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Broodstock management, induced breeding and the constraints in the larval rearing of an endemic Malabar butter catfish, *Ompok malabaricus* (Valenciennes,1840)

Arjunan, V.M.[1,3], Sajeevan, M.K.[2,3*] and Anvar, A.P.H.[2,3]

[1] *Faculty of Ocean Science and Technology, Kerala University of Fisheries and Ocean Studies, Kochi, India. [1]Orcid id -https://orcid.org/0000-0001-6358-163X*
[2] *Department of Fishery Resource Management, Kerala University of Fisheries and Ocean Studies, Kochi, India. [2]Orcid id -https://orcid.org/0000-0001-7204-7471;*
[3] *Professor Alikunhi Chair for Sustainable aquaculture Systems, Kerala University of Fisheries and Ocean Studies, Kochi, India. [3]Orcid id -http://orcid.org/0000-0003-2182-3017.*
**E.mail: sajeevanfsi@gmail.com*

ABSTRACT
Individuals of a silurid catfish *Ompok malabaricus* (Valenciennes 1840) were collected from the Periyar River using a traditional fishing trap known as koodu. Fish were carefully transported to the hatchery and quarantined for two weeks for disease screening. After the quarantine, the fish were conditioned as brood stock by administering formulated diet and clam meat. Matured broodstock was bred under captivity, using *Wova-FH* @0.5 ml/kg- body weight. Hatchlings were reared in the hatchery using zooplankton as feed. Developmental changes in the larvae were documented. Larvae show a high rate of cannibalism. Thus, the present study reveals the importance of broodstock management and constraints in the larval rearing of endemic Malabar butter catfish.

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1. Introduction

Being a rapidly expanding food-producing sector, one out of two fish produced may come from aquaculture by 2025(Bartley, 2005). The requirement for domesticated finfish species has increased with the rapid development of commercial aquaculture (Duarte *et al*., 2007). The ability to control the breeding cycle under captivity is essential for domestication and establishing sustainable aquaculture production (Mylonas *et al*., 2010). Broodstock acquisition or development has a great role in successful captive breeding programs. Collection of wild fish using harmless methods, transportation into the hatchery, and weaning them using formulated diet into broodstock is the easiest method of Broodstock development (Harrell, 1984).

After collection from the wild, broodstock can be transported into the hatchery and conditioned as broodstock by providing a diet that improves breeding performance (Sullivan *et al*., 1997). Two kinds of broodstock can be found, i.e., broodstock that has developed from captured fish – captive broodstock & domestic broodstock that are reared from fingerlings into an adult under hatchery (Sullivan *et al*., 1997). Transportation and handling procedures consist of several potential stressors, such as capture, on-loading, transportation, unloading, acclimatization, and stocking (Ashley, 2007; Finstad *et al*., 2003; Iversen *et al*., 1998, 2003, 2005; Portz *et al*., 2006;). Quarantine is an important action to be considered when developing national strategies for aquatic animal health management that help in the prevention of the spreading of severe diseases into the natural environment (Arthur, 1996; Arthur *et al*., 2008; FAO/NACA, 2000).

With essential fatty acids and amino acids for the growth and development of fish larvae, planktons can be termed living capsules of nutrition (Radhakrishnan *et al*., 2019). The jerking movements of plankton attract feeding response in larvae (Bengtson, 2007), and their variable size helps in ingestion and digestion (Kinne, 1997), and they ensure the bioavailability of essential nutrients for growth and development without affecting the water quality (Watanabe *et al*., 1978) in the larval rearing system. Weaning, a critical period in larval rearing, is the transition from live feed to formulated feed (Kestemont *et al*., 2003). Along with live-feed, early weaning of formulated dry feed is widely practiced for freshwater and marine species before metamorphosis (Aragão *et al*., 2004; Carvalho *et al*., 2003; Helland *et al*., 2003).

For improving the larval and juvenile quality, it is essential to have sound knowledge of the larval fish nutritional requirements, which would help in the activation and development of digestive enzymes, and enhance the digestion and absorption of micro-diets (Kolkovski., 2001).

