Captive breeding of a recently described endemic barbin, *Neolissochilus tamiraparaniensis* (Cypriniformes: Cyprinidae) from Kalakad Mudanthurai Tiger Reserve, Tamil Nadu, India.

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ABSTRACT

Neolissochilus tamiraparaniensis is described very recently from a east flowing river, Tamiraparani River basin, from the Kalakad Mundanthurai Tiger Reserve of the Agasthyamalai Biosphere of peninsular India. This big sized barbin attains 40 cm in length and 1.5 kg in weight which showed its distribution so far from the Tamiraparani River and its tributaries. This endemic species is attempted for induced to spawning by a intramuscular injection of hormone ovaprim, HCG and Pituitary separately. Spawning was complete in 10 - 11.5hr after hormone injection. The maximum eggs number and fertilization rate (84.67 %) were shown in Ovaprim injected fishes.

Keyword: Captive breeding, *fertilization* rate, hatching rate, human chorionic gonadotropin hormone, *Neolissochilus tamiraparaniensis* , ovaprim, pituitary gland extract, ovulation rate.

INTRODUCTION

Captive propagation and restocking programmes for threatened fishes have been started or proposed in several countries (Budihna and Ocvirk, 1990). Recently, however, several captive propagation and reintroduction are successful for, *Zacco pachycephalus* (Wang *et al.*, 1995), Mahseer, *Tor khudree* (Kulkarni and Ogale, 1978, 1979; *Tor putitora* (Hamilton) (Shrestha, 1998; Nilgiri rainbow trout (Gopalakrishnan *et al.*,1999).

The captive breeding study of an endemic fish species, *Neolissochilus tamiraparaniensis* is first of its kind. In southern India, carps naturally spawn during the south-west monsoon (July – August) and spawning was induced by manipulation of water levels. Ovulation can be induced during non- spawning season not only by environmental manipulation but also by hormonal stimulation. Like members of the family Cyprinidae, final maturation and ovulation normally do not occur under captive conditions in the wild carp, *N. tamiraparaniensis*. The most advanced method of ovulation stimulation is used more and more frequently in fish hatcheries, replacing the traditional hypophysation treatment.

Neolissochilus tamiraparaniensis is a recently described barbin from the Tamiraparani River basin of southern Western Ghat regions of Tamil Nadu (Arunachalam *et al.,* 2017). Viable populations of this species are in the up streams of Manimuthar River, Tamiraparani main stream at Inchikuzhi and in Gadana River tributaries of the basin. All the up streams are in the Protected Areas of Kalakad Mundanthurai Tiger Reserve. Currently, the up streams of Tamiraparani iRiver and two tributaries under Kalakad Mundanthurai Tiger Reserve are well protected and can be a fish sanctuary under this Protected Area (Arunachalam, 1998). This herbivorous fish is characterized by its rapid growth, larger size

and tasty flesh. The maximum size (38.4cm) is noted. (personal observation Arunachalam). The male has attractive (golden yellowish) colour than female.

MATERIALS AND METHODS

Live specimens were collected from up streams of Tamiraparani River and their tributaries such as Manimuthar and Gadana (5 km from Sri Paramakalyani Centre for Environmental Sciences of Manonmaniam Sundaranar University, senior author's doctoral study) below the Gadana Reservoir and were transported to the laboratory of the Centre for Environmental Science and also in the areas of breeding ponds by the Government Fisheries Farm. Fishes were stocked in cement tanks of 10 m × 6 m × 1.5 m size for acclimatization, using river water from Gadana Reservoir. During the period of acclimatization (20 days), supplementary feed was given as daily ration and it was placed in the tank at the rate of 5% body weight of the fish. The body of adult *N. tamiraparaniensis* is shiny yellowish with distinct pale-yellow fins; and the body of juvenile is silvery white in colour. Present distribution of *N. tamiraparaniensis* is known only from Tamiraparani River basin, South Tamil Nadu, India. Viable populations exist in Pampar, Kallar and Iluppaiyar streams of Gadana River. It is also found in Manimuthar River of Tamiraparani basin (Arunachalam, 2000).

