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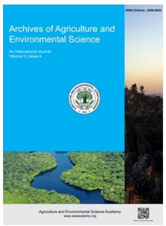

ORIGINAL RESEARCH ARTICLE

Nursing and management of early produced larvae of Thai pangas (*Pangasianodon hypophthalmus*) using greenhouse concept

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ABSTRACT

This experiment was conducted for the development of nursing techniques of early produced larvae of Thai pangus (*Pangasianodon hypophthalmus*) using Greenhouse concept for a period of 40 days from 10th February to 20th March 2020. The experiment was designed into two treatments (i) Greenhouse pond (GP) and (ii) Control or open ponds (CP) having three replicates each. Greenhouse concept was used for increasing the temperature during the winter month for proper growth and survival of the spawn. For this purpose, three ponds were covered with transparent polyethylene sheet fastened into bamboo frame and three ponds with no such covering. All the nursery ponds were stocked at a density of 20 g hatchling/decimal with 3 days old *P. hypophthalmus*. After 40 days of nursing period, the highest mean final length, weight gain and survival rate of fry were found to be 9.75 cm, 12.44g and 73.19% in greenhouse pond where in Control ponds it was 6.39 cm, 7.22g and 58.08%, respectively. A significantly higher ($p<0.05$) mean gross production of 6.07 kg/ decimal was found in greenhouse pond where in Control ponds it was 2.80 kg/ decimal in 40 days of nursing period. Water quality parameters were found to be better with good primary production in the green house ponds due to retaining day light temperature by polyethylene sheet. Results from the present experiment indicated that greenhouse technique can be suitable for the nursing and management of early produced larvae of Thai pangas with proper growth and good survival rate.

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INTRODUCTION

Pangasianodon hypophthalmus is locally known as pangas or Thai pangas in Bangladesh, an important fast growing catfish species in the Asian region particularly in Vietnam, Myanmar, Thailand, India, Indonesia, and Bangladesh (Phan *et al*., 2009). A total of 55 catfish species are found in inland waters of Bangladesh (Rahman *et al*., 2019), among which Thai pangas is one of the most popular fish species in aquaculture compared to other cultured species in Bangladesh because of its faster growth with high density culture, large size and a high market demand (Begum *et al*., 2012; Sarker, 2000). It was first introduced in Bangladesh in 1989 for cultivation purpose (Sarker, 2000) because of its spectacular growth but first commercial production of this species was started in 1993 (Ali and Haque, 2011) after successfully developed the induced breeding technique of this species (Rahman *et al*., 1993) and then it has rapidly been developed into an economically significant species in aquaculture industry (Ali *et al*., 2013). In Bangladesh, successful induced spawning of *P. Hypophthalmus* was first done by Rahman *et al*. (1993) through injecting the PG extracts at Riverine Station, Bangladesh Fisheries Research Institute, Chandpur on 8th May, 1993 and then in 2006, the first record of induced breeding of native pangas and their embryonic and larval development was observed by Rahman *et al.* (2006) in Bangladesh. They attain sexual maturity at the age of 2.5-3 years. Their captive breeding season starts from late March and continues up to August in Bangladesh. Peak spawning season of this species lies between May and June (Sah *et al.*, 2018). So, the fingerlings of pangas become available in May in the private hatcheries in Bangladesh which shortened the culture period of pangas. The main constraint of production of pangas is the late availability of fish seed due to low temperature in winter and lack of supply of quality seed. To ensure the availability of gravid and matured broods of Thai pangas in early season in January-February, a successful experiment was done by Das *et al.* (2021) where greenhouse technique was implemented to retain the optimum temperature in the pond. By using this technique, they found the fully matured males and females on 6th February 2016, which was two months earlier than the normal spawning season (Das *et al.*, 2021). Hence, for sustainable aquaculture, nursing and rearing of these early produced *P. hypophthalmus* seed (spawn) are very important to ensure reliable and regular supply of fry. If supply of gravid pangas could be ensured in early season of February and can be reared the early produced seed in special condition which become available in March that would increase the production by extending grow-out period. In such conditions, greenhouse is one of the technologies used to control temperature and humidity to boost fish growth and survival by preventing drastic drop or rise of temperature (Alex *et al.*, 2020). Greenhouse pond is a concept where sunlight shines during the day through the transparent polythene sheet into the pond and warms the air inside the greenhouse system. Furthermore, different studies indicate that water temperature in greenhouses can be increased by 3-9°C (Ghosal *et al.*, 2005). The present experiment has been undertaken to develop a nursing and management technique of the early produced larvae of Thai pangas by using a practical and economically viable methodology for mass seed production and rearing of *P. hypophthalmus* under greenhouse pond (GP) nursery management system to get a better understanding of the effects of greenhouse concept on the growth and survival of fry.

