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Comparison of reproductive performance of *Clarias batrachus* (Linnaeus, 1758) collected from three Indian rivers

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ABSTRACT

A study was conducted to compare the reproductive performance of *Clarias batrachus* (Linnaeus, 1758) collected from three different rivers viz., Krishna in Andhra Pradesh, Godavari in Maharashtra and Narmada in Madhya Pradesh. Twenty pairs of brooders from each location were randomly selected and induced with pituitary extract (P) and ovaprim (O). Reproductive performance in terms of stripping percentage, pseudo gonado-somatic index, spawning, fecundity, fertilisation rate, hatching rate, and survival rate as well as fry rearing performance in terms of percentage weight gain, specific growth rate (SGR) and survival rate were monitored. Brooders collected from Godavari exhibited significantly ($p < 0.05$) higher values for all the reproductive performances indicators compared to brooders collected from Narmada and Krishna rivers. But fishes collected from Krishna River exhibited significantly higher ($p < 0.05$) fry survival. Fry produced from Godavari brooders attained highest ($p < 0.05$) percentage weight gain (1085.88 ± 37.41), SGR (17.61 ± 0.19) and fry survival rate (43.67 ± 1.45) in comparison to Narmada. Results clearly indicated that reproductive performance varied significantly among the brooders collected from the three different rivers.

Keywords: Brooders, *Clarias batrachus*, Ovaprim, Pituitary extract, Reproductive performance

Introduction

Among the indigenous catfishes, *Clarias batrachus* (Linnaeus, 1758), commonly known as magur, is one of the commercially important species in India. Culture of this species has been identified as a national priority owing to its good demand, decline in fishery, hardy nature and aquaculture potential (Sharma *et al.*, 2010). Magur breeds once in a year during June to August in the natural environment. However, population of this species has been reduced drastically during recent years due to overfishing, habitat alterations and indiscriminate use of pesticides in preferred natural breeding grounds such as paddy fields and wetlands (Goswami, 2007; Khedkar *et al.*, 2010). This species is classified under threatened category (Argungu *et al.*, 2013), warranting the need for conservation and sustainable management.

Studies on different aspects in captive breeding of *C. batrachus* has been carried out, such as optimisation of breeding using different inducing agents (Sahoo *et al.*, 2005; Hossain *et al.*, 2006; Jagtap *et al.*, 2015), standardisation of latency period (Sahoo *et al.*, 2007; Jagtap *et al.*, 2015) and

stripping response with different inducing agents (Sahoo *et al.*, 2007). However the quality of seed produced and breeding performance in captivity cannot be assured due to poor quality of brooders and lack of sufficient knowledge on reproductive traits in wild condition, resulting in over dependency on wild seed for stocking in ponds which is uneconomical and less sustainable (Kiran *et al.*, 2013).

Knowledge on reproductive parameters is a prerequisite for designing proper management measures in sustainable aquaculture (Fostier and Jalabert, 2004; Morgan, 2008) and reproductive performance has been evaluated in several fish species (Kamler, 2005; Teletchea *et al.*, 2009). Inter-population variation in relation to reproductive traits in *Salmo trutta* (Linnaeus 1758) was analysed by Labie-lund and Hindar (1990). Bagenal, (1971) studied the effect of geographical variation on reproductive traits in fishes. Similarly several studies evaluated the reproductive parameters among various populations in order to find out the reproductively superior population (Estay *et al.*, 2004; Alonso-Fernandez *et al.*, 2013).

Genetic variation among magur populations from various rivers have been documented in India and Bangladesh (Islam *et al.*, 2007; Garg *et al.*, 2010 and Khedkar *et al.*, 2016). But no study has been carried out so far on the breeding performance of this species across different geographical locations in Indian waters. In this backdrop, the present study on reproductive performance of magur from three different rivers in India, evaluated the differences in reproductive potential of these geographically isolated fishes.

