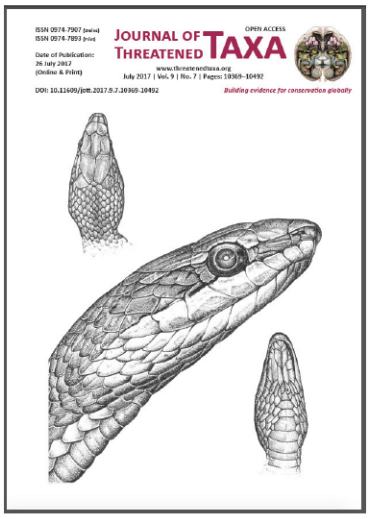

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COMMUNICATION

CAPTIVE BREEDING FOR CONSERVATION OF DUSSUMIER'S CATFISH (ACTINOPTERYGII: SILURIFORMES: CLARIIDAE: *CLARIAS DUSSUMIERI*) A NEAR THREATENED ENDEMIC CATFISH OF PENINSULAR INDIA

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Captive breeding for conservation of Dussumier's Catfish (Actinopterygii: Siluriformes: Clariidae: *Clarias dussumieri*) a Near Threatened endemic catfish of peninsular India

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Abstract: The peninsular Indian endemic Dussumier's Catfish *Clarias dussumieri* once abundant in the wetlands and other water bodies of Kerala is now in rapid decline. The present paper focuses on an approach towards the conservation of this rare catfish through artificial propagation. Fishes were bred in captivity by the administration of fish pituitary extract at the rate of 20–40 mg.kg^{-1}. Spawning occurred after 12–14 hr of injection and fertilized eggs hatched after 16:30hr. Larval rearing was carried out in cement cisterns and the larvae attained a mean size of 51.6±1.6mm in 60 days. Since brooders of this species have become extremely rare in nature, the present study on captive breeding by hormonal manipulation methods without sacrificing the male fishes, assumes significance for conserving this endemic species.

Keywords: *Clarias dussumieri*, endemic catfish, fish pituitary extract, hatching, induced breeding.

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INTRODUCTION

Freshwater aquaculture development in India has hitherto focused on the Indian major carps, and carp culture accounts for 95% of the total inland fish production (FAO 2016). Although many of the indigenous fishes have local preference and demand, such species have not been incorporated into the culture systems owing to lack of availability of fingerlings. Compared to other Southeast Asian countries, the 'Index of Biodiversity'in Indian aquaculture is quite low (Kutty 1999). In this context, there is an urgent need to standardize mass production of indigenous fishes by captive breeding so as to replenish and restore them in natural waters, and facilitate their sustainable utilization on commercial scale.

In Kerala, poor consumer preference for carps results in the culture of undesirable exotic species like African Catfish *Clarias gariepinus* (Burchell, 1822) and carnivorous Pacu *Piaractus brachypomus* (Cuvier, 1818), despite the ban imposed on its culture and their introduction to natural waters (Binoy 2010). The endemic Catfish *Clarias dussumieri* Valenciennes, 1840 is a candidate species that has high aquaculture potential, as it has been reported to grow to a maximum size of over 3kg in nature (Padmakumar et al. 2010).

Clarias dussumieri popularly known as 'naadan mushi' or 'naadan muzhi' (Image 1), is an endemic catfish of peninsular India that has been assessed as 'Near Threatened' in the IUCN Red List of Threatened Species (Abraham 2011). The species, once abundant in the rice polders, rivulets and temple ponds of Kerala, has now sharply declined as a result of increased use of pesticides and agrochemicals for intensive rice cultivation practices and land reclamation for construction (Padmakumar et al. 2010; Shaji & Kumar 2016). *Clarias dussumieri* has very high consumer preference as a food fish with medicinal and nutraceutical properties due to the presence of high concentration of physiologically available iron, necessary for the synthesis of haemoglobin and essential amino acids (Padmakumar et al. 2004). Much of the research in India, has been centred on the breeding and propagation of *Clarias magur* (Sahoo et al. 2008) and *Heteropneustes fossilis* (Alok et al. 1998; Bindu et al. 2009), and little work has been carried out on *C. dussumieri* (Padmakumar et al. 2004; Aneesh et al. 2013). Owing to their close morphological similarity with *C. magur,* the species is often wrongly identified and reported in publications implying their abundance. The situation calls for very sincere efforts for their propagation and popularization of culture practices. Studies on captive breeding of *C. dussumieri* was undertaken for the first time at the Regional Agricultural Research Station (R.A.R.S), Kumarakom as part of the National Agricultural Technology Project (Padmakumar et al. 2004) and the studies that followed assumes significance in this context. Present report is on the breeding of *C. dussumieri* in captivity through near natural means without sacrificing the male for milt collection and artificial breeding.