Many endemic species are found in the rivers originating from the Western Ghats biogeographic region (Daniels, 2001), and the torrential and perennial hill streams are the major water source of these rivers (Johnson and Arunachalam, 2009). The presence of endemic species has great significance, as it enhances the amount of genetic diversity and opens a path into the biogeographical questions regarding how the origin and distribution patterns have changed with time (Skarbek, 2008; Muderhwa and Matabaro, 2010).

Captive breeding of many freshwater catfish has been successfully achieved in India. The Asian catfish *Clarias batrachus* was successfully bred and reared in captivity by different workers (Cheah *et al.,* 1990; Goswami and Sarma, 1997; Das, 2002; Mahapatra, 2004; Hossain *et al.,* 2006; Sahoo *et al.,* 2007), *Clarias magur* (Sahoo *et al*., 2008) and *Heteropneustes fossilis* (Alok *et al*., 1993; Bindu *et al*., 2009), and little work has been carried out on *Clarias dussumieri* (Padmakumar *et al.,* 2004; Aneesh *et al.,* 2013;

Padmakumar *et al.,* 2017). Captive breeding of *Ompok pabda* has also been carried out successfully in different parts of India (Akhter uzzaman *et al.,* 1993; Kohinoor *et al.,* 1997; Bhowmik *et al.,* 2000; Mukherjee and Das, 2002; Mollah, 2003; Chakrabarty and Chakrabarty, 2005; Sarkar *et al.,* 2005; Mahmood, 2006; Hussain, 2006; Roy *et al.,* 2007; Chakrabarty *et al.,* 2008; Rahman *et al.,* 2008; Purkayastha *et al.,* 2012; Sarkar *et al*., 2005). The Indian butter-catfish *Ompok bimaculatus* which is also an endangered species was artificially spawned to propagate its existing population (Choudhury, 1962; Sridhar *et al.,* 1998; Raizada *et al*., 2013). Captive breeding of *Ompok pabo* was carried out by Bhowmik *et al.,* 2000; Mukherjee *et al.,* 2002; and Sarma, *et al.,* 2012. Though some successful attempts of breeding and larval rearing of *Wallago attu* has been taken up in the past in India (Ahmad, 1944; CIFRI, 1985; Parameswaran *et al*., 1988; Gupta *et al*., 1992; Sahoo *et al*., 2002; 2006; Raizada *et al*., 2015).

Ompok malabaricus is an endemic catfish found only in the western ghats and it belongs to the family Siluridae (Abraham, 2011). Alteration in the riparian habitat and destructive fishing practices are threatening the existence of many species, thus, it is essential to have captive breeding and ranching activities for the conservation of the species (Johnson and Arunachalam, 2009). Among the Genus *Ompok* present in India, captive breeding technology is standardized only for *O. pabda* (Bhowmick *et al*., 2000; Chakrabarty *et al*., 2005, 2007; Chakrabarti *et al*., 2009; Mukherjee *et al*., 2002; Purkayastha *et al*., 2012; Sarkar *et al*., 2005; Roy *et al*., 2021), and *O. bimaculatus*, (Banik, 2010; Banik *et al*., 2011; Raizada *et al*., 2013; Sridhar *et al*., 1998). The present study aimed to identify the challenges in broodstock procurement, induced breeding, and larval rearing of an endemic freshwater catfish from the Western Ghats.

2. Materials and Methods

2.1 Hatchery set up

2.1.1. quarantine set up

After the disinfection and washing, 500-liter (L)concrete rings were used as quarantine tanks. An air stone and PVC pipe with a diameter of 10 cm were provided in the tank. Wild-caught individuals were kept under observation for checking any incidence of disease symptoms for two weeks (Ponniah and Sood., 2002).

2.1.2. Broodstock maturation set up (Fig. 1)

A blue-coloured Fiber Reinforced Plastic (FRP) tank with a water holding capacity of 500 liters is disinfected, and collected 300 liter (L) of filtered fresh water is in it. Broodstock holding tank is connected to a canister filter Model No (Boyu – EF- 45). It is filled with sponge, charcoal, clay balls, and ceramic balls, which act as a substrate for bacteria that help maintain water quality. Broodstock holding tanks and submerged aquatic plants were provided with hideouts in the form of PVC pipe with a length and diameter of 10 cm and 20 cm, respectively. After completion of quarantine, the fish were reared to broodstock in the maturation tank.

