CIBTech Journal of Zoology ISSN: 2319–3883
Online, International Journal, Available at http://www.cibtech.org/cjz.htm
2024 Vol.13, pp.522-529/Mrigakhi et al.
***Research Article** (Open Access)*

INDUCED BREEDING PERFORMANCE AND LOW-COST SEED PRODUCTION OF CLARIAS BATRACHUS IN RAIN WATER AND POND WATER IN A FARMER FRIENDLY HATCHERY

***Mrigakhi Borah[1], Rimen Bordoloi [2], Rajeev Basumatary[3], Ashalata Devi[4] and Parijat Saikia[5]**
[1,4] *Dept. of Environmental Science, Tezpur University, Assam, India*
[2,3] *Dept. of Zoology, D. R. College, Golaghat, Assam, India*
[5] *ClimeTrek, London*
[*]*Author for Correspondence: mrigakhiborah@gmail.com*

ABSTRACT
The *Clarias batrachus*, commonly known as Indian magur, is highly valued for its nutritional and medicinal benefits and is in high demand in the market. The excessive use of chemicals and pesticides, along with other anthropogenic factors resulting to degradation of habitats, has significantly reduced the availability of this fish species in the natural water bodies of Assam. Moreover, the limited availability of magur fish seed supply for farmers poses an additional challenge in the production of magur fish in Assam. The present study focuses on the development of a cost-effective magur seed production facility, aimed at meeting the need and promoting local magur seed production. A thirty-days experiment on induced breeding of *C. batrachus* was conducted in Golaghat district of Assam, India for the purpose of producing magur seed. A farmer friendly, low-cost hatchery was used to hatch the eggs of magur fish. To assess their feasibility with different water sources, experiments were conducted using with pond and rain water. A total of ten breeding trials were conducted, with 5 trials each using rainwater and pond water. The fertilized eggs were kept in incubation trays, with equal amounts in both rainwater and pond water to assess hatching performance. The experiment resulted in successful magur seed production, with a significantly higher ($P<0.001$) hatching percentage (75%) and survival rate (70%) in rain water compared to pond water. In contrast, pond water had a maximum hatching percentage of 32% and a survival rate of only 7%.

Keywords: *Clarias batrachus, induced breeding, seed production, farmer-friendly, rainwater, low-cost hatchery*

INTRODUCTION
Clarias batrachus (L. 1758) (Indian magur), is an Asian catfish species that possesses significant nutritional and therapeutic values. It is very beneficial for the health of children as well as for pregnant and lactating women, particularly those suffering from anemia. This fish is highly digestible and rich in high grade protein, iron and beneficial lipid, which contributes to its potential as a medicinal fish (Chaturvedi *et al.*, 2013). The meat of this fish is rich in minerals like, Ca, Fe, Mn, K, P and all essential vitamins. Despite its nutritional and therapeutic significance, the majority of people in Assam are unable to get this fish because it is only found in rural areas. The application of pesticides in the paddy fields of Assam which serve as the main breeding grounds of *C. batrachus*, has led to a significant decline in the availability of magur seed from its natural sources. Studies have shown that Buprofezin, a compound found in the pesticides, is lethal to the embryonic development of *Clarias species* (Marimuthu *et al.*, 2013). Besides, increased temperature due to climate change and shifting rainfall patterns have a direct impact on metabolism, growth rates and may also lead to potentially shorter breeding season of the fishes (Ojanguren and Brana, 2003). Climate change can lead to alterations in wetland habitats where magur typically breeds and availability of shallow water bodies essential for spawning has also decreased due to

changes in rainfall patterns (Mitra *et al.,* 2023). Moreover, the African catfish (*Clarias gariepinus*), an invasive species, has threatened the survival of indigenous *Clarias batrachus* in India (Khedkar *et al.,* 2014) including Assam.

