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Induced spawning and larval rearing studies in butter catfish *Ompok bimaculatus* (Bloch, 1794): A candidate species for diversified aquaculture and stock enhancement

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Abstract

The butter catfish *Ompok bimaculatus* (Bloch, 1794), is a high-value freshwater species, with potential for aquaculture and conservation, though commercial-scale farming remains challenging. This study aimed to develop captive broodstock, induced spawning and evaluate larval growth as well as survival using four distinct diets. Captive broodstock was successfully raised to maturity. Mature females were induced to spawn with WOVA-FH (0.6-1.0 ml kg⁻¹ body weight) and HCG (5,000-10,000 IU kg⁻¹ body weight), while males with WOVA-FH (0.2-0.35 ml kg⁻¹) or HCG (1,500-2,500 IU kg⁻¹). Both hormones gave good results in full ripening of the gonads. WOVA-FH resulted in higher fertilisation (71.26-98.66%) and hatching (12.15-36.66%) rates, compared to HCG. Larvae reared for 30 days showed highest growth with *Tubifex* worms (SGR: 21.09±0.18%), while *Artemia* nauplii yielded the highest survival (51.11±3.38%). Results highlight the feasibility of induced breeding and the effectiveness of *Artemia* nauplii for early larval rearing, supporting the species' potential for aquaculture diversification.



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The butter catfish, *Ompok bimaculatus* (Bloch, 1794), is a high-value freshwater fish with significant potential for aquaculture diversification and conservation (Jayaraman, 2006). Despite its economic and ecological importance, the commercial-scale farming of *O. bimaculatus* remains constrained by challenges in induced breeding and high larval mortality, necessitating optimised spawning and larval rearing protocols. Native to South Asian freshwater systems, including rivers, wetlands and reservoirs, *O. bimaculatus* has experienced a severe decline in population due to overexploitation, habitat degradation and threats from invasive species (Banik, 2011). Its Near Threatened status (IUCN, 2021) underscores the urgency for developing captive breeding techniques

to support stock enhancement and sustainable aquaculture (CAMP, 1998; Mogalekar *et al.*, 2016). While previous studies have explored synthetic hormones (Ovaprim, Ovotide) for induced spawning, inconsistent results and low larval survival persisted, highlighting the need for comparative assessments of alternative spawning agents and larval diets (Basavaraja *et al.*, 2013; Raizada *et al.*, 2013). Larval rearing remains a critical bottleneck, with survival rates fluctuating drastically (10.4-78.7%) depending on feeding regimes (Pradhan *et al.*, 2014; Biswas *et al.*, 2023). Although live feeds like *Artemia* nauplii and zooplankton improve growth, cost-effective and sustainable alternatives must be evaluated to enhance hatchery productivity.

This study investigated the efficacy of two spawning agents WOVA-FH (Wockhardt India Ltd., Mumbai) and human chorionic gonadotropin (HCG, Ovidac, Bayer Zydus pharma, Mumbai) in inducing ovulation and improving fertilisation rates in *O. bimaculatus*. Furthermore, it evaluated the impact of various diets (*Artemia* nauplii, egg custard, Tubifex worms and chicken liver) on larval growth and survival. By addressing these knowledge gaps, the present investigation aims to develop and standardise protocols for the breeding and rearing of *O. bimaculatus*, supporting its commercial aquaculture and conservation.

Live specimens of *O. bimaculatus* (15-25 cm) were collected from Markonahalli (Tumkur) and Hemavati (Hassan) reservoirs, Karnataka, using gillnet and drag nets. Fish were anaesthetised with Quinaldine (20 ppm; Sigma, Germany) for 2 min before transportation in 10-15 l containers with aerated reservoir water. Upon arrival at experimental site, fish were disinfected with KMnO₄ (3 ppm, 2 min) and acclimatised in quarantine tanks (3 × 3 × 3 ft; 8 fish per tank) and later shifted to rearing tanks. Injured specimens were treated with KMnO₄ (5 ppm, 2 min) in quarantine tanks. Water quality was maintained primarily through aeration and a 50% water exchange daily. After 5 days, 40 females and 47 males were transferred to cement tanks (10 × 5 × 1 m with 0.5 m water depth) fertilised with poultry manure (1.5 kg per tank). After stocking, water depth was gradually increased from 0.5 to 1 m, with 30% water exchange fortnightly. Temperature (26-28°C), pH (6.7-7.3) and ammonia (0 ppm) were monitored daily. Fish were fed with chopped oil sardine and olive barb (15% of body weight per day). Unconsumed food and waste material, were siphoned out daily after feeding.