Materials and methods

Fish collection and maintenance

Adult *C. batrachus*, (~70 nos. each) were collected from three different rivers in India *viz.*, Krishna River from Vijayawada District in Andhra Pradesh (AP), Godavari from Chandrapur District of Maharashtra (MH) and Narmada from Bhopal District in Madhya Pradesh (MP) during April - May 2012. Breeding experiments were conducted in earthen ponds of the freshwater fish farm of the Kakinada Research Centre of ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Andhra Pradesh. The fishes were separated based on collection source as well as sex and maintained in earthen ponds of 200 m² (water depth 1 m) for one month and were fed with farm made feed containing 35% protein.

Breeding of magur by hormonal inducement

Breeding trials of magur collected from different locations were carried out during July 2012. Females having soft, bulging gravid abdomen with reddish vent, round button shaped genital papilla and males with increased vascularisation on the pointed genital papilla (Sahoo *et al.*, 2005), were used for the breeding programme. Twenty pairs of brooders from each habitat were selected for breeding. One set of ten pairs of brooders each from all the three locations were injected with carp pituitary extract (P) and a second set of ten pairs each were given ovaprim (O). Before giving injection, total length(cm) and total weight (g) were recorded. Carp pituitary extract was prepared according to Bhowmick (1990) and female brooders were given intra-muscular injection @50 mg kg⁻¹ body weight (Goswami and Sarma, 1997; Sahoo *et al.*, 2013). Synthetic inducing agent ovaprim (M/s Syndel Laboratories, Canada) was administered at a dose of 1 ml kg⁻¹ of body weight of female (Srivastava *et al.*, 2012). Males were given half the dose of respective inducing agent. After injection, males and females were kept separately in plastic tubs holding 25 l pond water.

Reproductive performance analysis

After the optimum latency period of 14-17 h (Sahoo *et al.*, 2007), stripping and artificial fertilisation were carried out. Males were sacrificed and the testes were carefully removed, cut into small pieces, squeezed and mixed with

physiological saline solution. Simultaneously the females were stripped and eggs were collected in small clean and dry plastic trays. A batch of small quantity of eggs was transferred to a pre-weighed petriplate and weighed in an electronic balance (with an accuracy of 0.002 g), for further analysis of breeding parameters. Before and after stripping, weight of the fishes was noted to aid in estimation of stripped egg weight, stripping percentage, pseudo gonado-somatic index (PGSI) and spawning fecundity. Stripping percentage was calculated according to Brzuska (2003) and PGSI was determined as per Abdurraheem *et al.* (2012). Spawning fecundity in each set was calculated as per Hossain and Islam (1990). Ova diameter studies were carried out using 30 eggs from each individual female using an ocular micrometer. Fertilisation rate was calculated following Adebayo and Popoola (2008).

Hatching and larval rearing of magur was carried out in the model magur hatchery at Kakinada Centre of ICAR-CIFE. Fertilised eggs were collected on *Eichhornia* roots and shifted to rectangular FRP troughs (4.1' x 1.6' x 0.65' having a steady water level of 8 cm) provided with flow-through system and aeration. Hatching was completed between 24 and 28 h post-fertilisation and the hatching rate was determined by counting the number of two days old hatchlings according to Haniffa and Sridhar (2002). Rearing of hatchlings was carried out in the flow-through system till 4th day of stocking with a stocking rate of 2000±200 hatchlings per trough, provided with newly hatched *Artemia* nauplii as feed @ 20-25 nauplii per larva, four times a day. Stocking density was reduced to 1000±100 hatchlings per trough till 12th day until the appearance of frequent vertical movements of fry and they were fed with mixed zooplankton (dominated by *Moina* sp.) @ 5 ml per trough (500 cells ml⁻¹) and tubifex worms at 15 % of body weight. Uneaten food was removed after 30 min. At the end of larval rearing, survival of fish was estimated according to Shinkafi and Ilesanmi (2014).