There have been a very few studies on magur seed production in India (Chaturvedi *et al.*, 2013; Dhara and Das, 2018); Mahapatra *et al.,* 2000; Sahu *et al.,* 2000; Rajbongshi *et al.,* 2020), and commercial seed production is still not common in the country. Much work is required in this sector to achieve commercial large-scale seed production of Indian magur. Moreover, farmers are uninterested in adopting aquaculture of this species because of insufficient breeding knowledge and necessary support required during fry rearing. Establishing a hatchery and collecting brood fish is a significant challenge for farmers, primarily due to the high cost involved. In Assam, the price of magur fish ranges from 800-1600 per kg. Presently, the magur seed supply in Assam is sourced from Bangladesh and West Bengal only.

Furthermore, water quality is crucial for producing healthy fish seed, as it can carry infectious diseases and contaminants harmful to fish. Additionally, the success of fish seed production, including fertilization and survival rates, depends on effective management strategies such as maintaining good water quality and proper nutrition (Faruk and Anka, 2017). This paper evaluates the use of two freshwater sources for a cost-effective and productive hatchery in Assam. In this study, we conducted an experiment to develop a farmer-friendly low-cost induced breeding and seed production technology of magur using an affordable hatchery (Figure 1), with the goal of establishing a profitable magur breeding facility in Assam. We utilized rainwater and pond water as two distinct fresh water sources for incubating the eggs in hatcheries to assess the feasibility of both the water types in magur breeding.

Figure 1: Low-cost hatchery prepared for farmers

MATERIALS AND METHODS

A low-cost hatchery was prepared using properly cleaned, used plastic saline pipes and plastic basins. The system was designed to provide the controlled flow of water from an overhead tank into the plastic basin through saline water pipes, thereby promoting the process of aeration for the fertilized eggs. The stoppers in the plastic saline pipes were used to regulate flow of water droplets as per requirements. Each plastic basin has a capacity of approximately 1,000 eggs. This affordable hatchery (Figure 1) is easily accessible

to even the poorest farmers. The structure can be modified by farmers according to their needs. Induced breeding trials were conducted in this low cost hatchery and experimented with rain water and pond water.