Uniform maturation was observed after rearing the fishes for three months on the broodstock diet. The present study successfully developed captive broodstock of *O. bimaculatus* using a diet comprising low-value fish (olive barb and oil sardine) @ 15% of body weight per day, which effectively supported gonadal maturation in captivity. This finding is consistent with the report by Qayyum and Qasim (1964), who described *O. bimaculatus* as a predominantly piscivorous species, with small fish comprising approximately 60% of its diet. Debnath et al. (2013) further observed that adult *O. bimaculatus* exhibited predatory and cannibalistic tendencies. Substantial growth has previously been recorded in broodstock fed with live prey such as mola carp, flying barb, crabs and molluscs. Banik et al. (2011) also demonstrated successful broodstock maturation using a combination of mass-cultured rotifers, small live prawns, trash fish and mustard oil cake in a 2:1 ratio at 5-10% of body weight daily. Similarly, Chakrabarthy et al. (2009) reported

that trash fish and boiled chicken viscera (5-10% body weight per day), coupled with regular water exchange, facilitated gonadal development in *O. pabo*. Effective and uniform maturation observed in the present study, confirm the use of low value fishes as an ideal broodstock diet. Identification, handling and management of brooders, including extraction of testes, preparation of sperm suspension, stripping of females, fertilisation techniques and egg hatching protocols were conducted following the methods described by Basavaraja et al. (2013) with minor modifications.

WOVA-FH (Wockhardt India Ltd., Mumbai) was administered to females at doses of 0.6, 0.8 and 1.0 ml kg⁻¹ body weight (non-replicated) and successfully induced ovulation, resulting in egg production ranging from 4,154 to 5,890 per female (Table 1). In males, doses of 0.2-0.3 ml kg⁻¹ body weight effectively induced spermiation. Human chorionic gonadotropin (HCG) was administered to females at 5,000, 7,500 and 10,000 IU kg⁻¹ and to males at 1,500, 2,500 and 3,500 IU kg⁻¹ (non-replicated). Among these, doses of 5,000-7,500 IU kg⁻¹ in females yielded 3,448-4,288 eggs, while the highest dose (10,000 IU kg⁻¹) failed to induce ovulation. In males, both the doses (1,500-2,500 IU kg⁻¹) were effective for spermiation. Relative fecundity ranged from 34,617-58,966 eggs kg⁻¹ body weight for WOVA-FH and 45,137-53,046 eggs kg⁻¹ for HCG.

Fertilisation rates were significantly higher with WOVA-FH (71.26-98.66%) compared to HCG (67.34-70.64%). However, hatching success was generally low in all treatments, with WOVA-FH yielding hatching rates of 12.15-36.66% and HCG resulting in rates ranging from 0 to 8.23% (Table 1). Hatching occurred within 22-24 h post-fertilisation. These findings corroborate previous studies, such as Sarkar et al. (2005) for *Anabas testudineus*, and Haniffa and Sridhar (2002), who successfully used HCG in *Heteropneustes fossilis*. The low hatching rates observed in this study could be attributed to poor gamete quality, ineffective fertilisation, or suboptimal handling of fertilised eggs, consistent with earlier findings in the same species (Sridhar et al., 1998; Vijaykumar, 2010; Basavaraja et al., 2013).

Larval rearing trials were conducted over 30 days using 4 days old fry (average length: 6 mm; weight: 3.6 mg) stocked at 45 fry per 100 l FRP tank, with four dietary treatments: *Artemia* nauplii, egg custard, *Tubifex* worms and chicken liver, each with three replicates. *Artemia* nauplii were administered @ 200-300 nauplii per fry, while the other diets were provided twice daily at 15% of body weight, with feed quantities adjusted biweekly (Basavaraja et al., 2013).