Fry rearing trials

Magur fry were reared in rectangular FRP tanks (6' x 2' x 1.5'), provided with aeration. Early fry were stocked at 250 nos. per tank in three replicates. The length and weight of 25 larvae were recorded using a measuring scale and electronic balance respectively, prior to stocking and at weekly intervals during rearing. A steady water level of 25 cm was maintained in the tanks and PVC pipes were placed in the tanks to provide shelter for the fishes. Every day tanks were cleaned and about 10 - 20% water was exchanged daily. The fishes were fed with mixed zooplankton (dominated by *Moina* sp.) @ 10 ml per trough (500 cells ml⁻¹) along with crumbled feed (150 µ) having 40% protein and tubifex worm at 10% of body weight three times a day) during entire rearing period. Faecal waste and dead larvae were removed before the next feeding. At the end of two weeks of rearing, all survived fry were collected and enumerated. The percentage

weight gain, survival rate and specific growth rate (SGR) were calculated according to Sahoo *et al.* (2004).

Physico-chemical parameters of water in the hatchery unit were analysed daily following standard protocols (APHA, 2000).

Statistical analysis

Statistical analysis of the data was carried out using Statistical Package for the Social Sciences (SPSS) software, version 16.0. Differences in breeding parameters among three groups of magur were tested using one-way analysis of variance (ANOVA) followed by a Tukey's test at 5% level of significance.

Results and discussion

Physico-chemical parameters of water in the breeding and larval rearing systems *viz.*, temperature, dissolved oxygen, total alkalinity and pH recorded were: 26-28°C, 7-7.5 ppm, 110-130 ppm and 7.5 - 7.8 respectively and were well within the optimum range (Tucker, 1991) and were uniform for all the stocks.

The study was conducted during the peak breeding period of the species and the reproductive indices such as weight of stripped eggs, stripping percentage, spawning fecundity, PGSI and ova diameter (Table 1) were recorded for the two different inducing agents *viz.*, ovaprim and pituitary extract separately. The pituitary gland produces and stores gonadotropin hormones (GtH), which play a decisive role in ovulation and spermiation (Rottmann *et al.*, 1991). Ovaprim, a synthetic gonadotropin releasing hormone (GnRH), triggers pituitary to produce GtH which is necessary for spawning (Rottmann *et al.*, 1991; Yanong *et al.*, 2009). Although the mode of action of these inducing agents are different, the results of all parameters indicating breeding performance, obtained in the present study were almost similar.

In the present study, the average spawning fecundity of magur was observed in the range of 3600±274.16 to 9539±746.50, similar to the earlier studies (Goswami, 2007; Sahoo *et al.*, 2007; Jatpat *et al.*, 2015). Higher weight of stripped eggs and spawning fecundity were noted for magur from Godavari followed by Krishna River for both pituitary and ovaprim inducement, whereas the value observed for magur from Narmada was found to be significantly lower

($p < 0.05$) for both the inducing agents. Stripping percentage also varied significantly ($p < 0.05$) among the fish collected from different rivers and was highest in magur from Godavari [14.57±1.026 (P); 11.82±0.184 (O)], followed by Krishna [9.74±0.286 (P); 9.90±1.149 (O)] and Narmada [10.04±0.498 (P); 9.17±0.457 (O)]. Similar values were observed in closely related species *Clarias gariepinus* (De Graaf *et al.*, 1995; Shinkafi and Ilesanmi, 2014). Since stripping process was involved in the breeding programme, PGSI is employed as an index in the present study according to Oyelese (2006) instead of GSI. From the Table 1, it is evident that PGSI was significantly higher ($p < 0.05$) in Godavari group compared to Krishna and Narmada. Ova diameter of magur collected from Narmada [110.84 ± 0.30 μ (P); 110.73±0.23 μ (O)] was found to be significantly higher ($p < 0.05$) than Krishna and Godavari. Cheah *et al.* (1990) reported an ova diameter of 99.0±0.11 μ to 127±0.18 μ during ovulation; while, Thakur (1980) reported an ova diameter of 150 μ indicating variation in ova diameter.