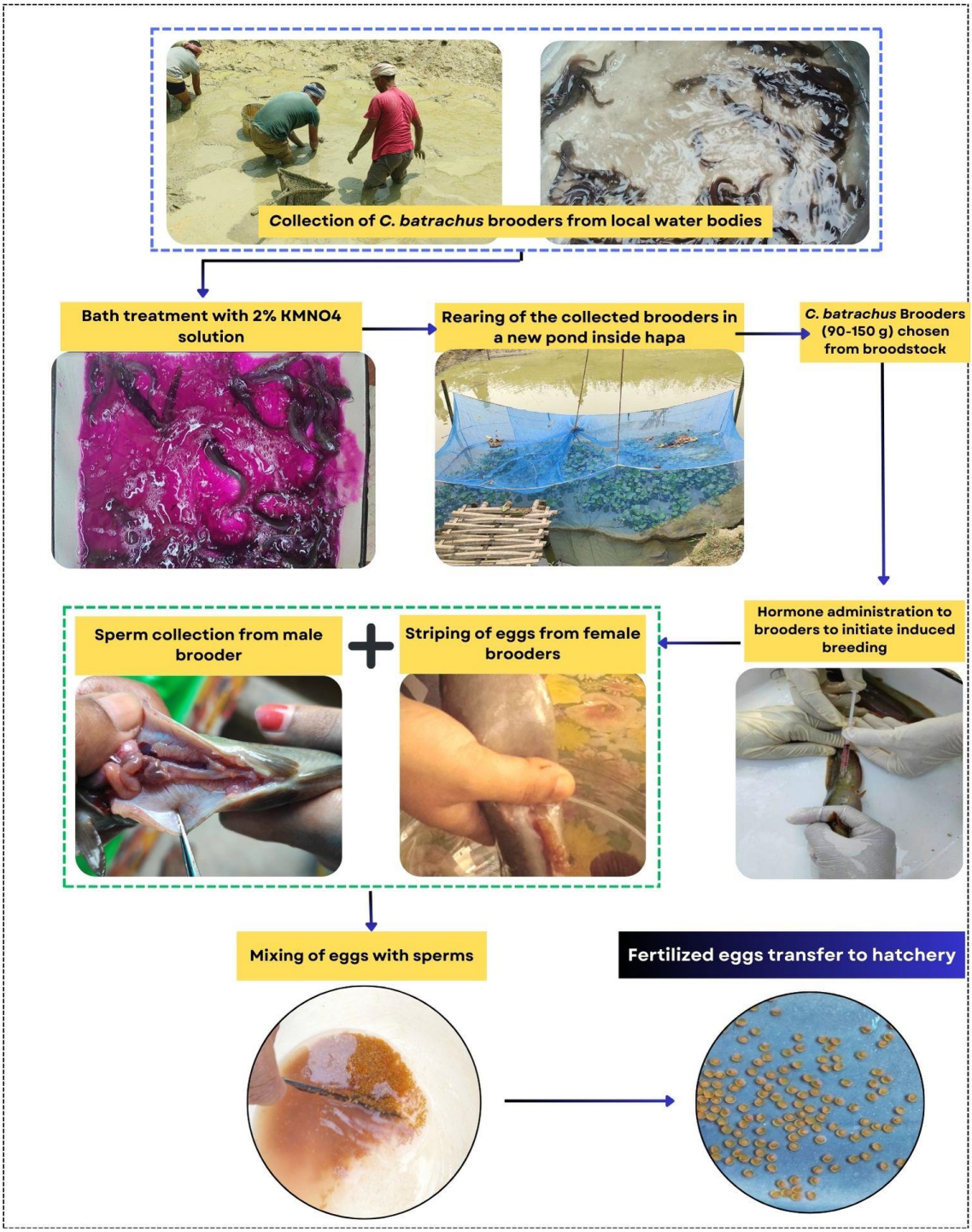

Figure 2: Figure showing stages of breeding techniques up to production of fertilized eggs

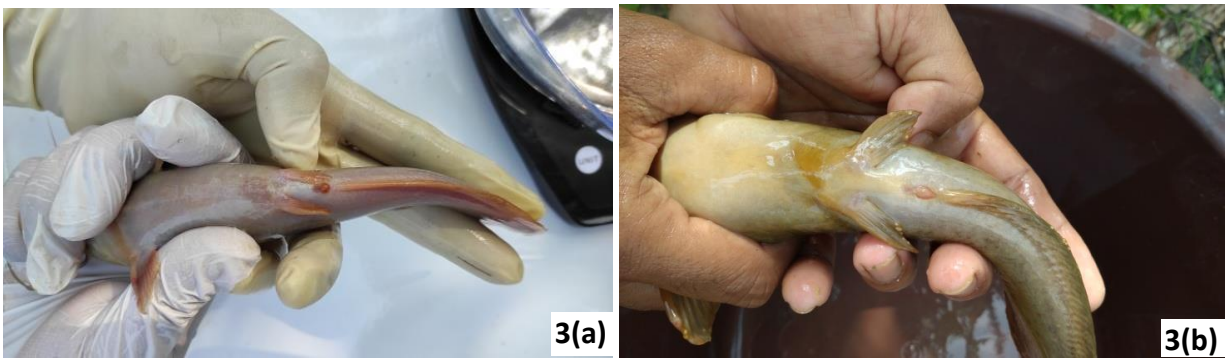

Figure 3: Matured brooders. Female *C. batrachus* brooder with rounded genital papillae (a) and

The breeding technique

The breeding technique involves various stages, which are outlined in the Figure 2, showing the process up to the production of fertilized eggs ready for the hatchery. During the breeding season (April to July), induced breeding trials were initiated in Golaghat district of Assam, India. Healthy *C. batrachus* were collected from natural water bodies from the villages and from nearby fish markets of Golaghat District. They were given bath treatment in 2% $KMnO_4$ solution. The collected brooders were reared in a newly prepared pond that was covered with plastic nets (8x6x5ft). A few brooders were kept in a natural pond inside a hapa measuring 8x8x8ft to enhance survival and to reduce stress. The male sacrificing breeding technique, developed by the Central Indian Fisheries Research Institute (CIFRI, 1983), was used with some modifications (Sahu *et al.*, 2010; Chaturvedi *et al.*, 2013). For breeding experiment, Magur brooders weighing between 90-150gm each were chosen from the broodstock. The females were identified by bulging abdomen and oval genital papilla (Figure 3a), whereas males had a conical and elongated genital papilla with pointed reddish tip (Figure 3b).

