

Breeding for Conservation: Case of an Endangered Catfish, Clarias dussumieri (Valenciennes, 1840)

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Abstract

In an investigation to estimate the natural stocks of an indigenous, threatened catfish Clarias dussumieri (Valenciennes) in Kerala, India in 2011, only 208 individuals were obtained, revealing its vulnerability to extinction, while the exotic African catfish Clarias gariepinus (Burchell) was obtained throughout the year. Induced breeding of C. dussumieri was successful in captivity without stripping or sacrificing males by administering a synthetic hormone Wova-FH, at the optimum dosage of 1.0 ml kg⁻¹ body weight for both males and females that resulted in fertilization and hatching rates of 97 and 99% respectively, and maximum mean effective fecundity of 5587± 321. Mass seed production by induced breeding would facilitate suitable aquaculture programmes for conservation of C. dussumieri involving natural stock enhancement to protect them from the imminent threat of extinction. It is also recommended to revise the IUCN red list category of C. dussumieri from "Near-Threatened" to 'Critically Endangered'.

Keywords: *Clarias dussumieri*, conservation aquaculture, fishery, induced breeding, threatened species

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Introduction

The Clarias dussumieri (Valenciennes), an air breathing catfish endemic to India (Jayaram, 2006) is variously categorized as a vulnerable (Molur & Walker, 1998), endangered (Lakra et al., 2010); and a near threatened (IUCN, 2012) fish. They are predatory, feeding on small fishes, insects and

detritus (Padmakumar et al., 2010). *C. dussumieri* differs from other congeners in having fewer dorsal fin rays and a broader snout (Jayaram, 2006), and can be easily distinguished from related species like *Clarias batrachus* (Linnaeus) and African catfish *Clarias gariepinus* (Burchell) by observing the occipital process (Talwar & Jhingran, 1991).

Three species of clariid catfish viz., Clarias magur (Hamilton), Clarias dussumieri dayi (Hora) and C. dussumieri dussumieri have been listed in India by Devi & Indra (2009). Of these C. magur had previously been classified as C. batrachus, which enjoyed a wider distribution covering most of the states of India (Talwar & Jhingran, 1991; Jayaram, 1999, 2006), while being absent in Kerala (Easa & Shaji, 1997). Easa & Shaji (1997) had found C. dussumieri as 'abundant' during 1993-1995 in the rivers of Kerala. However, many of the subsequent reports, except Kurup et al. (2004) have listed this species as 'very rare' based on relative abundance (Gopi, 2000) and included it under the 'vulnerable' category (Shaji et al., 2000; Dahanukar et al., 2004), 'endangered' (Lakra et al. 2010), or have not mentioned its occurrence in any of these rivers (Devi et al., 2005; Raghavan et al., 2008), indicating that the population of *C. dussumieri* had been declining alarmingly over a short period. Deterioration of its natural habitats and breeding grounds by reclamation of lands, aggravated by excessive use of chemical fertilizers and pesticides for agriculture in the wetland systems where this species naturally existed might have contributed to this decline (Padmakumar et al., 2010). In a survey conducted in 1215 ha of wetlands in Kerala for one year, Thomas et al. (2002) could not collect even a single specimen of the native Clarias species.

The African catfish *C. gariepinus*, an exotic species is presently a major farmed species in many parts of the country (Thakur, 1998), and has been farmed in Kerala since 1993 (Krishnakumar et al., 2011).

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This fish which reportedly escaped from farm ponds has been found from the extensive natural water bodies (Krishnakumar et al., 2011). Vidthayanon (2002) had suggested that the highly competitive *C. gariepinus* might have the potential to replace other species of *Clarias* from the freshwater habitats in Thailand.

The remarkable decline in populations of *C. dussumieri* over a short period of time necessitates urgent review of the status of its population in Kerala, while it is important to conserve this threatened species from further decline (Sundarabarathy et al., 2005; Sarkar et al., 2006). The present paper describes an assessment of the natural populations of *C. dussumieri* in the wetlands of Kerala, supported by an effort to standardize the protocol for its captive spawning and larval rearing, leading to discuss some feasible strategies for its conservation. The study also estimated the landings of the exotic species *C. gariepinus* as part of the survey from the natural habitats of the native catfish species.

