbing perch larval growth for 45-day period

No. of days	*Length (mm)*	*Weight (mg)*
0	0.389 ± 0.0422	0.0069 ± 0.0015
1	0.581 ± 0.0617	0.0349 ± 0.0008
3	7.699 ± 0.2981	0.911 ± 0.0087
5	8.84 ± 0.5420	1.906 ± 0.0195
10	14.12 ± 0.4491	44 ± 0.1763
15	17.12 ± 0.4049	80 ± 0.1763
20	22.8 ± 0.7795	970 ± 1.5634
25	23.61 ± 2.0538	980 ± 4.8283
30	24.759 ± 6.9541	1450 ± 4.7107
35	28.089 ± 1.4955	1600 ± 2.3945
40	39.49 ± 3.6259	1840.1 ± 1.6633
45	50.07 ± 1.6779	3700 ± 1.7638

Note: Values were measured from 30 larvae

Table 5. Total number of larvae and survival rate (%) of climbing perch produced

Days	*Total larvae count (average)*	*Overall survival rate (%)*
1–15	1270.33 ± 34.222	84.69%
25–35	1164 ± 15.8219	77.60%
45	1087.67 ± 34.9131	72.51%

tive technology, availability, and cost-effectiveness.[20] The use of sGnRHa and the dose of 30 µg/kg were chosen based on established literature. The study closely correlates with the findings obtained by Levavi-Sivan et al.[21] using sGnRHa at varying doses. Based on their study, the optimal dose for achieving a higher spawning percentage in silver perch lies between 30 and 40 µg/kg of GnRHa.[21] Additionally, the decision to choose sGnRHa was influenced by a desire to minimize side effects, prioritize the health and behavior of the fish subjects, and consider factors such as availability, cost-effectiveness, potential advancements in hormonal induction techniques, and ethical or regulatory considerations.

A dosage experiment in a previous study (unpublish data) was conducted to determine the optimal dose of sGnRHa for induced spawning of climbing perch. Each spawner was injected intramuscularly with different doses of sGnRHa (10, 20, and 30 µg/g) and a saline solution as a control treatment. The highest spawning fecundity, fertilization, and hatching rates were obtained using the highest treatment dose of 30 µg/g sGnRHa at an incubation period of 20 h. The administration of sGnRHa at 10 and 20 µg/g in female climbing perch was seen to be insufficient for ovula-

Table 6. Economic parameters of climbing perch reared in tanks and cages

A. Personel Expenses	Unit	Unit Cost	Quantity	Total cost	Total Cost/ cycle
Field Assistant/ Laborer (3 hours/day for 45 days)	pax	8,437.50	1	8,437.00	8,437.50
Sub total				8,437.00	**8,437.00**
B. Pond Preparation Expenses					
Lime	sack	100	3	300	300
Chicken Manure	sack	100	3	300	300
Fine mesh net	roll	4,000.00	2	9,000.00	8,000.00
Rope, 3.5mm	roll	500	1	500	500
Gi Pipes, Sch. 40, 60cm	piece	300	10	3,000.00	3,000.00
Sub total *(depreciation cost per cycle for 3 years)*				**13,100.00**	**545**
C. Broodstock Development and Maintenance					
Breeder	kilogram	250	4	1,000.00	1,000.00
Commercial Feed Pellets	sack	1,900.00	1	1,900.00	1,900.00
Breeding tank	piece	125	11	1,375.00	1,375.00
Sub total				4,275.00	**4,275.00**
D. Induce Spawning Expenses					
Hormone	vial	2,800.00	1	2,800.00	350
Syringe	box	400	1	400	400
Sub total *(depreciation cost per cycle)*				**3,200.00**	**750**
E. Larval & Fry Rearing Expenses					
Chlorella Paste	kilogram	1,500.00	1	1,500.00	1,500.00
Rotifer, 1L	liter	100	3	300	300
Commercial Fry Mash	sack	1,800.00	1	1,800.00	1,800.00
Air blower	unit	1,500.00	1	1,500.00	187.5
Airhose	roll	500	1	500	500
Sub total *(depreciation cost per cycle for 3 years)*				**5,600.00**	**4,287.50**
F. Operational and Other Valiable Expenses					
Fuel	liter	68	5	340	340
Electricity	kWh	8.76	34.25	299.86	299.86
Sub total				**639.86**	**639.86**
Grand Total				**30,976.86**	**18,934.36**