Male *C. batrachus* brooder with elongated genital papillae (b).

In the month of April, the brood fishes were stocked in a specially prepared fish pond. The fish were fed daily with a protein rich diet consisting of a mixture of trash fish and rice bran in a ratio of 9:1, which is equivalent to 10% of the fish's body weight. Both female and male magur were administered a single dose of hormone 'Ovafish' for induced breeding. The male was administered a dose of 0.03ml/100g and the female received a dose ranging from 0.05-0.08ml/100g of body weight simultaneously. Small-gauge hypodermic syringes were used to inject hormones into the muscle of broodstock. Male and female fishes that had been treated with hormones were housed in separate tanks. After a period of 18-21 hours post injection, the fishes were physically examined for ovulation by hand stripping method. The eggs were extracted by applying gentle pressure to the abdomen towards the vent and collecting them on a glass dish. The injected males were sacrificed to prepare a sperm suspension in clean water. The testes were removed and cut into small pieces using a sterile blade and small scissors, and then homogenized with 0.9% NaCl solution in a glass homogenizer. The sperm suspension was spread evenly over the eggs and clean water was added. The eggs and sperm were mixed by gently moving the tray for 4-5 minutes. The fertilized eggs (Figure 4a) were cleaned and transferred to the low cost hatchery that was specially developed for the purpose of the present study. Eggs that were not fertilized or dead (indicated by an opaque or white colour) were removed immediately to prevent fungal infection.

Two water sources, rainwater and pond water, were used in the hatchery to assess their suitability for hatching *Clarias batrachus* larvae. To ensure the water quality in the hatchery, regular monitoring of the water in the nursery ponds, rearing ponds and tanks were done. Rainwater used was freshly stored and pond water was used after passing through a filtration unit from an overhead tank to reduce the presence of planktons and Total dissolved solids (TDS). Pond water exhibited higher TDS with 18.75 mg/L compared to rainwater (0.75mg/L). The important water quality parameters of rainwater and pond water

are given in Table 1. In the year 2022, ten breeding trials were conducted with 5 trials (T1-T5) each with rainwater and pond water. Equal numbers of fertilized eggs (1000 eggs per tray) were placed in incubation tray supplied with both rain water and pond water supply to assess their hatching performance. The observations were made for a period of 30 days starting from the day of fertilization. Student's t-test was performed to test the significance of the difference in hatching rate and survival rates in rain water and pond water.

Table 1: Water quality parameters in rain and pond water.

Parameters	Pond water	Rainwater
pH	7.2 ± 0.24	7 ± 0.23
Average Temp (°C)	31.5 ± 0.63	30.3 ± 0.81
TDS (mg/L)	18.75 ± 1.07	0.75 ± 0.85
DO (mg/L)	6.7 ± 1.1	8.2 ± 2.3
Alkalinity (mg/L)	114.96 ± 3.4	77.52 ± 2.54
Ammonia (ppm)	>0.1	>0.1

RESULTS AND DISCUSSION

All the breeding trials were successful in both the water sources. Brooder individuals weighing between 100-150 gms of body weight and reared inside a hapa showed higher breeding performance. The hatching percentage of rainwater ranged from 55- 75%, which was significantly higher (P=0.0001) than the hatching percentage of pond water, which ranged from 5-32%. The pH of pond water (7.2) and rainwater (7) were with the optimal pH range for breeding, which is between 6.8 and 7.5.