Materials and Methods

Surveying the stocks of Clarias species

Assessment of the populations of C. dussumieri in Kerala, the southern peninsular state of India was done primarily by surveying the fish markets and landing centres. A preliminary survey was carried out twice a month for a period of one year (June 2009 - May 2010) in the 124 inland fish markets and 56 major fish landing centres that were identified in Kerala to determine the availability of *C. dussumieri* and other *Clarias* species. The collected fishes were identified using the taxonomic keys of Jayaram (2006), Talwar & Jhingran (1991) and Ng & Kottelat (2008). Primary data were collected by personal visits by enumerators to markets and landing centres wherever possible, but in a few places, because of manpower and budget constraints, predetermined brokers or commission agents were relied for obtaining information on availability of the species in the data sheet provided. From the data collected for C. Gariepinus, the average daily availability in each centre was calculated and an estimate of the total quantity of this species marketed in Kerala was arrived at by multiplying the average daily landing in each centre surveyed with the total number of working days in the respective centres.

In those areas where at least one specimen of the target species (C. dussumieri) was reported, detailed investigations were carried out with the help of traditional fishermen by regular sampling trials twice in a month for a period of 12 months (June 2010- May 2011) in water bodies (swamps, canals, river stretches, public and private ponds, rice fields, etc.) suspected for the presence of *C. dussumieri* by cast netting, hand picking, using catfish traps made of split bamboo poles and battery operated, lowvoltage electric fishing devices which were used by indigenous fishermen in the region. Locations traditionally associated with the presence of C. dussumieri in inland fishery catches, which were ascertained by interviews with fishermen were also subject to detailed investigation. Data regarding the number of fish caught, source, method of collection, total length and weight as well as the apparent sexual condition of the fish were recorded after they were transported live to the laboratory at College of Fisheries Panangad, Cochin. A transparent calibrated ruler was used to measure the total length of fish up to the nearest 1 mm (Sundarabarathy et al., 2005) and an electronic balance (Sartorius Mechatronics, India) with sensitivity of 1 g was used for measuring the body weight.

Collection and maintenance of broodstock

Live *C. dussumieri* (22.53 \pm 2.89 cm and 166 \pm 12.23 g) collected from wetland systems and ponds in Thrissur district, Kothamangalam in Ernakulam district and Kaliyar, Adimali and other isolated areas in Idukki district were transported to College of Fisheries, Cochin, in plastic containers of 50 1 capacity (10 fish in each), half-filled with water without aeration, and maintained in circular cement cisterns of 75 cm diameter and 90 cm height filled to half the level with freshwater, each holding approximately 200 l. The bottom of these tanks were provided with a 10 cm layer of washed sand as substrate and short pieces of PVC pipes (10 cm diameter) to facilitate hiding habbit of the fish. The tanks were covered using nylon net to prevent the fish from jumping out. A total of 8 such tanks were maintained indoors holding 48 fish which were used as broodstock. The fish were fed ad libitum with a mixed diet consisting of clam meat, prawn meat, trash fish and formulated feed of 35% protein twice daily. Fifty percent of water in all the tanks was replaced with good quality well water once in a week. The important water quality parameters in broodstock tanks such as pH, temperature, dissolved oxygen, total alkalinity and total hardness were monitored regularly using a CyberScan Multi parameter portable meter (Eutech Instruments, Fisher Scientific, UK), and ammonia (NH₃-N) using a test kit (Aquatic Ecosystems, USA).

Breeding trials

Brood fishes were stocked in tanks during the months of February to March 2011, and development of the gonads was monitored regularly. Breeding experiments which began in the month of June 2011, when the fish were observed to have attained complete maturity by the onset of southwest monsoon rains, were conducted using rain water stored in rectangular fibreglass containers of size $120 \times 60 \times 75$ cm (500 l capacity) with rounded corners.