Fertilisation rate (Fig. 1) of magur from Godavari was highest ($p < 0.05$) (69.33±1.99); whereas that of Narmada was the lowest (46.67±1.79). Fertilisation rate of 60-75% and 70-72% were reported in magur by Rao and Jankiram (1991) and Srivastava *et al.* (2012) respectively. Hatching rate (Fig. 2) for magur from Godavari was found significantly higher ($p < 0.05$) (59±2.09), while magur from Narmada had lowest values (38.67±0.75). Rao and Jankiram (1991) reported a hatching rate in the range of 30-45%, while Srivastava *et al.* (2012) and Sharma *et al.* (2010) reported a range of 50-55% in *C. batrachus*. Survival rate of fry

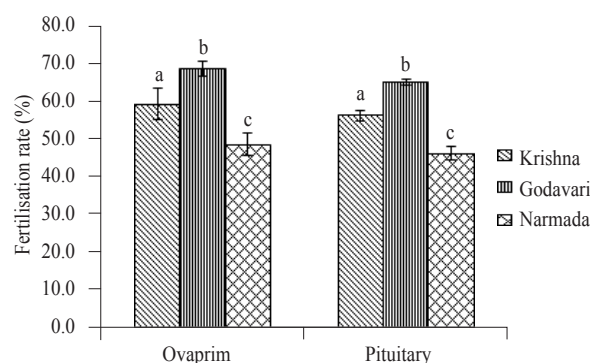


Fig.1. Fertilization rate (mean ± SE) of *Clarias batrachus* collected from different rivers. Bars with same superscripts do not differ significantly ($p > 0.05$)

Table 1. Reproductive indices of *Clarias batrachus* collected from different Indian rivers.

Rivers	Length (cm)		Weight (g)		Weight of stripped eggs (g)		Stripping %		Spawning fecundity		PGSI		Ova diameter (μ)	
	PE	Ovaprim	PE	Ovaprim	PE	Ovaprim	PE	Ovaprim	PE	Ovaprim	PE	Ovaprim	PE	Ovaprim
Krishna	28.12±0.36 ^a	28.60±0.32 ^a	200.67±7.41 ^a	195.67±3.07 ^a	20.08±1.15 ^a	19.50±2.49 ^a	9.74±0.286 ^a	9.80±1.149 ^a	8910±624.71 ^a	8169±1011.16 ^a	10.60±0.34 ^a	11.10±1.46 ^a	100.2±0.23 ^a	100.03±0.29 ^a
Godavari	25.0±0.30 ^b	26.17±0.23 ^b	152.50 ± 2.51 ^b	165.67±3.17 ^b	22.13±1.39 ^a	19.57±0.47 ^a	14.57±1.026 ^b	11.82±0.104 ^b	9539±746.50 ^a	8114±179.67 ^a	17.20±1.48 ^b	13.41±0.27 ^b	102.6±1.66 ^b	103.34±1.06 ^b
Narmada	26.08±0.60 ^b	27±0.30 ^b	154.33±3.85 ^b	166±1.86 ^b	15.42±0.47 ^b	15.20±0.66 ^b	10.04±0.498 ^b	9.17±0.457 ^b	5160±147.48 ^b	3600±274.16 ^b	11.26±0.72 ^a	10.16 ± 0.61 ^a	110.8±0.30 ^b	110.73±0.23 ^c

The values (mean ± SE) bearing same superscript do not differ significantly ($p > 0.05$)

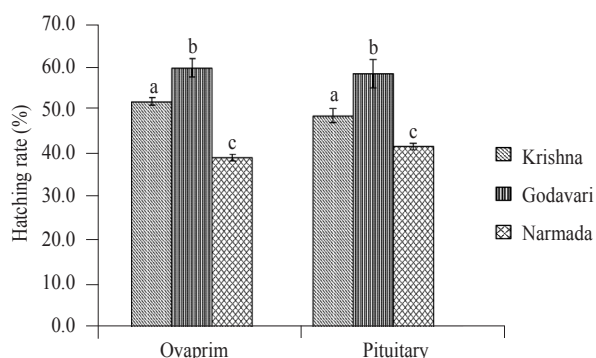


Fig. 2. Hatching rate (mean \pm SE) of *Clarias batrachus* collected from different rivers. Bars with same superscripts do not differ significantly ($p > 0.05$)

(12th day) produced from magur collected from different rivers is given in Fig. 3. Survival rate was highest in magur from Krishna (65.81 \pm 0.9) ($p < 0.05$) followed by Godavari (56 \pm 0.79) and the lowest value was observed in Narmada group (44.87 \pm 1.06).