Note: Values calculated in (Philippine peso) PhP/cycle/pond(165m2); 1PhP= 0.0176 USD; Hormone concentration=1.17ml/cycle @ 3 pairs of breeders

Table 7. Cost return analysis for climbing perch production

Cost Return Analysis	
Depreciation Cost	1,920.00
Variable Cost	17,951.86
Total Production/Cycle	11,418.00
Total Annual Production (8 cycles)	91,344.00
Gross Profit @PhP 2.00/fingerling	22,836.00
Net Profit	4,338.64
Payback period (cycles)	4.26
Total Cost	18,497.36
ROI	23.45546361

tion, which resulted in low fertilization and hatching rates. The data suggest that low or suboptimal doses of a reproductive hormone do not promote the development of eggs and the subsequent release of eggs during ovulation.[22]

Mandal, Kumar, and Jayasankar[18] conducted a similar experiment on the efficacy of sGnRHa at 6 different doses using a single intramuscular injection of GONOPRO-FH^{TM} at 0.002, 0.005, 0.01, 0.015, 0.02, and 0.03 µg/g body weight. They have observed that parameters like fecundity, fertilization and hatching percentages had improved at an optimal dose. Their findings revealed that female climbing perch treated with 0.015 µg/g body weight had the highest relative fecundity (715.13 ± 15.0 eggs/g) and fertilization rate (93.1 ± 8.0%). However, the female fish that received the highest dose of sGnRHa (0.03 µg/g body weight) had lower relative fecundity and hatching percentages.

Furthermore, a few other tests have been conducted on the effects of different hormones used for induced spawning of climbing perch. The assessment of developmental stages in this study significantly deviates from previous research on the early development pattern of *A. testudineus*, spanning from cleavage to subsequent divisions as illustrated in **Table 8**.

As subsequent stages unfold gradually, progressing in terms of both minutes and hours, they eventually culminate in the emergence of a newly hatched larva, which typically occurs at around the 20–21-h mark. Some studies were already conducted on the reproduction, early development, and larval rearing of climbing perch.[15,23-25]

As an attempt at breeding and developing culture production of climbing perch in the Philippines, Aya, Gutierrez and Garcia[15] found that the initial cleavage emerged half an hour after post-fertilization. The maturation of the egg extended for a period of 20–24 h post-fertilization (HPF) under a temperature of 29°C, employing a combination of hCG and 0.5 mL Ovaprim. In a related study, Ismail[23] investigated the use of luteinizing hormone-releasing hormone analogue (LHRHa) in climbing perch, finding that 200 µg/kg of LHRHa significantly increased egg production, while 2 µg/kg resulted in the highest hatching rate at 65.33%. Furthermore, Morioka et al.[24] reported a 100% fertilization rate with 14,000 fertilized eggs produced by a 50 g female climbing perch. Hatching occurred approximately 10.5–11.0 hours after spawning, with a hatching rate close to 100% at 27.2 to 29.1°C. The total length of newly hatched larvae ranged from 1.7 to 2.0 mm, with an average of 1.9 ± 0.1 mm. Amornsakun et al.[25] found that female climbing perch reach sexual maturity at 15.20±1.24 cm and 61.10±17.32 g, producing 24,120.5±3,328.24 ova/fish. Fertilized eggs recorded with a 92.67% fertilization rate and an 87.44% hatching rate at 27.0-30.5°C. Newly hatched larvae measured 2.02±0.20 mm.

Notably, in the study of Rahmadi et al.,[26] the developmental stages displayed certain discrepancies, which were potentially influenced by the use of Ovaprim as the inducing agent. This hormonal treatment has the potential to introduce variations in the progression of development. Rahman et al.[27] ascertained that the developmental timeline followed a distinct path due to the specific environmental conditions and the application of sGnRHa. Furthermore, Hassan, Okomoda, & Sanusi[28] incorporated triploidy and the administration of Ovaprim in their study, where a highly distinctive approach was adopted. These unique factors had a substantial impact on the developmental timeline of climbing perch. In the case of Sarkar et al.,[29] they have observed that developmental stages closely resemble those in this present study. However, it is worth noting that there may be minor variations in the timing of these developmental milestones when compared to our study. Additionally, the combination of specific temperature conditions and hormonal treatment resulted in noticeable differences in the timing of developmental stages, setting it apart from other studies,[30] as presented in **Table 8**.