Image 1. Brooders of *Clarias dussumieri*

MATERIALS AND METHODS

Live *C. dussumieri* were collected from the lowland areas and rivulets (9.3473–9.3835^{0}N and 76.3473–76.5741^{0}E) of the river Pampa. Male and female fishes were raised separately in experimental tanks (5x3x1m) for one year at R.A.R.S., Kumarakom. During this period they were fed with commercial pellet (Higashi fresh; crude protein content 20%), supplemented with fishmeal and chopped trash fish at the rate of 5–6 % of body weight. The morphometric characteristics and key biological features such as gonadosomatic index, fecundity and egg size were monitored.

Mature fishes having distinct secondary sexual characters were selected as brooders (Padmakumar et al. 2004) and were subjected to intra-muscular administration of hormone. Continuous showering and natural stimulation using filtered river water was provided in the tank with a closely simulated flow regime for 6–8 hr before the hormone injection. Altogether 15 breeding trials were conducted during the years 2001–2005. Both synthetic and natural hormonal extracts were utilized for breeding stimulation and final maturation of gonads. Hormonal analogue Ovaprim® containing 20mg SGnRHa and 10mg Domperidone in 1ml was used at the rate of 0.8–1.0 ml.kg^{-1} in the first three trials and crude carp fish pituitary extract, at the rate of 20–40 mg.kg^{-1}

was administered for the remaining trials, in a single dose. After the injection, both male and female fishes were kept together in an experimental tank in single and double pairs. They were kept under close observation for breeding behavior. All responses and exact time of egg laying were closely monitored for 12hr post-injection and further up to 20hr for fishes which failed to develop any visible courting responses.

Fertilised eggs were collected and observed continuously and images captured at 15 minute intervals, under a trinocular microscope (CETI, Belgium) at 10X magnification for documenting the embryonic development. This was done with the help of Magnus Imaging System supported by PixelView software, connected to a computer monitor. Time of fertilization was denoted as 0:0h. Dead eggs were removed from the bottom of the spawning tank at each stage of development.

RESULTS AND DISCUSSION

The total length (T_L) and total weight (T_W)of the fishes that appeared in the collections (n=52) ranged from 17–54.0 cm (38.85±10.5 cm) and 40g to 1130g (432.9±293.7 g) respectively. The highest G.S.I observed in this species was 24.4 and fecundity was 65,258 eggs.

Induced breeding

Mature males having an average size of 299.67±96.4g and females having 326±100.6g were observed to be suitable for breeding. Ripe females were identified by easy extrusion of uniform sized eggs when pressed on the abdomen. Being a monogamous fish forming a 'one to one' mating pair, one male and one female was observed to be a suitable set for breeding. Whenever more females or more males were placed together in one breeding pool, the fishes were observed to be seriously injured and even resulted in mortality. Hence a 1:1 ratio was strictly maintained in subsequent trials. Breeding trials were undertaken close to the onset of monsoon coinciding with first monsoonal showers-the identified spawning season in nature (Padmakumar et al. 2010). In the breeding trials using ovaprim as inducing agents, even fully matured and gravid females failed to respond, although the fish exhibited vigorous sex play. The broodfishes underwent mortality in all such trials. There were many reports on breeding other clariids using diverse hormonal regimes, viz., ovaprim (Mahapatra et al. 2000), carp pituitary extract (Clemens & Sneed 1971; Hogendoorn & Vismans 1980), HCG (Mollah & Tan 1983; Zairin et al. 1992; Szyper et al. 2001). Hence for further trials, a varying dose of pituitary alone was administered to the females while male received half of the female dose. Fertilization was successful in six trials and hatching was successful in three (Table 1). Recently, Aneesh et al. (2013) reported breeding of this endemic catfish using synthetic hormone Wova-FH.