The fry rearing performance indices such as final length and weight, percentage weight gain, SGR and survival rate were recorded after 14 days of rearing (Table 2). Magur fry from Godavari group exhibited higher performance compared to Narmada group ($p < 0.05$).

Our results from breeding trials revealed that the reproductive performance of fish collected from Godavari is better than Narmada and Krishna ($p < 0.05$). In fry rearing trial, Godavari and Krishna groups exhibited no significant difference between them, whereas both the populations exhibited significantly different performance as compared to Narmada population. Various studies have reported on the intra-specific variation in different reproductive parameters

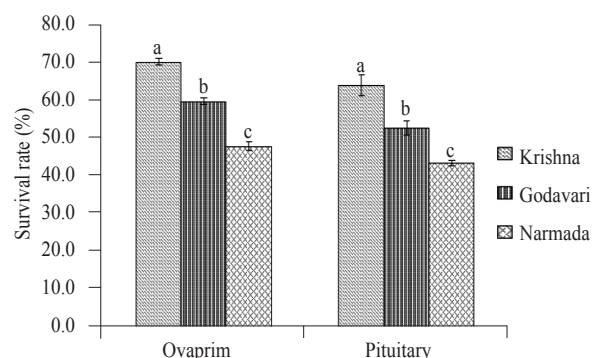


Fig. 3. Survival rate (mean \pm SE) of fry produced from *Clarias batrachus* collected from different rivers. Bars with same superscripts do not differ significantly ($p > 0.05$)

in fishes viz., *Poecilia reticulata* (Peters, 1860) (Svendsen *et al.*, 2013); *Lacerta vivipara* (Von Jacquin, 1787) (Bauwens and Verheyen, 1987) and in several European freshwater fishes (Blanck *et al.*, 2007). The present findings in magur from Indian rivers indicate variations in reproductive parameters between populations of same species.

Control of reproduction is a prerequisite for domestication of fish populations. Hence it is essential to evaluate differences in selected reproductive parameters between populations of same species, prior to undertaking large scale breeding and domestication programmes for aquaculture. Our study highlights the differential performance of reproductive parameters in magur populations from three Indian rivers indicating better reproductive capacity of brooders from river Godavari in comparison to brooders from Krishna and Narmada rivers. The knowledge generated will be of use in choosing brooders of magur, *C. batrachus* with superior reproductive traits for designing breeding plans for sustainable aquaculture development in the country.

Table 2. Fry rearing performance of *Clarias batrachus* from different rivers during two weeks of rearing period.

Rivers	Initial length (mm)	Initial weight (mg)	Final length (mm)	Final weight (mg)	% Weight gain	SGR (%)	Survival rate
Krishna	12.27 \pm 0.88 ^a	19.76 \pm 0.66 ^a	26.75 \pm 1.27 ^a	210.91 \pm 24.29 ^a	975.21 \pm 29.14 ^a	16.93 \pm 0.24 ^a	39.16 \pm 1.4 ^a
Godavari	13.3 \pm 0.71 ^a	20.01 \pm 0.64 ^a	28.37 \pm 1.39 ^a	238.64 \pm 27.09 ^a	1085.88 \pm 37.41 ^a	17.61 \pm 0.19 ^a	43.67 \pm 1.45 ^a
Narmada	13.56 \pm 1.26 ^a	20.77 \pm 1.34 ^a	24.3 \pm 1.42 ^b	174.41 \pm 15.64 ^b	740.32 \pm 59.86 ^b	15.20 \pm 0.16 ^b	29.86 \pm 2.18 ^b

The values (mean \pm SE) bearing same superscript do not differ significantly ($p > 0.05$)

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