The larval rearing of climbing perch is challenging during their early life stages due to the limited availability of food organisms that are the right size for their tiny mouths, which constitute only about 20.93% of their mouth height.[25] An initial diet for most freshwater fish typically consists of either rotifers or Moina sp.[31] In the Philippines, a study has already been carried out on the reproductive processes, initial development, and rearing of climbing perch by Aya, Gutierrez, and Garcia.[15] They found that the highest recorded survival rate occurred at a stocking density of 25 larvae per liter, reaching 64% fed with a combination of Artemia nauplii and microparticulate diet.

In this study, both food and aquaculture were optimized to ensure rapid growth and increase survival rate. The assistance provided by the nutritional components of microalgae plays a crucial role in augmenting the growth and survival of larvae. Under optimal conditions, with appropriate feeding, climbing perch can exhibit noticeable growth with an increased survival rate of 72.51% within 45 days. As juveniles, they are commonly fed with artificial feeds in the form of finely crushed particles that are suitable for their size. During the developmental phase, *Brachionus* sp. was introduced as the starter food source for climbing perch larvae on 3rd day, and this feeding lasted for 2 weeks. Days 11–14, a blended concentration of *Moina* sp. was introduced, as indicated in **Table 1**. The feed intake in this study was determined through previous experimental trials and observations. Various feeding trials with different amounts and formulations were carried out, observing responses in growth, survival, and overall performance. The authors evaluated the optimal feed intake by examining physiological and growth parameters and analyzing data to identify favorable outcomes.

It incorporates microalgae, rotifers, and Moina to fulfill the specific dietary requirements of the fish. The adjustments in feeding parameters at each stage reflect a consideration of the growth and nutritional needs of the climbing perch. Concrete tanks of size 100 m^2 were taken for larval rearing of the climbing perch. All the necessary management practices were adopted in the usual rearing management of other studies.

A study by De Wolf et al.[32] suggests that microalgae yield a shading effect within tanks, diminishing the light-induced stress in larvae. Microalgae confer nutritional advantages by directly enhancing larval digestion and immunity, or indirectly serving as sustenance for rotifers within larval tanks. Furthermore, microalgae contribute to environmental optimization by stabilizing water quality and acting against detrimental bacteria present in both the water and the larvae.

Another study conducted by Chattopadhyay et al.[33] in India revealed enhanced growth and survival rates in hilsa larvae. This improvement was achieved by utilizing *C. vulgaris*, cultivated as green water, and incorporating it into larval feeding in combination with other zooplankton sources. This extensively tried method is employed in the rearing of hilsa shad larvae, as indicated by its success in achieving larval survival rates exceeding 88%. This accomplishment is attributed to the utilization of green water, combined with the co-feeding approach involving other plankton species and a formulated diet. Our strategy aimed

Table 8. Comparison of the duration of *A. testudineus* development from fertilized eggs to newly hatched larvae with other studies using different hormones.

Stage of development	*Present study (28–30°C) sGnRHa*	*Aya, Gutierrez, & Garcia[15] (29°C)–(hCG)+ 0.5 mL Ovaprim*	*Rahmadi et al.[26] (28°C)–Ovaprim*	*Rahman et al.[27] (26 ± 0.5°C)–sGnRHa*	*Hassan, Okomoda, & Sanusi[28] (28.6°C) triploid-Ovaprim*	*Sarkar et al.[29] carp PG*	*Zalina et al.[30] (26°C)–LHRHa*
Blastodic stage	18–20 min	32 min	34 min–1 h	18–25 min	4 min	20 min	1 h
2-cell stage	1 h	48 min	25 min	30–40 min	30 min	35 min	1 h:30 min
4-cell stage	1.25 h	1 h	41 min	1 h:15 min	1 h:45 min	1 h:5 min	2 h
8-cell stage	1.5 h	2 h:15 min	25 min	1 h:30 min	2 h:10 min	1 h:25 min	2 h:30 min
16-cell stage	2 h	3 h	15 min	1 h:45 min–1 h: 55 min	2 h:20 min	1 h:50 min	3 h
32-cell stage	2 h:50 min	3 h:50 min	45 min	2 h:30 min	2 h:30 min	2 h:15 min	3 h:30 min
Morula	3 h:50 min	5 h:22 min	4 h:30 min	3 h:10 min–4 h	4 h:30 min	2 h:40 min–4 h	5 h
Blastula	5–6 h	6–7 h	5 h:40 min	4 h–5 h: 40 min	6 h	4 h–5 h:30 min	6–7 h
Gastrula	7–9 h	12.5 h	8 h:47 min	5 h:50 min–7 h:40 min	8–12 h	5 h:30 min–7 h:30 min	8–11 h
Neurula	10 h:50 min	12–13 h	15 h:39 min	7 h:40 min–9 h:25 min	13–14 h	7 h:30 min–9 h	12–13 h
Somite	11–16 h: 50 min	14–18 h	6 h:8 min	9 h:30 min–19 h	16–21 h	9 h:30 min–19 h	14–19 h
Newly hatched larva	20–21 h	20–24 h	18 h:38 min	19 h–22 h	18 h–23 h	21 h–22 h	20 h