2.1.3. Larval rearing set up

15 number of High Density Poly Ethylene (HDPE) crates with a water holding capacity of 75 liters were used for conducting larval rearing experiments (Fig. 2). Tanks were provided with air stone and a submerged aquatic plant *Ceratophyllum demersum*.

2.2. Broodstock collection, transportation, and conditioning

2.2.1. Broodstock sampling methods

The *Ompok malabaricus* broodstock was collected from the River Periyar (10°07'50.2"N 76°45'29.0"E) using koodu-a traditional fishing trap made up of bamboo used by the tribal people. Fish traps were filled with fish attractants like chicken viscera and deployed in the evening time in the deep pools of River Periyar, with 75% canopy cover.

2.2.2. Broodstock Packing and Transportation

50 L HDPE tank was used for the transportation of the broodstock. 40 L of freshwater from the stream is collected in the tank, and brooders were carefully transferred into the transportation tanks. Aeration tubes were connected to the tank from a portable aerator. Transportation was carried out in the evening time.

2.2.3. Broodstock Quarantine and Conditioning

On reaching the hatchery, fish were transferred into the quarantine tank after a water exchange of 100% in the transportation tank. Fish were observed for possible

Fig. 1. Broodstock maturation tank

Fig. 2. Larval rearing tanks

incidence of pathogens and parasites for two weeks. Feeding started from the second day of quarantine, and the broodstock was fed with clam meat in the evening.

2.3. Broodstock development, induced breeding, and larval rearing

2.3.1. Broodstock diet and maturation and water quality management:

Following the quarantine, fish devoid of any injury and parasitic infections were transferred into broodstock holding tanks and reared as broodstock. Considering the nocturnal behaviour of the species, they were fed with clam meat and formulated diet of CP -45%, at 2% of their body weight in the evening time after 7.00 PM, and the uneaten feed particles were siphoned out after one hour of feeding, and weekly 10 % of water exchange is carried out. Water quality parameters were recorded daily and analyzed using standard procedures (APHA, 2012), and weekly 10 % of water exchange is carried out (Table 1).

2.3.2. Induced Breeding Experiments

Matured broodstock of *O. malabaricus* was injected with *Wova-FH* at 0.5 ml/kg body weight during the night and released to the spawning tanks (Ramaswamy and Sundararaj., 1955).

2.3.3. Larval Rearing

Hatchlings were collected from the spawning tank and transferred into the larval rearing tanks at a density of 7 larvae /liter (Sahoo *et al*., 2010). Zooplankton was collected from the pond using a plankton scoop net with a mesh size of 100 microns twice a day for feeding the larvae. The experiment is continued for 21 days and changes were recorded.

Table 1. Water quality of the Broodstock rearing tank

Water quality parameters	Broodstock tank (Mean±SD)
Temperature (°C)	29±1
pH	7.5±0.6
Alkalinity (ppm)	35.8±4
Total Hardness (ppm)	58.12±9
Ammonia (ppm)	0
Nitrite (ppm)	0.01±0.04

3. Results

The method of fish collection using bamboo traps has useful in getting fish without injuries. Transportation of live fish in the evening protected them from temperature shock and helped minimize the transportation stress. The quarantine was effective in the prevention of entry of pathogens into the hatchery. The provision of hideouts in the quarantine tank, as well as the broodstock holding tank, helped in the maintenance of fish without stress. Canister filter and aeration helped maintain water quality and dissolved oxygen at optimum levels in the broodstock maturation tank. The addition of submerged aquatic plant "*Ceratophyllum demersum*" in the tanks helped in maintaining the water temperature at 27°C and it worked as a hideout for fish. A total of 6 breeding experiments were carried out, in which 4 experiments successfully produced hatchlings. *O. malabaricus* has a latency period of 10 Hrs and a hatching time is 22-24 Hrs. The spawning time, latency period, and hatching time were recorded (Table 2.).

In the larval rearing tank, adding submerged aquatic plant *Ceratophyllum demersum* helped the larvae find a hideout beneath the leaves. Plankton was collected from the pond twice a day using a plankton scoop net with a mesh size of 100 microns. Details of larval rearing have been attached in the table (Table 3). larvae have attained complete adult characteristics by 8th Day Post Hatching (DPH) (Fig. 3). The average growth rate of fry to larvae was statistically analyzed (Table 4).