The initial experiment using rainwater resulted hatching 30 hours post fertilization (Fig. 4b), with a hatching rate of 55%. Subsequent trials achieved a maximum hatching rate of 75% and survival rate of 70% which was also significantly higher (P=0.003) than pond water (Table. 2). In natural conditions, *C. batrachus* breeds during the rainy season. Rainwater provides ideal pH levels and water hardness that is necessary for the successful hatching and early development of cat fish eggs (Oladele *et al.*, 2007; Shireman and Smith, 1983). Moreover, rainwater is also devoid of chemical contaminants and provides optimum temperatures for the eggs (Bagenal, 1978). On the other hand, hatching rate in the pond water was very low with a maximum of 32%, whereas the survival rates ranged from 0% to7% only (Table. 2). Fungal infection has a substantial impact on hatching and survival rate of pond water spawning. Fungal infections in eggs are primarily caused presence of opportunistic fungi in pond water, as they colonize on the egg surface (Roberts, 2012; Boyd, 1998).

Table 2: Hatching % and Survival rate (%) in representative experimental trials T1-T5.

Breeding trials	Type of water	No. of Pairs	Weight of brooders (gms)	No. of fertilized eggs per tray	Breeding response	No. of hatched eggs	Hatching %	Survival rate (%)
T1	Pond water	1	90-100	1000	Successful	50	5	0
	Rainwater	1	90-100	1000	Successful	550	55	30
T2	Pond water	1	110-105	1000	Successful	200	20	1
	Rainwater	1	110-105	1000	Successful	700	70	35
T3	Pond water	1	110-130	1000	Successful	320	32	7
	Rainwater	1	110-130	1000	Successful	650	65	50
T4	Pond water	1	95-120	1000	Successful	240	24	3
	Rainwater	1	95-120	1000	Successful	750	75	70
T5	Pond water	1	120-100	1000	Successful	250	25	4
	Rainwater	1	120-100	1000	Successful	700	70	70

Hatchlings carry their yolk sac for three days (Figure 4c). All the hatchlings were adequately cared for and fed with planktons and *Artemia nauplii* during the initial week after absorption of the yolk sac. Subsequently, they were given a diet consisting of boiled egg yolk, prawn powder and food with high protein content. After duration of one month, they were being given antibiotic free nursery fish feeds and floating catfish feeds containing over 30% protein. Figure 5 represents eight days old hatchlings (5a) and one month old (5b and 5c) hatchlings. The fries and fingerlings showed healthy growth and are currently under investigation for their growth and development.