Table 1. Details of breeding trials of *Clarias dussumieri* using fish pituitary extract

Trial No.	Sex	W_T (g)	Dose (mg)	Fertilisation (%)	Hatching (%)	Survival (%)
1	F	300	4.5	60	--	--
	M	250	7.5			
2	F	400	15	63.3	38	37
	M	200	10			
3	F	200	10	90	90	80
	M	200	5			
4	F	350	21	49	--	--
	M	250	12			
5	F	300	18	96.5	23	20
	M	250	15			
6	F	300	10	40	--	--
	M	300	20			

Clariids have a natural tendency to hide in dark corners away from light (Offem et al. 2010) utilizing any hideouts available within the tank, a tendency seen even in the broodfish. Hence a PVC pipe (0.4m long and 4" diameter) was provided as hideout in the spawning tank. After hormonal administration, the male chases the female and swims underneath her and due to their natural affinity to dark corners settle in the hideouts. In trials when more than one hideout was provided the stimulated fishes enter into separate hideouts with occasional union with the prospective mate, again returning to their respective chosen hideouts. With elapse of time in all such instances, the spawning response was negative. Hence in subsequent trials, only a single hideout was placed in the spawning tank. Both the hypophysed fishes, i.e., male and female went into the same hideout chamber. The diameter of the hideout was so chosen, as just enough for the mating pair to wriggle in. When larger sized hideouts were provided, the fish was found to freely move in and out, most restlessly prior to spawning and with elapse of time response was negative. The selection of a single hideout with diameter sufficient enough for the mating pair to wriggle in with no free space, the response was positive. It appeared that the hideouts facilitated immediate physical contacts of the hypophysed brooders resulting

in courting and successful natural spawning. Hence, in all the subsequent trials, this method was meticulously employed with success. In all previous reports of induced breeding of clariids, the standard procedure has been stripping the ripe eggs after hormonal manipulation and fertilization of the stripped eggs utilizing milt from the male fish. As the males among clariids do not exude milt by usual procedures, the common method employed is to surgically collect the testis of the hypophysed male, prepare sperm extract and utilize the suspension for in situ fertilization of stripped eggs from the hypophysed females. This procedure necessitates sacrificing male fishes for artificial fertilization of the eggs (Rao et al.1994). Among catfishes, males are generally not free milting and most of them are considered not amenable to stripping (Pandian & Koteeswaran 1998) probably due to the unique anatomy of their tubular testis. Violent juxtaposition of the mating pair and wriggling of the body of male and female fishes facilitated by contraction and relaxation of successive myotomes, recall the act of stripping of gametes (Pandian et al.2001).

Clarias dussumieri is a broadcast spawner and no parental care was observed in these trials. Spawning occurred after a latency period of 12–14 hr with 40–96.5 % fertilization. The fertilized eggs were spherical, heavily yolked and orange in colour. The diameter of mature eggs varied between 1.3 and 1.6 mm (1.5±0.096), closer to the lower limit of most other catfishes like *C. magur, Pangassius sutchi, H. fossilis, Horabagrus brachysoma* and *C. gariepinus* (Thakur & Das 1985; Rao et al. 1994; Islam 2005; Bindu et al. 2009; Padmakumar et al. 2011; Olaniyi & Omitogun 2014). The mating pair always keeps away from the released eggs and this helps to protect the eggs from damages. The eggs are slightly sticky and adhesive but can easily be separated by gentle water flow. The fertilized eggs were transferred to a flow-through incubation system in shallow trays with a feeble flow of water, approximately 5cm sec^{-1}. Better aeration facilities and timely removal of dead and unfertilized eggs improve hatching rate and hatchling survival by preventing the deterioration of water quality.