In the current study, length (cm), length gain (%), weight (g), weight gain (%), biomass (mg) and biomass gain (%) were recorded

Table 1. Details on weight (g) and length (cm) of brooders, dosage of hormones, breeding response, quantity of eggs obtained, fertilisation rate, hatching period and hatching rate recorded for different treatment groups (Mean±SE)

Treatment groups	Weight (g) and length (cm) of male	Weight(g) and length (cm) of female	Spawning agent	Dosage (ml kg ⁻¹ body weight)	Breeding response	Quantity of eggs obtained	Fertilisation rate (%)	Hatching period (h)	Hatching rate (%)
I	110 22.8	100 22.5	WOVA-FH	0.6 (F) 0.2 (M)	Partial	5896	98.66 ±1.15	22-24	36.66
II	125 24.9	110 23.4	WOVA-FH	0.8 (F) 0.3 (M)	Partial	4824	96.60 ±0.81	22-24	16.27
III	85 23.2	120 24.4	WOVA-FH	1.0 (F) 0.35 (M)	Partial	4154	71.26 ±4.00	22-24	12.15
IV	55 17.3	95 20.3	HCG	5000 IU (F) 1500 IU (M)	Partial	4288	70.64 ±13.03	22-24	8.23
V	65 18.2	65 17.6	HCG	7500 IU (F) 2500 IU (M)	partial	3448	67.34 ±1.50	n.a	n.a
VI	65 18.5	55 16.8	HCG	10000 IU (F) 3500 IU (M)	n.a	n.a	n.a	n.a	n.a

n. a: Not available, Mean values having different superscripts in the same column are significantly different at p<0.05, n = 3

fortnightly following the methods of Basavaraja *et al.* (2013) and the results are presented in Table 2 as well as Fig. 1 and 2. Specific growth rate (SGR) differed significantly among treatments ($p < 0.05$), with fry fed *Tubifex* worms exhibiting the highest growth ($21.09 \pm 0.18\%$), followed by chicken liver ($20.92 \pm 0.03\%$), *Artemia* nauplii ($19.87 \pm 0.16\%$) and egg custard ($16.78 \pm 0.59\%$) (Table 2; Fig. 3). Total biomass gain was highest with chicken liver, followed by *Artemia* nauplii, *Tubifex* worms, and egg custard (Fig. 4). The superior growth gained by feeding *Tubifex* worms aligns with earlier reports attributing its high protein (approx. 65%) and lipid content to enhanced larval development in species like *Clarias batrachus* and *H. fossilis* (Sinha *et al.*, 2014; Saxena, 2014). Likewise, chicken liver, with its high calorific value ($6,185 \text{ cal g}^{-1}$ dry weight), has been found effective in promoting larval growth in *Wallago attu* (Vivekanandan *et al.*, 1977; Sahoo *et al.*, 2006).

Survival rates also varied with diet. The highest survival rate was observed in fry fed *Artemia* nauplii ($51.11 \pm 3.38\%$), followed by chicken liver ($48.88 \pm 3.38\%$). The lowest survival rate occurred in fry fed *Tubifex* worms ($20.0 \pm 1.15\%$) (Table 2; Fig. 5). These trends may be explained by variations in feed palatability, incidence of cannibalism and water quality. Previous studies have shown that microbial load from uneaten feed and deteriorated water can negatively affect the survival (Mahmood *et al.*, 2004; Dhawan and Kaur, 2006). Lower survival in the *Tubifex* group may have been caused by rapid decomposition of uneaten biomass and resultant bacterial proliferation, as also reported by James *et al.* (1993) as well as by Pradhan and Barman (2014). Egg custard is not a suitable feed for larvae and resulted in the lowest growth and poor