The larvae were actively predating on zooplankton and differential growth and cannibalism can be found from 12th DPH. With the support of zooplankton, larvae show a survival rate of 100% up to 11 DPH. Phase I- Cannibalism can be observed from 12 DPH, with signs of biting of larvae on the fins and gradually it transformed to the phase-II cannibalistic stage which can be identified by the behaviour of complete ingestion of smaller larvae by 15th DPH. Differential growth in the population can be found with the presence of the small, medium, and large larvae (Fig.4). After 21st DPH the larval survival rate has been decreased significantly to 3.6%.

Table 2. Details of induced breeding

Exp. No	Sex	ABW	Hormone Dosage @ 0.5 ml / kg	No Divisions	Injection Time	Spawning Time	Latency Period	Hatching Time	Remarks
1	Male	44	0.022 ml	1 Div	23.15	9.15			Development arrested, eggs were attached with dust particles.
	Female	72	0.036 ml	1.5 Div			-------	---------	
2	Male	44	0.022 ml	1 Div	21.3	7.3	10 hrs	7.30 - 8.30	Successful Hatching
	Female	72	0.036 ml	1.5 Div					
3	Male	44	0.022 ml	1 Div	10.3	0	0	0	Without Hideouts, Shade net, and plants
	Female	72	0.036 ml	1.5 Div					
4	Male	44	0.022 ml	1 Div	19.3	5.3	10 Hrs	5..30-6.30	Successful Hatching
	Female	72	0.036 ml	1.5 Div					
5	Male	44	0.022 ml	1 Div	18.2	4.2	10 hrs	4.00 - 5.00	Successful Hatching
	Female	72	0.036 ml	1.5 Div					
6	Male	44	0.022 ml	1 Div	23.15	9.15	10 hrs	9.30 - 10.30	Successful Hatching
	Female	72	0.036 ml	1.5 Div					

Table 3. Details of spawning and larval rearing experiments of *Ompok malabaricus*

Experiment	Latency period (Hrs.)	Fecundity (Number)	Fertilization rate (%)	Hatching Rate (%)	Survival After 7 Days (%)	Survival after 21 Days (%)
E1	10	5000	0	0	0	0
E2	10	5000	96	100	100	0.26
E3	10	5000	0	0	0	0
E4	10	5000	99.86	100	100	0
E5	10	5000	98.8	100	100	0.72
E6	10	5000	99.96	100	100	3.6

Table 4. Average growth rate of larvae upto 30 DPH

Sl No	Days Post Hatching (DPH)	Average Total Length TL (mm)	Average body weight ABW (g)
1	1	4.61±0.02	0.001
2	2	5.98±0.13	0.005
3	3	6.05±0.03	0.006
4	4	6.45±0.06	0.007
5	5	7.94±0.08	0.008
6	6	8.35±0.09	0.009
7	7	9.05±0.06	0.011
8	8	10.34±0.09	0.016
9	9	10.94±0.08	0.021
10	10	11.37±0.10	0.027
11	11	11.86±0.09	0.033
12	12	12.32±0.08	0.038
13	15	20.00±1.18	0.048
14	20	28.50±1.08	0.23
15	25	35.65±1.42	0.58
16	30	42.25±1.24	1.2