Four different doses viz., 0.5, 1.0, 1.5 and 2.0 ml kg⁻¹ 1 (T₁, T₂, T₃ and T₄, respectively) of an ovulating hormone Wova-FH (Biostadt Agrisciences, Wockhardt Life Science, Mumbai, India) containing synthetic analogue of gonadotropin releasing hormone with three replications for each treatment, and control were used for inducing spawning. Healthy female with freely oozing egg and ripe male of almost the same size were selected at the ratio of 1:1. Single, intramuscular injections were given near the base of dorsal fin above the lateral line of male and female using an insulin syringe at 17 00 h. Control fish were maintained in the same water conditions, but were not given hormone injections. Important water quality parameters in the treatment tanks were also recorded.

Immediately after administering the hormone injections, each pair of brooders was released into the breeding tank in which water level was maintained at a depth of 45 cm, provided with mild aeration and secured at the top with nylon net to prevent fish from jumping out of the tank. Adequate quantity of the aquatic weed Hydrilla verticillata (L.f.) Royle was also provided as cover for the fish to hide, thus facilitating a natural breeding habitat. During preliminary trials, it was observed that C. dussumieri displayed prolonged and intermittent spawning act extending up to 6 h, leading to considerable damage to the earlier-laid eggs because of vigorous movement of the breeders during subsequent courtship and breeding play. Therefore, perforated rubber panels of 5 cm thickness with a number of holes each of 3 cm diameter were placed covering the entire bottom of each breeding tank facilitating free fall of the eggs onto the tank bottom, thereby preventing egg damage.

Hatching

The spent parents, aquatic plants and rubber mat were removed from the breeding tank soon after spawning. The fertilized eggs were then carefully siphoned out and transferred to a specially designed flow-through hatching system comprising of a series of circular fibreglass basins each of 90 cm diameter and 15 cm depth. Continuous freshwater flow at a rate of 30 l h⁻¹ was maintained in each basin to keep the demersal eggs in slightly floating condition to avoid crowding of the eggs. Breeding parameters such as the effective fecundity (total egg output of each female fish), egg diameter, latency period, fertilization rate, and hatching rate were determined according to Sarkar et al. (2005).

The water flow in the hatching tanks was reduced after hatching, retaining the non-feeding, early yolk sac fry. After absorption of the yolk sac, viz., from the fourth day onwards hatchlings were fed ad libitum with newly hatched Artemia nauplii (INVE, Thailand) three times a day for a period of one week. On eight day post-hatching, the fry were transferred to outdoor fibreglass tanks (1500 l) for subsequent rearing, before they were gradually weaned to formulated feed containing 45% protein. outdoor rearing tanks were maintained under shade at a water depth of 12-15 cm with sufficient number of hideouts to facilitate the obligatory habit of aerial respiration and hiding by C. dussumieri. Uneaten food materials and excreta were carefully siphoned out from the bottom of rearing tanks periodically, and the water quality parameters were monitored in regular intervals.

Statistical analyses to test the significance of the effects of different doses of the hormone on various breeding traits considered for the induced-bred C. dussumieri viz., the effective fecundity, latency period, fertilization rate, and hatching rate were carried out by means of software package SPSS (version11.5). Data analysed using one factor ANOVA at a significance level of p<0.01 and wherever the effects were found to be significant, least significant difference (LSD) was calculated and the significant effects were identified (Snedecor & Cochran, 1956).

Results and Discussion

Population Survey of Clarias species

During the first stage of the survey, large number of individuals of the exotic *C. gariepinus* were obtained throughout the year from 22 fish markets and other landing centres (Table 1), while *C. dussumieri* was represented by only one or two specimens in those stations which were subject to detailed search in the second stage of the survey from June 2010 – May 2011. Only 208 specimens of *C. dussumieri* of size ranging from 8 to 48 cm and 32 to 1300 g were caught during the second stage of the survey (Table 2).