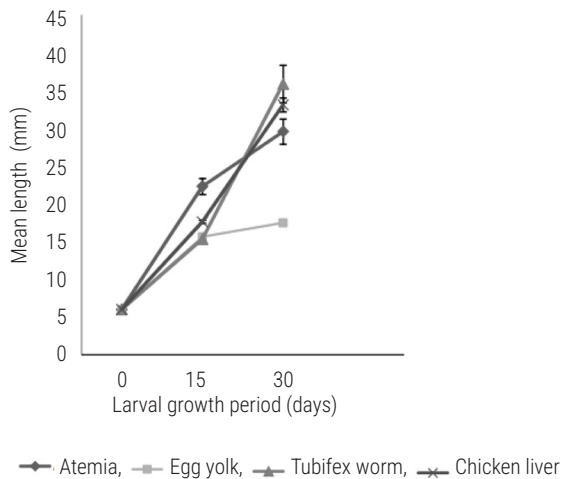


Fig. 1. Mean length (mm) of *O. bimaculatus* fed for 30 days

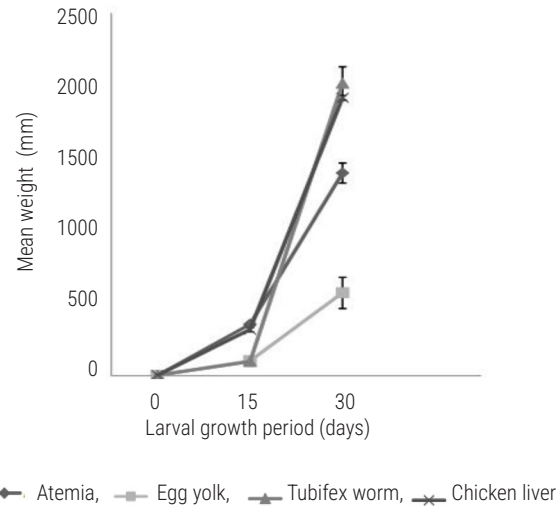


Fig. 2. Mean weight (mg) of *O. bimaculatus* fed for 30 days

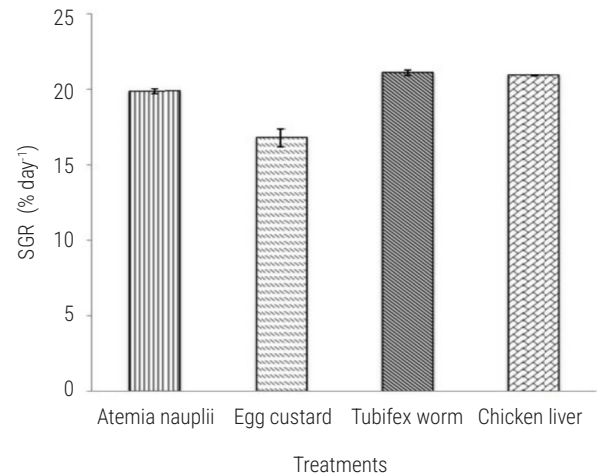


Fig. 3. Specific growth rate (%/day) of *O. bimaculatus* fed on different diets (Mean±SE)

survival. Although *Tubifex* feed gave the highest growth, it cannot be recommended for further evaluation or feeding due to the poor survival rate. Chicken liver can be considered as a better larval feed for *O. bimaculatus* because of the moderate growth and survival of larvae observed in this study. More detailed investigation is needed to the impact on water quality in large scale production systems using chicken liver as a larval feed. *Artemia* emerged as the best

Table 2. Details of length (mm), length gain (%), weight (mg), weight gain (%), biomass (mg), biomass gain (%), specific growth rate (% per day) and survival (%) of *O. bimaculatus* recorded at the end of the experiment (Mean±SE)

Treatment	Length (mm)	Length gain (%)	Weight (mg)	Weight gain (%)	Biomass (mg)	Biomass gain (%)	Specific growth rate (% day ⁻¹)	Survival (%)
<i>Artemia</i> nauplii	29.55±1.66 ^b	492.5±27.874 ^b	1398.53±68.77 ^b	38848.05±1910.08 ^b	32375.66±3634.16 ^c	19884.97±2243.30 ^c	19.87±0.17 ^b	51.11±3.38 ^b
Egg custard	17.47±0.39 ^a	291.16±6.541 ^a	572.1±107.36 ^a	15891.66±2982.12 ^a	6604.1±1032.521 ^a	3976.60±637.36 ^a	16.78±0.56 ^a	25.17±1.47 ^a
<i>Tubifex</i> worm	35.86±2.48 ^c	597.66±41.67 ^c	2016.66±114.65 ^c	56018.33±3184.69 ^c	18500.00±4255.67 ^b	11319.75±2626.96 ^b	21.09±0.19 ^c	20.00±3.38 ^a
Chicken liver	33.10±0.91 ^{bc}	551.66±15.14 ^{bc}	1916.66±16.67 ^c	53240.55±462.95 ^c	42150.00±2807.27 ^c	25918.51±1732.88 ^c	20.92±0.03 ^c	48.88±3.38 ^b