MATERIALS AND METHODS

Study area

The study was conducted in the Flood plain sub-station, Bangladesh Fisheries Research Institute, Santaher, Bogura, Bangladesh for a period of 40 days from 10th February to 20th March 2020. The study area was located between 24.7762° N latitude and 88.9940° E longitude (Figure 1).

Early brood development and induced breeding of *Pangasianodon hypophthalmus* using greenhouse technique

The experiment was conducted in rain fed ponds having an area of 30 decimal each with an average depth of 1.5 m. For the early development of broods of Thai pangas, two ponds were fully covered with transparent polyethylene sheet fastened in frame, made of bamboo which we called as “Greenhouse Pond”. Then the ponds were stocked with adult and healthy Thai Pangas at the rate of 990 nos/ha corresponding to 12 kg/decimal in October 2019. Commercial pellets containing 35% crude protein supplemented with 1-2 ml cod liver oil /kg feed and vitamin premix were supplied to stocked fishes for enhancing the maturation of eggs during the rearing periods of 4 months from November to February.

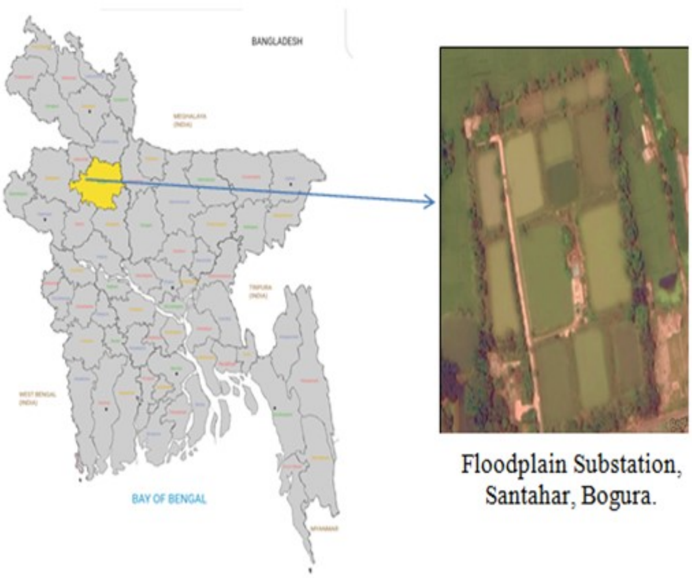

Figure 1. *Study area of Floodplain Substation of Bangladesh Fisheries Research Institute.*

Table 1. Design of the experiment of induced spawning for the early developed brood.

	Male	Female	Month
Pre-maturing agent (mg and ml/kg BW)	1 mg PG 0.2 ml (Ovaprim)	2 mg PG 0.8 ml (Ovaprim)	December February
1st Dose (mg /kg BW)		2 mg PG+200 IU HCG	February
2nd Dose (mg /kg BW)	2 mg PG	12 mg PG+250 IU HCG	