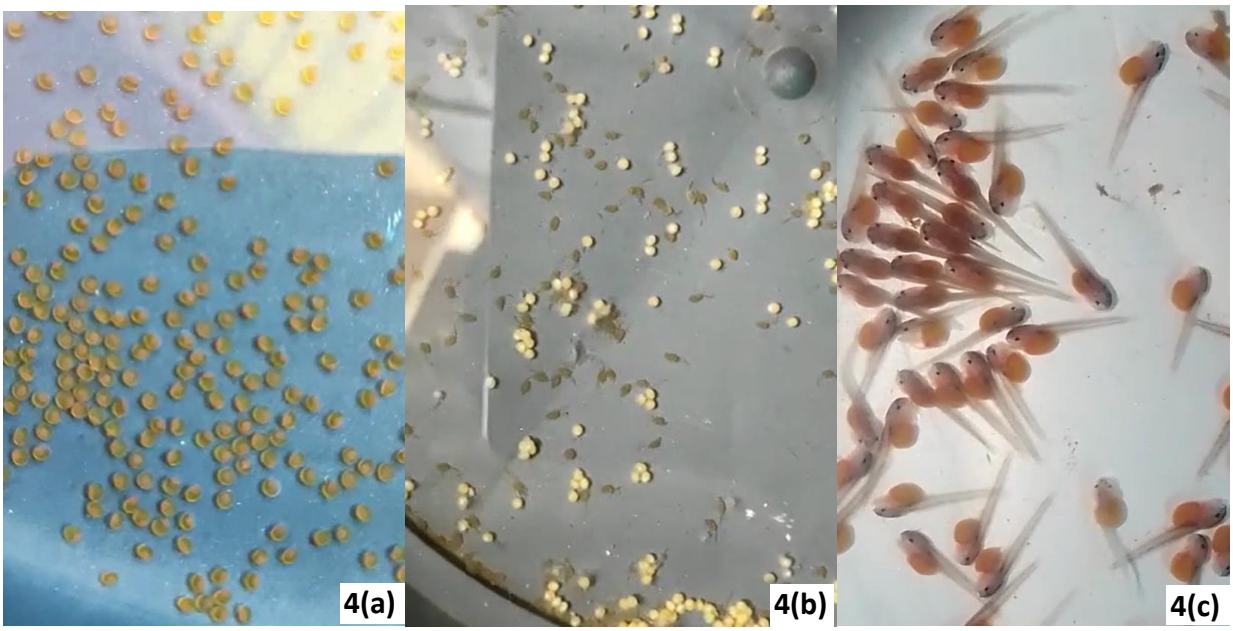

Figure 4: Different stages of breeding of *C. batrachus* Fertilized eggs (a), Hatching of eggs (b) and Two days old hatchlings bearing yolk sac (c).

Figure 5: From eight days old (a) hatchlings to one month old (b and c) magur fries.

During the breeding season of the year 2022 and 2023 in Golaghat district of Assam, around 15,000 fry were produced through induced breeding trials of magur fish in the low cost hatchery itself. The fish in their fry stage were released to a newly established nursery pond at the selected study site. It has been reported that seasonal stresses caused by changes in climatic variables may affect overall productivity of the fish seeds in both natural and aquaculture systems (Comte and Olden, 2017). The fish species' seed

production was below expectations in the research region due to the late development of eggs in the females caused by the delayed pre-monsoon rainfall in 2023. Most of the fishes carried immature or damaged eggs due to the seasonal stress. Studies have confirmed that changes in temperature and rainfall pattern have an impact on metabolism, growth rates of the fishes which are potentially shortening the breeding season (Ojanguren and Brana, 2003).

Conclusion

The present investigation on breeding trials has confirmed that Indian magur (*Clarias batrachus*) can be successfully produced and reared using low-cost and simple technology. Farmers in Assam now have the option to utilize this technology to produce magur seed according to their needs, as these seeds are currently not available commercially in the market. To ensure successful seed production, it is imperative that the brood fish are healthy and weigh over 100g. Maintaining good water quality is crucial, as it influences the hatching and survival of magur seed. The hatching rate was found to be higher in rainwater compared to pond water. It is not advisable to use pond water for egg incubation in a hatchery, as it can cause infection in eggs leading to unsuccessful breeding outcomes. The optimal water temperature range between 27-31^{0}C and pH should be maintained within 6.8-7.5 for successful large-scale hatching and larval survival. Ensuring sufficient oxygenation and prompt removing eggshells and debris is vital to maximize the survival of hatchlings. The cost-effective technique developed in this study can be easily adopted by farmers to improve their livelihoods through magur seed production locally, directly supporting the UN SDG goals such as SDG 1, SDG 2, and SDG 8. Adopting this cost effective breeding technology, native magur breeders have the potential to significantly contribute to meet the growing demand for magur fish seeds in Assam in the near future.

ACKNOWLEDGEMENTS

The authors are grateful to Department of Science and Technology (DST) for providing financial assistance under DST Women Scientist-B scheme to carry out research work in induced breeding of Clarias batrachus and to train unemployed people of Assam for entrepreneurship generation. They are also grateful to Mr. Utpal Bora, farmer from Golaghat district of Assam, India for providing space for nursery ponds and broodstock management and Dr. Pratibha Deka, Tezpur University for providing valuable insights in the manuscript.

Statements and Declarations:

***Funding*:** The work is funded by the **Department of Science and Technology (DST)** under Women Scientist - B Project under the Principal Investigation of **Dr. Mrigakhi Borah** and mentorship of **Dr. Ashalata Devi.**

Competing interests: The authors declare that there is no financial or non-financial interest regarding the publication of this paper.