* Mean values having different superscripts in the same column are significantly different at $p < 0.05$, $n = 3$

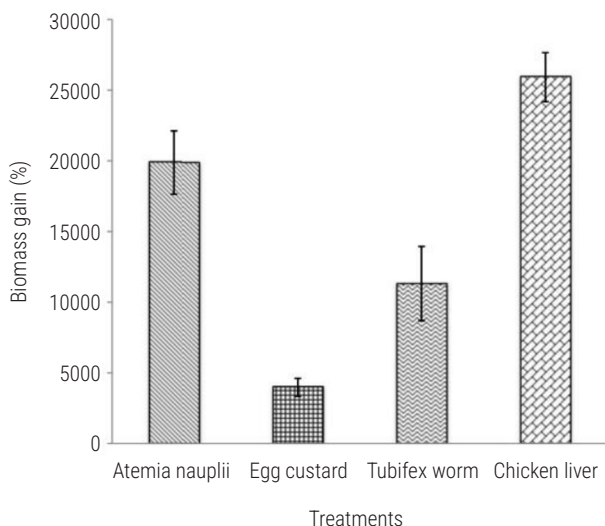


Fig. 4. Biomass gain (%) of *O. bimaculatus* fed on different diets (Mean±SE)

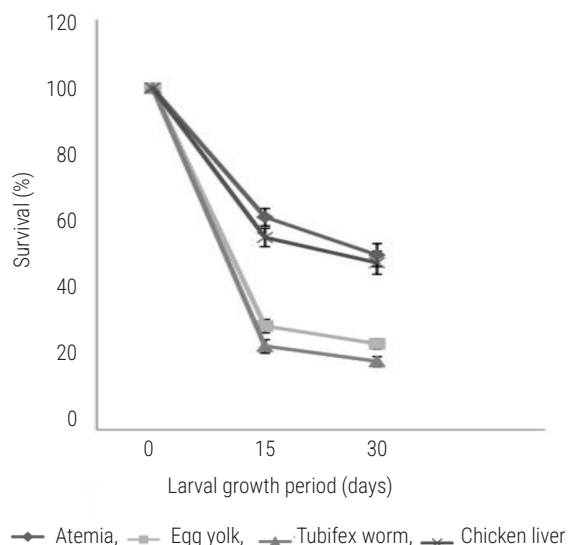


Fig. 5. Survival (%) of *O. bimaculatus* fed for 30 days

live feed for initial larval rearing because of the better growth and survival of larvae compared to the other live feeds tested.

Water quality parameters during larval rearing remained within optimal ranges in all the trials: temperature (27-28°C), pH (6.86-7.03), dissolved oxygen (6.3-6.7 mg l⁻¹), ammonia-N (0.014-0.022 mg l⁻¹) and total alkalinity (41.1-43.8 mg l⁻¹). These values are within recommended limits for catfish larviculture (Rao et al., 1994; Sahoo et al., 2014, 2015), supporting healthy larval development. Deviations from these parameters are known to increase stress and mortality (Mahmood et al., 2004; Dhawan and Kaur, 2006).

In conclusion, the study demonstrates that feeding low-value fish and maintaining optimal water quality can successfully induce gonadal maturation in *O. bimaculatus*. Induced spawning was achieved using both WOVA-FH and HCG, with WOVA-FH proving more effective. Based on the feeding trials, *Artemia* nauplii

is recommended as a suitable starter diet for *O. bimaculatus* larvae. This research supports the development of standardised protocols for induced breeding and larval rearing of *O. bimaculatus*, contributing to species conservation and diversification of aquaculture practices.

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