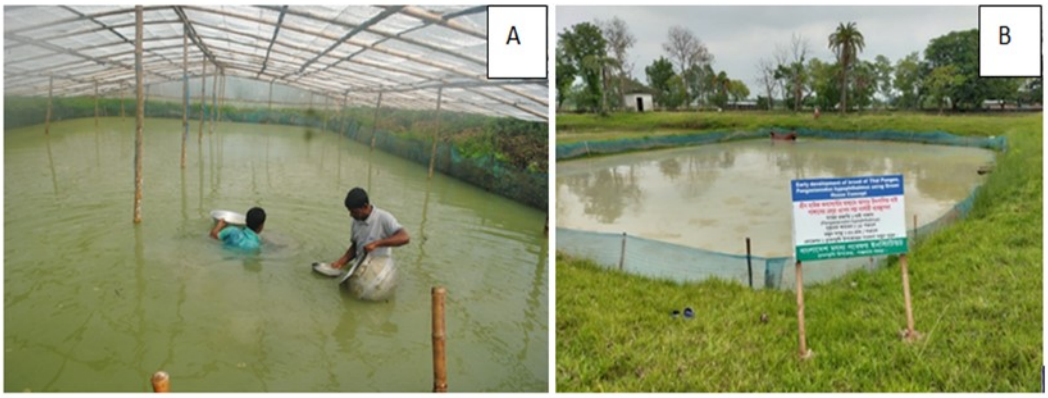

Figure 2. *(A) Greenhouse pond (GP), (B) control pond (CP).*

Feed was given twice a day in the morning and evening at the rate of 10 % in the first month, 8% in the second month, 5% in the third month and 3% in the fourth month. The stocking ratio of female: male was 2:1 weighing of 4.5-5.5 kg and 3.0-3.5 kg, respectively. Water quality parameters such as temperature (°C), pH, total alkalinity, dissolved oxygen, hardness and ammonia of water were recorded weekly basis using a commercial kit box (Model: FF-3, USA) with partial exchange (not more than 10% at a time) of water at 7 days interval. Aerator was used in the ponds to maintained dissolved oxygen and proper circulation of water. After stocking, fishes were regularly monitored to assess gonadal maturation by observing secondary sexual characters. In 6th February 2020, when the broods became ready to spawn, induced breeding programme was undertaken with the broods developed under greenhouse technique by using synthetic gonadotropin releasing hormone analogue (SGnRH) commercially known as "Ovaprim" and Pituitary gland (PG) as pre-maturation agent. Pituitary gland (PG) and Human Corionic Gonadotropin (HCG) were used as the inducing agent for induced breeding of Thai Pangas (Table 1).

Early maturation of Thai Pangas is related to the gonado somatic index, length-weight relationship of gonad, ova diameter and fecundity of fishes (Ranjan *et al*., 2018; Goswami *et al*., 2007). The initial length, weight, and gonad weight during stocking of broods were 61.4 ±2.74 cm, 2.5 ±0.91 kg, 156±0.26 g, respectively which become 67.1±0.59 cm, 3.9±0.31 kg and 376±0.86 g after four months of rearing periods in greenhouse ponds. Under Greenhouse condition, fishes got suitable environment for their gonadal maturation and spawned earlier at beginning of February where the normal spawning period started from late April (Das *et al*., 2021). After getting ready to spawn brood, standard protocol was followed to induce the breeding of pangas reared under Greenhouse condition. The fertilization rate (%), hatching rate (%) and survival rate (%) were calculated accordingly which was found to be 84.46 ± 4.11%, 71.20 ± 1.69% and 60.40 ± 0.59%, respectively which was more or less similar to the results of induced breeding of Thai pangas of other studies (Rahman *et al*. 1993; Kabir *et al*. 2013).

Preparation of nursing pond for early produced larvae of Thai pangas

Nursing of the larvae was carried out in the ponds having an area of 15 decimal each with an average depth of 1 m for a period of 40 days from 10th February to 20th March, 2020. The experiment was designed into two treatments (i) Greenhouse pond (GP) and (ii) Control or open ponds (CP) having three replicates each and was assigned into a Completely Randomized Design (CRD). Among six ponds, three ponds were fully covered with transparent polyethylene sheet fastened into frame, made of bamboo that was treated as "Greenhouse pond" (GP). The other three ponds were kept open and were treated as "Control Pond" (CP) (Figure 2). All the ponds were prepared by drying, excavating bottom soil to increase depth and the dykes were repaired properly by cleaning aquatic vegetation. All the ponds were made free from undesirable fishes by applying rotenone at the rate of 16 kg/ha. After 15 days of poisoning, lime (CaO) was applied at the rate of 125 kg/ha followed by cow dung at the rate of 750 kg/ha and waited for a week. After that ponds were filled up with water up to a depth of 1.5 m. Inorganic fertilizers such as Urea and TSP were applied in the ponds at the rate of 63 kg/ha, respectively to promote plankton growth and waited for another week to allow the water become suitable for stocking.