Table 1. Occurrence of African catfish *Clarias gariepinus* in different landing centres and markets in Kerala surveyed for one year during June 2009 to May 2010

Name of the Centre	Annual landings of African catfish (kg)					
Aluva	8 736					
Angamali	7 488					
Chalakkudy	8 112					
Champakkara	8 736					
Changanacherry	6 240					
Chempu	4 680					
Irinjalakkuda	4 368					
Kaduthuruthy	3 120					
Kothamangalam	3 120					
Kottayam	6 240					
Kuttippuram	3 432					
Kumali	3 120					
Mankompu	7 800					
Mundakkayam	4 992					
Nilambur	3 744					
Parumala	8 112					
Thiruvalla	5 616					
Thottappally	7 800					
Thrissur	8 736					
Tirur	4 992					
Vaikom	11 232					
Vellayani	3 120					
Others	11 856					
Total	145 392					

It will be worthwhile to consider the possible reasons for decline in populations of C. dussumieri in Kerala, in view of a series of probable events that could have contributed to the reduction in their numbers, including the epizootic ulcerative syndrome (EUS) that had infected the water bodies in catastrophic proportions. Tonguthai (1985) observed that the fish species mostly affected by EUS in Thailand were snakeheads and catfishes. The EUS that was first reported in Kerala from Pookote Lake in Banasurasagar reservoir area in Wayanad in June 1991 and had rapidly spread to all parts of Kerala (Anon, 1992), possibly had a significant impact particularly on C. dussumieri which is perhaps one of the most susceptible species. In the present study, no specimen of *C. dussumieri* could be obtained from the Kuttanad region, the biggest wetland system of Kerala (Narayanan et al., 2011), which once supported a major fishery of C. dussumieri. Intensive agricultural practices followed in Kuttanad wetlands have necessitated extensive application of agrochemicals for paddy cultivation. It was reported that huge quantities of fertilisers (20 000 t) and toxic pesticides (500 t) had been sprayed in Kuttanad (Anon, 1992). Consumption of pesticides reached 1 381 t in 1994-95 and 1328 t in 2001-02 (Devi, 2010). The adverse effects associated with the presence of these chemicals and the impact of EUS might have played a key role in the perceived rapid decline in population of the species in Kerala.

Lal & Singh (1987) reported the adverse effects of pesticides on lipid metabolism in C. Batrachus. Certain pesticides or components of pesticide formulations may have negative impact on fish as, endocrine disrupters (Pait & Nelson, 2002), reproductive endocrine disruption in catfish (Singh & Canario 2004), reproductive dysfunction and abnormal development in fish (Khan & Law, 2005). The exposure to pesticides at sublethal concentrations during pre-spawning phase affected the reproduction in catfish (Singh & Singh, 2007). Invasive species might affect the native gene pool by hybridisation and introgression of genes, thereby sometimes leading to extinction of native flora and fauna (Rhymer & Simberloff, 1996). The African catfish which have escaped into the wild could reproduce with the native catfish, and such hybrids are known to have contributed to the decline of native C. batrachus (Welcomme & Vidthayanon, 2003). However in the present study, the population of C. dussumieri was not found even in areas where C. gariepinus had not yet been reported, and there

Table 2. Number, length (range and mean), and weight (range and mean) of *Clarias dussumieri* individuals collected from various landing centres in Kerala surveyed during June 2010 – May 2011

								Length (cm)		Weight (g)	
	^a Jun - Sep	^a Oct - Jan	Feb	Mar	Apr	May	Total	Range	Mean ±SD	Range	Mean ±SD
Aluva			2				2	25-48	36.5±16.26	200-1300	750±777.82
Thrissur		4		2			6	9-26.4	16.83±6.51	32-182	99.83±61.56
Adimali	9		27	17			53	8-28	17.18±4.88	32-188	120.72±47.46
Kumali			4				4	17.9-24.2	21±2.66	148-172	159.5±10.12
Kaliyar	11	10	2	45	37	7	112	9-28.2	20.03±4.19	32-215	153.85±29.27
Vaikom					2		2	23-24	23.5±0.70	167-170	168±2.12
Kothamangalam				14	5		19	15-25.3	21.05±2.73	132-174	159.26±11.26
Kallada	2			2	3		7	15-28.8	22.11±4.27	132-188	161.71±17.69
Vellayani		3					3	14.9-18.3	16.33±1.76	132-146	137.67±7.37
Aggregate	22	17	35	80	47		208	8-48	21.61±6.11	32-1300	212.28±202.88

^a Since no appreciable number of fish was obtained during the period June 2010 to January 2011, these data were compiled into two groups

was no firm evidence with the limited data available to suggest that the invasive species had significantly contributed to the rapid population decline of *C. dussumieri*. *C. dussumieri* in Kerala appear to naturally occur in the derelict ponds, paddy fields, and many other stagnant water areas associated with wetlands (Abraham et al., 2011). Indiscriminate use of paddy fields and other wetlands coupled with aquatic pollution might have led to reduction or loss of natural habitats as well as breeding and rearing grounds for this species. This is often aggravated by exploitation for fish as food and ornamentals causing rapid decline in their wild populations (Ali et al., 2007; Padmakumar et al., 2010).