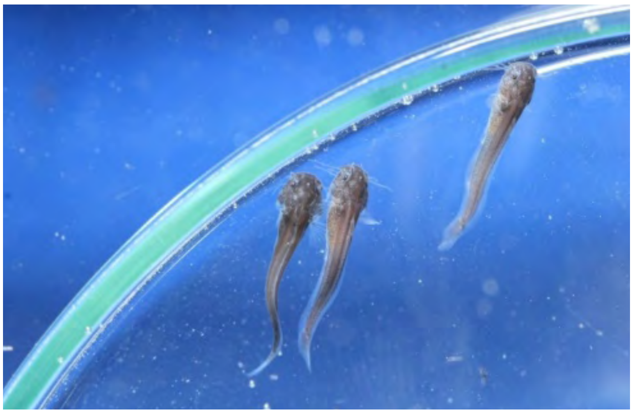

Fig. 3. Larvae after 8 DPH

Fig. 4. Larvae of *Ompok malabaricus* with differential growth after 21 DPH

4. Discussion

A good number of workers had taken up trials on captive breeding of *Ompok pabda* (Bhowmick *et al*., 2000; Chakrabarty *et al*., 2005, 2007; Chakrabarti *et al*., 2009; Mukherjee *et al*., 2002; Purkayastha *et al*., 2012; Sarkar *et al*., 2005; Roy *et al*., 2021); however, a few attempts have been made on *Ompok bimaculatus*, where captive breeding has been carried out either by stripping or using aquatic substrate like *Eichhornia crassipes* and *Hydrilla verticellata* (Banik, 2010; Banik *et al*., 2011; Sridhar *et al*., 1998).

Poor survival rate during the early rearing stage is a bottleneck in the larval rearing of *Ompok* sps. (Biswas *et al*., 2019). Chakrabarti *et al*. (2007) and Debnath *et al*. (2013) have pointed out that the cannibalism & heterogenous growth in the early larval stage of *Ompok bimaculatus* could be due to genetical. The importance of feeding larvae with live feed is evident from the works on various catfish species (Bairage *et al*., 1988; Haq and barua., 1989; Hirano and Hanyu., 1990; Gheyas., 1998; Hung *et al*., 2002; Giri *et al*., 2003; Srivastava *et al*., 2012; Malla and banik., 2015). Onwuteaka and Prince (2015) suggested the selective removal of shooters from the same year class to reduce cannibalism.

The function and development of the digestive tract are directly proportional to the methodology of co-feeding with micro-diet and the accuracy of the feeding regime (Cahu and Zambonino-Infante., 2001; Vega-Orellana *et al*., 2006; Engrola *et al*., 2007, 2010; Kamarudin *et al*., 2011; Liu *et al*., 2012). Faster development of internal organs can be found in butter catfish larvae fed with live feed (Gisbert *et al*., 2008), whereas abnormalities are found in larvae fed only with micro diets. Improper weaning might delay the development of the stomach (Hamza *et al*., 2007; Liu *et al*., 2012).

Intra-cohort aggression and cannibalism have a major role in the survival of larval rearing in carnivorous fish species (Baras 2013; Kumar *et al*., 2017). In the hatchery conditions, larvae with uniform size show type 1 cannibalistic behaviour of biting on the abdomen and tail of their siblings (Baras 2013; Baras and Jobling 2002), whereas type II cannibalism (complete prey ingestion) starts with an increase in size heterogeneity (Hecht and Pienaar 1993). Decreased stocking density, regular size-

-grading, and addition of hide-outs with weaning protocols are the common practices followed to reduce cannibalism in *Ompok* spp. (Debnath *et al*., 2013). Application of anti-aggression dietary supplementation (Tryptophan), in rainbow trout, grouper, and Atlantic cod was found to be an ideal strategy for reducing cannibalism and improving the survival rate (Wilson, 1989; Hoglund *et al*., 2005; Hseu *et al*., 2003; Krol and Zakes., 2016; Winberg *et al*., 2001 Johnston *et al*., 1990; Pedro *et al*., 1998). Pradhan *et al*., 2014 reported poor survival and retarded growth on *Ompok bimaculatus* larval rearing with micro diets & zooplankton and suggested weaning should carry out only after 7th DPH. Biswas *et al*., 2019 has reported reduced feed intake by larvae in their analysis on the effect of tryptophan in the feed of *Ompok bimaculatus* larvae and recommended the addition of 2% tryptophan in feed to reduce the cannibalism.

In the present study, broodstock management and induced breeding procedures were effective for developing a captive breeding program, but the survival rate of larvae after weaning has significantly reduced to 3.6%. For the initial 12 days zooplankton was supplied ad libitum helped to decrease the cannibalistic behaviour, but during the feed transition phase there was a reduced acceptance of the formulated diet. This has significantly affected the survival rate. Hence further research is essential on larval nutrition to improve the survival rate of *Ompok malabaricus* larvae and optimization of dietary protein requirements is essential for the evaluation of the aquaculture potential of *Ompok malabaricus.* Ompok malabaricus is a local delicacy for the tribal people due to its taste and the aquaculture potential of the species is yet to be evaluated.

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