Stocking of spawn

After growing sufficient plankton, 3 days old larvae were stocked at the rate of 20 g/ decimal (666 fry in number/decimal) in both treatments in 10th February 2020. The stocking was done preferably during morning hours by acclimatizing them to the new environment. The feeding rate was 18-6% body weight which was gradually decreased weekly by adjusting their body weight. The feeding schedule during the experimental period was shown in (Table 2).

Table 2. Feeding chart of the stocked *P. Hypophthalmus* spawn.

Days	Feed ingredients	Feeding rate (% BW)	Feeding Frequency (times/day)
1-5	Chicken egg yolk, V-plex (15ml) + Vit. C	18	6
6-10	Milk powder, flour, fine powder of feed, Vitamin premix	13	6
11-20	Floating Nursery feed (0.3 micrometer)	10	5
21-30	Crumble (sinking) nursery feed	8	5
31-40	Floating Nursery (Starter) feed (0.5 micrometer)	6	4

Monitoring water quality and fish growth

The water quality parameters such as temperature (°C), dissolved oxygen (mg/l), pH, transparency (cm), ammonia (mg/l), alkalinity (mg/l), hardness (mg/l) were recorded at weekly interval throughout the experimental period at 9.00-10.00 hours using centigrade thermometer, portable DO meter (Model Lutron PDO-519), portable pH meter (HANNA HI 8424), Secchi disc, Ammonia test kit (Freshwater, HANNA) and Aquasol test kit, respectively. The fishes (n≈25) from all ponds were collected using a surrounding net for measuring individual length (cm) and weight (g). Samplings were done four times at 10 days interval throughout the nursing period to check the development and physical conditions of the larvae. Standard wooden measuring board and portable digital balance (Denver-xp-3000) were used to measure length and weight, respectively.

Estimation of yield parameters

The individual length (cm) and body weight (g) of fishes (n ≈ 60) and number of harvested fish were recorded for estimating different yield parameters as follows:

Survival rate (%) = Number of fish stocked ÷ Number of fish harvested × 100

Specific growth rate (SGR, %day^{-1}) = [In (final weight) – In (initial weight) ×100]/ No. of days of the experiment

Final weight gain (g) = Final individual weight – Initial individual weight

Average daily gain (ADG, g/day) = (Mean final weight – Mean initial weight) ÷ Culture days

Feed conversion ratio (FCR) = Feed applied (dry weight)/ Live weight gain

Net production (kg/m^3) = Total biomass at harvest – Total biomass at stocking

Analysis of experimental data

The data were analysed through student's t-test using the statistical software Statistix 10. Standard deviation in each parameter and treatment was calculated and expressed as mean ±SD.

RESULTS AND DISCUSSION

Water quality parameters

The water quality parameters showed variations during the experimental period in both greenhouse pond (GP) and control pond (CP), but the value of temperature in greenhouse pond were significantly (*P<0.05*) higher than the control pond (Table 3). The temperature within the greenhouse pond was in the recommended range for the proper growth performance of brood fish and larvae of Thai pangas as temperature is the main triggering factor for the development of broods of *P. hypophthalmus* as well as the nursing of fry in greenhouse system.

The average temperature of water in the greenhouse ponds was found to be 27.21°C where in the control ponds it was about 22.32°C during the experimental period (Table 3). Temperature of water in both types of ponds was higher in the evening than that of morning. During 40 days of larval nursing, there was no need of regulation of temperature in the GP as the temperature did not increase above 31°C. When the temperature became high in the afternoon in the greenhouse pond, it was regulated by opening the polythene sheet at two opposite corners of each pond. It is remarkable to note that water temperature of GP was always higher to some extent (2.50-3.00°C) than CP (Table 3). Higher mean temperature recorded in the greenhouse pond might be due to the 'greenhouse effect'. During sunshine, total solar radiation received by the greenhouse cover is partly reflected, absorbed and transmitted inside the greenhouse through walls and roofs. A large portion of this transmitted radiation is absorbed by water hence increased temperature (Mohapatra *et al.*, 2007). Temperature from the present study were found to be higher that has been recorded by Josiah *et al.*, (2012) where temperature range within 22.67-27.33 ˚C with a mean value of 24.19 ˚C while studying temperature in greenhouse aquaculture pond at Eldoret Kenya.