The largest specimen of *C. dussumieri* collected from Kerala in the present study was 48 cm and 1.3 kg, which is larger than the maximum size of 25 cm (Talwar & Jhingran, 1991) previously reported for *C. dussumieri*, and that of 41.5 cm and 450 g reported for the related species, *C. batrachus* (Thakur & Das, 1986). Maximum availability of *C. dussumieri* was observed during March - April, while the catch was the lowest during October-January.

Induced Spawning

The breeding season of *C. dussumieri* was found to coincide with the southwest monsoon rains (June – August) as observed in the reproductively active specimens collected from the wild, as well as in the

broodstock held in cement cisterns which is in confirmative with the observations of Padmakumar et al. (2010).

In *C. dussumieri*, sexes can be easily distinguishable by observing the genital papillae (Fig. 1). In male the body was slender and streamlined the genital papilla was conical and pointed, becoming protruded and highly vascularised during the breeding season, while the female had a shorter and blunt papilla which became swollen when matured with freely oozing eggs on applying slight pressure on the highly distended abdomen. However, the ripe male was not found to discharge milt by applying pressure on the abdomen. Brooders exhibited spawning activities after 6 to 8 h of hormone administration, and displayed prolonged and intermittent spawning and courtship behaviour extending for about 6 h. The eggs were slightly adhesive, but were found scattered all over the bottom without getting attached to Hydrilla. The fertilized eggs were golden-yellow in colour, spherical and with substantial yolk content, with size varying from 1.2 to 1.5 mm. The eggs hatched out between 17 - 20 h of incubation after fertilization at a temperature of 27°C, and the yolk sac fry were found clinging to the tank bottom with vigorous tail movements for the first two days because of the heavy yolk reserve. The physio-chemical parameters of water in the broodstock tanks and rearing tanks are given in Table 3, all of which were found to be within the optimum range.

Table 3. Water quality parameters (Mear	SD) in brood stock tanks and	l rearing tanks of Clarias dussumieri
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	Temperature (°C)	Dissolved pH Oxygen A (ppm)		Alkalinity (ppm)	,	
Broodstock tanks	26±3.2	7 ± 0.85	5.0 ±2.6	35.8 ± 4.0	58.12 ± 11.3	0.06 ± 0.07
Rearing tanks	27±1.79	7 ± 1.26	6.0 ±2.2	35.8 ± 5.0	56.66 ± 10.18	0.08 ± 0.08
Breeding tanks	27±1.16	7±0.58	6.16±0.26	15.5±0.51	6±0.59	0



Fig. 1. Mature *Clarias dussumieri* female (left) and male (right) showing difference in the genital papilla

The results obtained from the captive breeding trials are presented in Table 4. Among the four treatments tested, T_2 (1.0 ml kg⁻¹) and T_3 (1.5 ml kg⁻¹) doses of the hormone resulted in complete spawning, but in the T_3 set of fish, swelling and subsequent infection at the injected sites were observed. No spawning activity was noticed in T_1 (0.5 ml kg⁻¹) and the control sets, although a few of the T_1 fish showed pre-spawning behaviour. Partial spawning occurred in one out of the three replicates in T_4 (2.0 ml kg⁻¹), while no spawning resulted in rest of the fishes which were found morbid with excess slime on the body.

The latency period in all the treatments ranged between 10 to 12 h. The maximum mean effective fecundity of 5587 ± 321 per fish was obtained for T_2 , followed by T_3 with $5\,503\pm327$. The lowest effective fecundity of 2420 per fish was recorded in T_4 , the

lone fish which spawned in this treatment. Similarly, the mean percentage fertilization and hatching rates of 97% and 99% were the highest in T_2 , closely followed by T_3 which recorded mean values of 95% and 99%, respectively. In T_4 both percentage fertilization and hatching were the lowest (50% and 24%, respectively) in the partially spawned individual. The maximum number (from three replicates) of 16 166 hatchlings of *C. dussumieri* was produced from T_2 , followed by T_3 with 15 508 hatchlings. Only 285 hatchlings were obtained from the set of T_4 fish.