Embryonic and larval development

In *C. dussumieri*, yolk occupies 75% of the egg mass. The blastodisc appears 2–3 hr after fertilization and the germinal ring appeared as a ridge encircling the globular yolk in 4–5 hr (Table 2). As the development advances, the embryo appear more and more elongated and the tail appeared overlapping the head. The embryonic stages recorded in the present study are found to be similar to other tropical catfishes (Thakur & Das 1985; Arockiaraj et al. 2003; Islam 2005). Incubation was completed in 16.30hr at temperature 27±1^{0}C and pH 6.5 to 7.0 and within 20hr after fertilization all the eggs hatched. By this time, the egg membrane becomes conspicuously thinner and tail emergedout first by its vigorous twitching movements (Image 2). Hatching time among catfishes showed wide variations (Arratia et al. 2003). In catfishes like *C. magur, H. fossilis* and *H. brachysoma* it varied between 16–24hr (Thakur & Das 1985; Bindu et al. 2009), whereas in *C. gariepinus* and *P. sutchi* it ranged between 21–36hr (Bruton 1979; Islam 2005).

Size of the hatchlings varied between 4.9–5.1 mm

Table 2. Stages of embryonic development in *Clarias dussumieri*

HPF[a] (h: min.)	Morphological development
01.00	Multi celled stage
03.00	Morula stage
05.00	Formation of germinal ring
06.00	Embryo coiled around yolk
09 .00	Differentiation of head region, notochordal somites appeared
13.00	Movement of embryo started
14.00	Heart beat started
15.00	**Blood circulation visible**
16.30	Hatching

[a] Hours of post fertilization

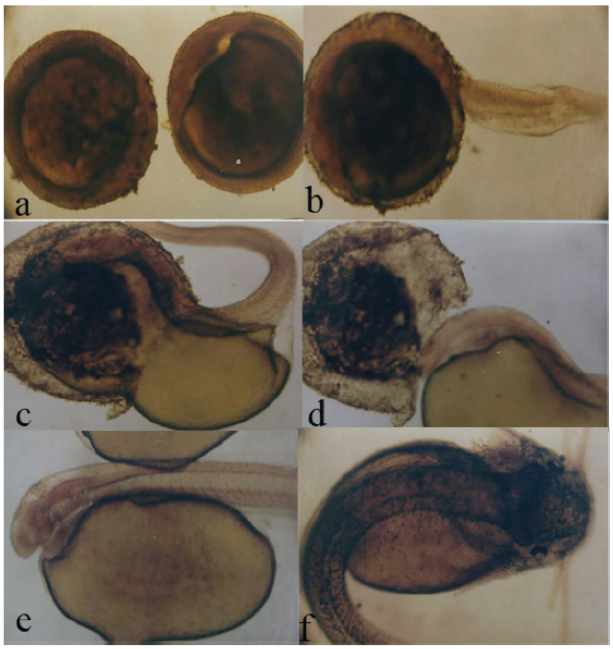

Image 2. Different stages of development and hatching in *Clarias dussumieri*

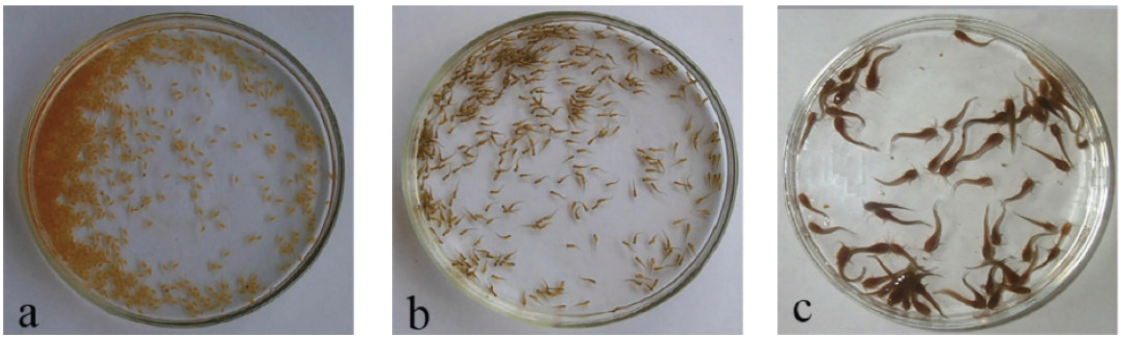

Image 3. a - hatchlings; b - two day old; c - fry of *Clarias dussumieri.* © K.G.Padmakumar