Though dissolved oxygen (DO) of both types of ponds tends to decrease with the progress of culture period, it did not decrease below critical level and no significant difference of DO values were recorded in the two pond systems (t-test, *p>0.05*). In the greenhouse pond, DO concentration was between a maximum and minimum value of 5.91 mg l^{-1}and 5.15 mg l^{-1}respectively with a mean DO concentration of 5.53 mg l^{-1} where in control pond the maximum and minimum value of 6.73 mg l^{-1}and 5.49 mg l^{-1}respectively with a mean DO concentration of 6.11mg l^{-1} were recorded (Table 3). Dissolved oxygen values recorded in the greenhouse and open pond were within the recommended

Table 3. Mean and range values of water quality parameters in greenhouse pond and control pond during the nursing period of 40 days.

Water quality parameters	Greenhouse pond (GP)		Control pond (CP)	
	Mean ± SD	Range	Mean ± SD	Range
Water Temperature (°C)	27.21 ± 1.55 b	25.66 – 28.76	22.32±2.45 a	19.87 – 24.77
pH	8.22 ± 0.11 b	8.11 – 8.33	7.51±0.22 a	7.29–7.73
DO (mg l^{-1})	5.53 ± 0.38 a	5.15 – 5.91	6.11±0.62 a	5.49–6.73
Alkalinity (mg l^{-1})	109.54±2.51 a	107.03–112.05	98.31±3.21 b	95.10–101.52
Transparency (cm)	28.12 ± 1.22 b	26.90 – 29.34	35.83±1.51 a	34.62–37.04
Total ammonia (mg l^{-1})	0.41 ± 0.05 a	0.36 – 0.46	0.28±.06 b	0.22–0.34

range of 4-8 mg l^{-1} as suitable for aquaculture production (Adekoya *et al*., 2004). The daily mean oxygen concentration recorded in the open pond was higher compared to the greenhouse pond. Higher oxygen concentration in the open pond could be resulting from mechanical aeration by wind as opposed to greenhouse pond which was sheltered hence wind effect was absent (Ehiagbonare and Ogunrinde, 2010).

In the greenhouse pond, pH value was range from 8.11 to 8.33 with a mean pH value of 8.22, where in control pond it was range from 7.29 to 7.73 with a mean pH value of 7.51 (Table 3). The pH ranges recorded in the two culture systems were within the recommended range of 6.7 to 9.5 as suitable for aquaculture and the ideal pH for optimal growth of fish between 7.5 and 8.5, pH above and below this could be stressful to the fish (Santhosh and Singh, 2007). The pH values recorded in the greenhouse ponds were more alkaline compared to control pond. The differences in the results could be accounted for by the dilution effect of precipitation (Peyami, 2016) or use of lime on the greenhouse pond as opposed to control pond. In the greenhouse pond, Total ammonia concentration was range from 0.36 mg l^{-1} to 0.46 mg l^{-1} with a mean pH value of 0.41 mg l^{-1} where in control pond it was range from 0.22 mg l^{-1} to 0.34 mg l^{-1} with a mean ammonia concentration of 0.28 mg l^{-1} (Table 3).

In the current experiment, the ammonium ion levels recorded were within the recommended range of below 0.5mg/L (Santhosh and Singh, 2007). However, mean ammonium ion concentration in the greenhouse was higher compared to the open pond. The high concentrations can be due to relatively higher temperature present in the greenhouse than in the control pond. Higher warmer temperatures favour de-nitrification process by the microbes which reduce NO_{2-} and NO_{3-} into NH_4.

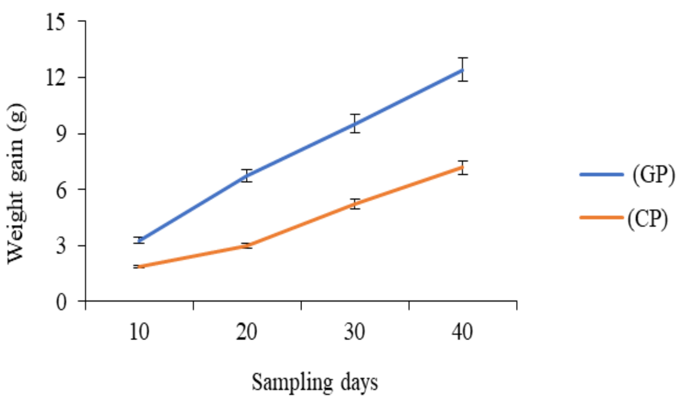

Figure 3. *Weight gain of larvae in greenhouse pond and control pond during the nursing period of 40 day (s).*