Reproductive characters of *N. tamiraparaniensis* were studied by Arunachalam and Sankaranarayanan (1998) and showed that the peaks occur during the post monsoon seasons [after south–west monsoon (July–August) and north–east monsoon (October – November)] and they also found out the sexual characters in mature males, horny tubercles present in the snout of males during breeding seasons. Adult males have 40–78 tubercles which are present below the orbit up to the tip snout region.

Collection of fishes

The number of fishes collected was twenty five females and 33 males. Fishes were collected from (size 12 to 23 cm) Gadana River and from Pabanasam upstream in Tamiraparani River, by using wide mesh gillnet and cast net during February to March (Plate a). These nets are usually spread at dusk in the study area near the inflowing streams and are inspected every hour. These were gently and carefully removed from the net, sometimes even by cutting the meshes of nets, to avoid injury to the fish. Then they were placed in flow-through concrete tank of 10 m × 6 m × 1.5 m size for acclimatization for each species. The tank was covered with net of 90% light occluding monofilament mesh.

Stock maintenance

After acclimatization, both male and female fishes were released into the earthen pond (size 15.5 m × 10 m × 2.5 m) for six months, after that they were segregated based on the identification character.

Bloodstocks management and selection

Initially brood fishes were kept in a earthen pool having water area of 15.5 m × 10 m × 2.5 m. At the advent of breeding season, the brood fishes were checked by observing gonadal condition (Linhart *et. al.,* 1995) and manipulated for better management aspects. To provide plentiful sustained supply of plankton and benthic organisms, brood ponds were fertilized at recommended dose using inorganic and organic fertilizers. Three weeks prior to spawning externally matured female and male fishes were segregated and kept in a small round earthen pond (10 × 10 × 2 m3 size). The pelleted feed was administered twice daily at the rate of 5% body weight of the total biomass during the experimental period.

Fertilization of the pond was carried out two times (1st week and 4th week) during the experimental period. After segregating, the male and female fishes were transferred into cement tanks of 10 m × 6 m × 1.5 m size each for conducting the induced breeding experiments. Females and males were finally selected examining their stage of ripeness among the collected brood and transferred into rectangular breeding hapa (2 m × 1 m × 0.75 m size) suspended into the chosen back pool along the course of the river. Ripe females were recognized by palpating the female's abdomen which is bulging and soft to touch and swollen. The genital papilla is slightly reddish and blunt with a reddish dot. Males, on the other hand have deep-pit-like vent and more slender body and ooze out milt when it is gently pressed.

Induced breeding

The segregated fishes were fed with artificial diets contain optimal protein on daily ration basis (at 5% body weight) until the brood stock reached an age of one year. Sexual maturity of fish was determined by visual examination of the gonad *i.e.* whether or not the

fish oozed milt or hand ripe eggs during the spawning period (May – Jul.) (Oct. – Dec.). One day before each experiment, required fishes were transferred to cement tank in female to male ratio of 1:2.

The substratum has mixed gravel bed with vegetations in tanks and hapa to induce breeding. In addition, spawning substrates like, slabs of black plexiglas, section of PVC pipe, cobbles, and vegetations were provided as cover objects. Three types of natural and synthetic hormones (pituitary extract, human chorionic gonadotropin, ovaprim) were used for induce spawning. For hypophysation, acetone dried carp pituitaries were obtained from a commercial fish farm, Dindugal, Tamil Nadu. Ovaprim was purchased from Glaxo India Limited, Chennai and HCG are obtained from Agarivet farm Care Glaxo India Ltd., Mumbai. Pituitary extracts were injected in two instalments for females with an interval of 6 hr between the injections and one dose for males whereas the other two hormones (HCG and ovaprim) were injected in a single dose to both the sexes (Table 1). For purposes of comparison, control fishes were given corresponding amounts of distilled water (Plate b).