Table 3. Growth of *Clarias dussumieri* hatchlings in captivity

Age (days)	T_L (mm)		W_T (mg)		Feed provided
	Range	Mean ± SD	Range	Mean ± SD	
1-3	7.0–9	8.33±0.69	---	---	---
6	9.5–10	9.78±0.21	---	---	Egg yolk, Artemia nauplii
10	11–13	11.7±0.06	10–30	0.16±0.10	Artemia nauplii, tubifex
20	14–18	14.75±2.38	30–50	31.5±12.26	tubifex, powdered pellet feed
30	18–31	20.42±4.96	40–100	61.2±21.76	prawn meat, tubifex, pellet feed
55	48–52.5	51.6±1.56	115–117	116.5±0.71	Pellet feed, trash fish

(5.0±0.1mm) and was larger than most other catfishes, viz., *Mystus montanus*, *M. cavasius, C. gariepinus, H. fossilis* and *H. brachysoma* (Arockiaraj et al. 2003; Rahman et al. 2004; Islam 2005; Bindu et al. 2009; Padmakumar et al. 2011) but smaller than *C. magur* (Sahoo et al. 2010). Hatchlings appeared rolling on the bottom with their attached spherical yolk sac on the ventral side. The early hatchlings look slender and transparent with unpigmented eyes and without a distinct mouth. They congregate in the tank corners, away from the aeration points. As the hatchling development proceeds (Image 3a–c), heavy pigmentation begin to appear on the dorsal portion of the head from the second day of hatching. The yolk gets fully absorbed by the 3rd day and the young fry were fed with freshly hatched nauplii of *Artemia salina*. This was followed till 10th day and then gradually weaned to tubifex worms, powdered pellets and minced prawn meat suspension. Mixed zooplankton, *Artemia nauplii, tubifex* and egg custard were found to be acceptable as larval diets during hatchery rearing of catfish larvae. These feed items containing 41–65 % protein (Sahoo et al. 2010) were reported as good diets. In two months, the hatchlings reached 51.6±1.6 mm and were transferred to open nurseries and fed on pellet feed (Higashi starter). Fish meal is the major ingredient of this commercial pellet. The survival rate of larvae can be improved if boiled egg yolk and live feed are provided (Table 3). Early feeding is most crucial in survival rate of hatchlings. As the youngone reaches fry stage, i.e., 25mm in size they were gradually weaned to commercial feed formulations. Since cannibalism is most common among catfishes sufficient supplementary food and rapid transfer of larger young ones by periodic sorting was found essential for success in fry nursing of this species.

CONCLUSION

In the context of their rapid population decline in the wild (Shaji & Kumar 2016), it has become imperative to develop suitable conservation strategies for *C. dussumieri*. Development of artificial breeding protocol for *C. dussumieri* assumes relevance in this context. A novel method for near natural spawning was evolved by providing horizontal hideouts that ensured intimate physical contact for the monogamous mating pairs. This method avoids the necessity of sacrificing the male fish for artificial fertilization of the stripped eggs in clariids. It is also crucial to take up studies on population structure and dynamics of this species. In the context of ambiguity in correct identification and taxonomy, studies on genetic characterization of this species (Devassy et al. 2016) assume relevance. Further population genetic studies coupled with captive breeding for successful translocation and reintroduction of this species is essential (Cohn 2001; Null & Lund 2011). More importantly, there is an urgent need for restoration of the natural habitats of this threatened

species, as habitat disturbances and intrusion of exotics has largely contributed to the current state of decline. Standardization of simple protocol for hatchery production of seeds is a breakthrough not only for conservation management of this endemic species but also for their potential use as a candidate species in aquaculture systems.

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