Ali (2002) reported that ammonium levels increase with warmer temperatures. Findings by Makori *et al.* (2017) obtained similar results of concentration in the range of 20-50µgL^{-1} in fish ponds within Busia County-Kenya. In the greenhouse pond, alkalinity was range from 107.03 mg l^{-1} to 112.05 mg l^{-1} with a mean alkalinity value of 109.54 mg l^{-1} where in control pond it was range from 95.10 mg l^{-1} to 101.52 mg l^{-1} with a mean alkalinity value of 98.318 mg l^{-1} (Table 3). Mean alkalinity in the greenhouse was higher compared to the open pond. The high concentrations can be due to addition of lime in the greenhouse which elevated the total bicarbonate alkalinity and increases the pH than in the open pond.

Growth, feed utilization and production of stocked spawn

The initial weight, final weight, weight gain, average daily weight gain (ADWG), specific growth rate (SGR), survival and gross yield of Thai pangas fry are presented in (Table 4). The survival rate was 73.19±1.05 % and 58.08±0.66 % in greenhouse ponds and control ponds, respectively, with significant differences between the treatments (*$P < 0.05$*). The greenhouse pond resulted a significantly higher survival rate than that of the control pond which was higher that has been reported for 21 days of larval rearing period by Chakraborty, (2020). Greenhouse pond in the present experiment showed better survival than in the control pond which is supported by the result of Josiah *et al.* (2014) where they reported the survival rate ranging from 82-97.33% for African Catfish (*Clarias gariepinus*) fry inside greenhouse tank and 30.6-55% outside the greenhouse tank. Yongphet *et al.* (2016) also found higher survival rate of 86.11 to 90.30 % for 120 days of culture periods in the greenhouse tank than in the open tank which is also coincide with the present study.

The larvae grew steadily in greenhouse pond at the rate of 0.31±0.01 g/day during the entire nursing period and reached to an average weight gain of 12.41 g with significant difference *(P < 0.05)* compared to control pond where the average weight gain was 7.19 g (Table 4; Figure 3). Mean weight gain in the present experiment was found to be higher in the greenhouse pond than control pond which was supported by the result of Josiah *et al.* (2014) and Yongphet *et al.* (2016) where they found better weight gain in the greenhouse tank rather than open tank for the fry of African Catfish and Climbing perch, respectively. The average daily weight gain in Thai pangas in greenhouse system and control pond was within the range of 0.30 to 0.32g and 0.17 to 0.19 g for the same species. The ADWG of Thai pangas in the present experimental in greenhouse pond was lower that has been reported by Yongphet *et al.* (2016) for 120 days of culture periods where they found ADWG of Climbing perch (*Anabas testudineus*) range from 0.99-1.18 g/day in green house tank in Thailand but was higher that has been reported (0.18 to 0.27 g/day) for 21 days of larval rearing period with different diets in Bangladesh by Chakraborty, 2020.

The value of specific growth rate (SGR) ranged from 14.97 to 15.17 % day^{-1} in green house ponds and 13.61 to 13.81 % day^{-1} in control ponds with a declining rate, progressively (Table 4; Figure 4). The SGR of pangas fry in green house ponds was significantly higher *(P < 0.05)* than that of control ponds. The SGR of Thai pangas in the present experiment in greenhouse condition was higher that has been reported (3.22 to 3.36 % day^{-1}) for 120 days of rearing period of Climbing perch by Yongphet *et al.* (2016) and 4.77 to 6.07 % day^{-1} reported by Josiah *et al.* (2014) for 42 days of fry nursing period of African Catfish in greenhouse tank. The SGR in the present study was also higher that has been reported (11.87 to 12.25% day^{-1}) by Chakraborty (2020) in earthen pond for 21 days of larval rearing period of Thai pangas.