Singh et al. (2002) reported that the number of ovulated eggs per fish was significantly higher at higher dose of ovaprim for catfish *Heteropneustes fossilis* (Bloch), while for *C. dussumieri* in the present study partial spawning resulted at a higher dose of 2 ml kg⁻¹body weight of Wova-FH, perhaps because of the impeding effect of high doses of hormone on the physiological functions of fish and the consequent stress it caused. This was evidenced by excessive slime production coupled with swelling and inflammation at the site of injection in fish administered with higher levels of 1.5 and 2.0 ml kg⁻¹ of hormone in the present study.

The fertilization and hatching rates of 97% and 99%, respectively obtained in the present study were high compared to the fertilization and hatching rates (84% and 72%) reported for *C. batrachus* by Sahoo et al. (2005).

Statistical analyses using one factor ANOVA showed that the dosages of the hormone WOVA-FH administered had significant effects (p<0.01) on the effective fecundity, latency period, fertilization rate and hatching rate in the breeding trials of *C. dussumieri*. The effect of dosages at levels of 0.5 and 2 ml kg⁻¹ of the hormone injected in fish were significantly lesser than that for 1.0 and 1.5 ml kg⁻¹, while the difference was insignificant between

Table 4. Effect of various doses of Wova-FH on the effective fecundity, latency period, percentage fertilization, and percentage hatching of *Clarias dussumieri*

	Weight of female (g) (Mean±SD)	Weight of male (g) (Mean±SD)	Effective fecundity (No.) (Mean±SD)	Latency period (h) (Mean±SD)	Percentage fertilization (Mean±SD)	Percentage hatching (Mean±SD)	Remarks
T_1	189.33±13.65	201.67±14.43	-	-	-	-	No spawning; injuries over the body due to aggressive pre- spawning behaviour.
T_2	202.67±11.02	204.33±9.81	5587.33±321.01	11.17	96.99±0.43	99.44±0.58	Complete spawning.
T ₃	199±13.53	200.33±10.79	5503.33±327.16	11.5	95.36±1.29	99.06±0.9	Complete spawning; but swelling and infection at the injected site.
T_4	190.67±4.04	195.67±4.04	2420 ^a	10.5	49.58	23.75	Partial spawning or no spawning; brooders found morbid with excess slime production.
С	189±5.2	195.33±0.58	-	-	-	-	No spawning.

^aOnly one fish spawned in T₄

the dosages of 1.0 and 1.5 ml kg⁻¹. While there was no difference between 1 and 1.5 ml kg⁻¹ dose, the effective lower dose could be treated as optimum as the desired results could be induced at the lower dose itself. Higher dose of 1.5 ml kg⁻¹ resulted in swelling and infection at the injected site of fish.

Conservation

It is concluded from the present study that the wild stocks of C. dussumieri in the wetlands of Kerala have reached a critically low level and the species is currently facing an imminent threat of extinction. The alarming declines in the natural stocks of C. dussumieri warrant urgent measures for its conservation and efforts to prevent further loss of the scanty patches of its population in wetland habitats. It is also recommended to revise the IUCN red list category of C. dussumieri from "Near-Threatened' to 'Critically Endangered'. The indiscriminate application of toxic chemicals and high doses of fertilizers for paddy in wetlands would have taken an obvious toll of the available stocks of this vulnerable fish. Promoting organic farming which dispenses with the need to use harmful inorganic chemicals in the farming system could be a feasible option to support their population thereby providing them with the most compatible habitats and favourable breeding grounds.

The present paper describes the first successful attempt of induced spawning of *C. dussumieri*. Adopting the protocol outlined for captive breeding will lead to establishing a feasible strategy for conservation aquaculture of *C. dussumieri*, by ranching in suitable wetland habitats that will facilitate conservation and restoration of the species. This would satisfy its high local demand as a favourite food fish, and ease the fishing pressure on this critically endangered indigenous fish from the wild.

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