Note: h=hour; min=minutes

to strike a balance between controlling algae overgrowth and maintaining a sustainable and healthy pond environment. These methods were adapted based on best practices in aquaculture and pond management specifically: regular manual removal of excess algae to maintain optimal biomass levels; use of screens or barriers to limit sunlight penetration and control algae growth and introduction of natural algae-consumers, such as certain species of herbivorous fish or microorganisms, to help control algae populations.

Climbing perch species are usually farmed in monoculture systems, and stone-pitched ponds are ideal for farming the species as they exhibit climbing behavior.[34] However, the precise methods and factors to consider when moving fish from tank-based rearing to pond culture can differ based on the species and local circumstances. It is generally advisable to employ proper acclimatization and a gradual shift to reduce stress and facilitate a smooth adjustment to the new surroundings.

In Bangladesh, an examination of climbing perch aquaculture in a pond setting revealed that the primary determinant affecting productivity is the cost of industrial feed pellets. Likewise, an intensive farming approach marked by a comparatively elevated stocking density of fish population was demonstrated to be economically feasible.[9] There is still limited information available on the economic capability of climbing perch using natural food (live food).

In the present study using economic analysis, an increased survival rate and a reduced rearing duration at harvest correlated with increased net income. By incorporating depreciation cost or the gradual deterioration of capital assets over a period of time on the materials belonging to the fixed costs, the results of the economic analysis of the study accurately reflect the economic impact of capital investments on the overall cost structure of climbing perch production, providing a more realistic assessment of profitability and long-term viability.

Given that the breeding tanks are operational for 10 years, while the nets and GI pipes are anticipated to be useful for 3 years. Additionally, considering the 1.17ml of hormone concentration to the 3 pairs of breeders/spawning cycle, a vial of hormone for 8 cycles can be utilized. The depreciation cost incorporated into the formula is based on this eight-cycle span, as indicated in **Table 6**.

It is important to note that the initial capital outlay for materials and supplies for the study may be subject to fluctuations in prevailing market prices. The cost per unit of fry was determined based on the established price for catfish at the BFAR–NFTC, which ranges from PhP 1.00–2.00. There is no recognized market standard yet for the pricing of climbing perch fry in the country. Using this approach, economic indicators demonstrate that cultivating climbing perch is cost-effective and yields profitable outcomes over a production cycle. Specifically, rearing climbing perch directly in net cages or ponds resulted in a higher cost return of 23.45, as shown in **Table 7**.

The study findings reveal that it is feasible to rear climbing perch larvae in tank-based systems for initial development and then transfer them to cage-based systems within ponds for sustained nursery cultivation and production. Tank rearing offers controlled environments for climbing perch while employing cages present opportunities for enhanced fish growth and improved survival. These results lead to a shorter rearing period, accelerating production cycles, and increasing overall productivity in climbing perch farming. Economically, the cost return analysis showed that the climbing perch production venture was financially viable based on the provided results of the study. However, further assessments may be necessary to ensure the sustainability and profitability of long-term operations.

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ETHICS STATEMENTS - IACUC

The research followed the rules and regulations on the conduct of scientific procedures using animals stated in Administrative Order 40 series of 1999 of the Department of Agriculture of the Republic of the Philippines.

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