Author Contributions: The authors confirm their contribution in the present study as follows: **Mrigakhi Borah:** Principal investigator of the project and was responsible for carrying out the experiment, trained the farmers and paper writing; **Ashalata Devi:** Mentor of the project, assisting in conducting the experiment, continuous monitoring and writing the paper; **Rimen Bordoloi:** helped with the breeding process and trained the farmers; **Rajeev Basumatary:** helped with testing water quality parameters and the writing process; **Parijat Saikia:** contributed to the writing of the paper. The first draft of the manuscript was written by **Mrigakhi Borah** and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

REFERENCES

Bagenal TB (1978). Aspects of fish fecundity. In: *Fish energetics*, edited by Tytler P. and Calow P. (Boston, Springer) 75-101.

Boyd CE (1998). Water quality for pond aquaculture. (Alabama Agricultural Experiment Station, Auburn University).
Chaturvedi CS, Somdutt and Pandey AK (2013). Successful induced breeding of *Clarias batrachus* by Ovaprim administration under agro-climatic conditions of Lucknow (Utter Pradesh). *J. Exp. Zool. India*. **16**(1) 163-167.
CIFRI (1983). Annual report, CIFRI, ICAR, (Barrackpore, West Bengal).
Comte L and Olden JD (2017) Climatic vulnerability of the world's freshwater and marine fishes. *Nature Climate Change* **7** 718–722.
Dhara K and Das UN (2018). Breeding performance of the walking cat fish *Clarias batrachus* (Linnaeus, 1758) using synthetic inducing agent, Alpa-FH with special reference to the occurrence of morphological deformities in their early stage of life. *Journal of Entomology and Zoology Studies* **6**(6) 957-963.
Faruk MAR and Anka IZ (2017). An overview of diseases in fish hatcheries and nurseries. *Fundam Appl Agric* **2**(3) 311-316.
Khedkar GD, Tiknaik AD, Shinde RN, Kalyankar AD, Ron TB and Haymer D (2014). High rates of substitution of the native catfish *Clarias batrachus* by *Clarias gariepinus* in India. *Mitochondrial DNA* **27**(1) 1-6.
Mahapatra BK, Sengupta KK, De UK, Rana GC, Datta A, Basu A and Saha S (2000). Controlled breeding and larval rearing of *Clarias batrachus* (Linn.) for mass scale propagation. *Fishing Chimes* **19** (10-11) 97-102.
Marimuthu K, Muthu N, Xavier R, Arockiaraj J, Rahman MA and Subramaniam S (2013). Toxicity of Buprofezin on the survival of embryo and larvae of African Catfish, *Clarias gariepinus* (Bloch). *PLoS ONE* **8**(10), e75545. doi:10.1371/journal.pone.0075545.
Mitra A, Gawad FKA, Bassem S, Barua P, Assisi L, Parisi C, Temraz TA, Vangone R, Kajbaf K, Kumar V and Guerriero G (2023). Climate Change and Reproductive Biocomplexity in Fishes: Innovative Management Approaches towards Sustainability of Fisheries and Aquaculture. *Water* **15**(4), 725.
Ojanguren AF and Brana F (2003). Effects of temperature and food availability on growth, survival, and yolk conversion efficiency of brown trout, *Salmo trutta* fry. *Environmental Biology of Fishes* **66**(1), 33-40.
Omotosho OO, Fawole AA and Olayemi JB (2007). Aquaculture and Fisheries management. (Spectrum Books Limited, Nigeria).
Rajbongshi A, Ali A, Chakravarty M, Deka M, Mazumdar H and Baishya S (2020). Studies on induce breeding performance of magur (*Clarias batrachus*) in FRP hatchery installed at Nalbari district, Assam. *Journal of Entomology and Zoology Studies* **8**(4) 1818-1822.
Roberts RJ (2012). Fish Pathology, 4th edition, (John Wiley & Sons, USA).
Sahu AK, Sahoo SK and Ayyappan S (2000). Seed production and hatchery management: Asian Catfish, *Clarias batrachus*. *Fishing Chimes* **19** (10-11) 94-96.
Sahu SK, Giri SS, Chandra S and Sahoo AK (2010). Management in Seed rearing of Asian Catfish of Asian Catfish, *Clarias batrachus*, in hatchery conditions. Aquaculture Asia. **16**, 23-25.
Shireman JV and Smith CR (1983). Fish and hatchery management, 2nd edition, (US Fish and Wildlife service, United States).