The mean values of FCR were significantly different *(P<0.05)* between the treatments (Table 4). The lowest average FCR value of 0.98 was observed in green house treatment where the highest average FCR value of 1.55 in control pond system. In the present study, the FCR value was more or less similar in green house ponds (0.98) but higher in control ponds (1.55) compared to the values of 0.68 to 0.83 which has been reported by Chakraborty (2020) in case of larval rearing of the same species in pond condition.

Corresponding to higher growth and survival, green house treatment resulted in the significantly higher *(P < 0.05)* gross mean production of 6.07 kg/decimal and net production of 6.06 kg/decimal for 40 days of nursing period (Table 4). The mean gross production rate of pangas larvae in control pond treatments was 2.80 kg/decimal and the net production was 2.79 kg/decimal. The overall results of the present study demonstrated that the highest growth, survival and production in the green house technique might be attributed due to retain of optimum temperature, which provided suitable environment for proper growth of this catfish species. The highest survival (73.19%) and lowest FCR (0.98) in green house treatment indicates that there was less stress of fishes for the proper growth and survival due to optimum water quality parameters. Temperature was the prime triggering factor for the early development of these larvae. As greenhouse polyethylene sheet retained higher temperature, it was provided a better environmental condition for primary food production and water quality parameters was remain in optimum condition for the metabolism and physiological development than the control pond. In the present study, it was observed that during feeding most of the fishes in green house ponds, compared to fishes in control ponds system was come surface and feed actively which proved that greenhouse ponds might have provided a less stressful environment to the fry of *P. hypophthalmus* for their growth and survival.

Table 4. Growth parameters and survival rate of Thai Pangas fry in greenhouse pond (GP) and control pond (CP) during 40 days of nursing periods.

Parameters	Treatments	
	Greenhouse pond (GP)	Control pond (CP)
Initial length (cm)	0.51±0.02	0.53±0.01
Initial weight (g)	0.03±0.03	0.04±0.02
Final length (cm)	9.75±0.39 a	6.39±0.41 b
Final weight (g)	12.44±0.51 a	7.22±0.30b
ADWG (g)	0.31±0.01a	0.18±0.01b
Weight gain (g)	12.41±0.51a	7.19±0.51b
SGR (% / day)	15.07±0.10a	13.71±0.10b
FCR	0.98±0.10a	1.55±0.35b
Survival (%)	73.19±1.05a	58.08±0.66b
Production (number/dec)	487.95±6.97a	387.17±4.40b
Gross production (kg/dec)	6.07±0.33a	2.80±0.14b
Net production (kg/dec)	6.06±0.33a	2.79±0.14b

Mean values with different superscript letters in the same row are significantly different *(P < 0.05)*.

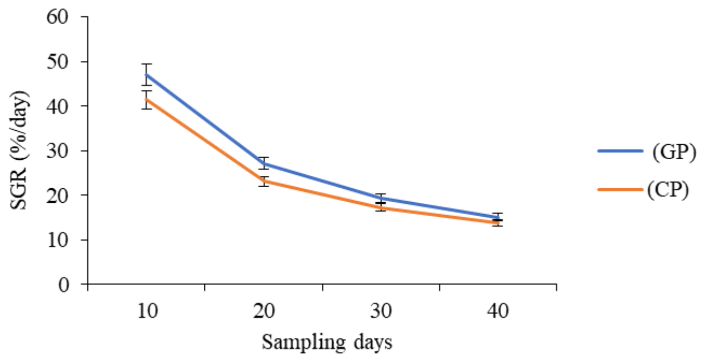

Figure 4. *Specific growth rate (SGR) of larvae in greenhouse pond and open pond during the experimental period of 40 day (s).*

Conclusion

Fish culture inside greenhouses facilities is an important alternative method of maintaining water temperatures within the acceptable range for fish growth and survival with low feed conversion rates, due to the effect of temperature on fish metabolism and consequently on food consumption. Results from the present study clearly demonstrated that the use of greenhouse technique resulted in significant increase in temperatures within the greenhouse pond which enhanced the growth and survival of Thai pangus fry. This technique might be ensuring the hatchery owner and fish farmers the supply of mature brood pangas and early produced fry during the winter months of January to February which will lengthen the fish culture period and will shorten the time which was waste in winter due to low temperature. By this way, farmers will be benefited economically by cultivating pangas multiple times in a year.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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