Injections were administered intramuscularly in the dorso-lateral region of the body. The injections were given in the late afternoon or early evening (15-18 hr). Early evening was found to be better as cent percent response was shown. In each hormone treatment, three doses were used to find out the response of fish and to observe the variation in latency period, egg number, fertilization percentage, latency period (hr), number of egg, egg diameter (mm), fertilization rate (%), Incubation period (hr), hatching percentage, survival at first feeding, survival at fry stage and fingerlings stage (%) (Table 3.1-3.3). Recognizing the dosage of Fermin (1992a) as the middle dose, a lower dose and a higher dose were fixed for the present experiment to induce *N. tamiraparaniensis.* Eggs were placed and incubated in plastic trays through which water circulated. Hundred eggs were randomly sampled an hour after incubation and they were observed under the microscope to assess the rate of fertilization. Fertilization rate was estimated from the ratio of eggs sampled. Triplicates were maintained for each of the dose for all the hormones used in the experiments.

Experimental design

The breeding design is shown in Table 1. The hapa for *N. tamiraparaniensis was* made out of fine meshed marlin cloth which allows flowing of water through it. The width lid net hapa was fixed at the back pools along the length of the river and small smooth river pebbles (size 24 cm in diameter) and gravels were spread on bottom of the hapa (2 m x 1 m x 0.75 m). Aquatic macrophytes like *Hydrilla verticiellata, Eichhornia crassipes* and fibre mesh were introduced into the hapa (1 m x 0.5 m x 0.25 m).

Breeding

Immediately after administering the hormone injections, the breeding pairs were transferred to the breeding hapa/tank for spawning (Plate 1). Sex ratios and substrates mentioned in experimental design were tabulated (Table 1). Courtship behaviour like pair swimming smoothly, chasing of females by the male and jumping little up the surface of the water were observed before spawning. As per expectation hormone induced breeders spawned in the next day morning within 11.00-13.00 hr after injection. In the case of breeders injected with low dosage spawned 36.00-38.00 hr after injection. The control fishes (distilled water) did not spawn any egg up to 3 days. The fishes were allowed to spawn completely about 4-6 hr after breeding started and were removed and records were maintained for the weight of individual females and the total weight of the eggs released. After spawning the breeders were transferred to the small tank for further observation. The mean range of water quality parameters during the study period were as follows: water temperature 27-29°C; dissolved oxygen 6.6-7.5 mg/l; pH 7.1-7.3 and salinity 1.01-1.03%.

Immediately after the breeding is over, the spawners were given deep bath in 1% saline solution for 15-20 min, followed by a deep bath in freshwater, with a mild current if possible, for about ½ an hour. Fishes were then maintained separately sex wise in two small ponds, which were free from any other feeding with powdered rice bran and oil cake (1 :1 ratio). Then they were released back in to the experimental ponds with other stock of fishes under scheduled brood fish management programme (Chondar, 1994). Males, if they are good in health and conditions, once attaining full maturity were utilized for subsequent

breeding after 10-15 days of fine rearing only one.

Egg counting and Hatchery operation

The submerged eggs were collected from the gravel floor of the breeding hapa by waving the water after removing the artificial substratum and transferred directly to the glass hatchery unit for *N. tamiraparaniensis*. Dull, unfertilized dead eggs were separated from transparent, living ones. The percentage of live embryos was calculated for the first time within 24hr of the fertilization of the eggs. A sample of eggs was taken using wide mouth pipette from the central part of the hatchery unit and 100 of these were placed on a petridish. The developing embryos were counted under a magnifying glass (×2). For the first 24 hr water flow was maintained at 0.6 l/min to keep the eggs rotating inside the hatchery jar (Vijayakumar, 1998). After 8 hr water flow was increased to 1.5 l/min to facilitate the removal of undeveloped embryos and to increase oxygen supply to the developing embryos. The undeveloped (dead) embryos came out through the outlet with the water flow. The hatchability was determined as the percentage of normal larvae from the total number of live eggs in each sample. The hatched larvae propelled themselves to the surface of the water and were carried into the plastic trough of outer hatchery jar.

Like the ordinary hatching hapa it has also got two rectangular cloth troughs, an outer fine-meshed muslin hapa of size 2 m × 1 m × 1 m and an inner round meshed mosquito net hapa of size 1.75 m × 0.75 m × 0.5 m, both with their upper ends open were used (Plate c). All hatched larvae were collected and transferred to separate fibreglass tanks of 1500 l capacity (tank with water flowing) covered with thick mesh to minimize light and by which time yolk absorption had taken place. Larvae were fed with rotifers, *Brachionus* spp. (days 3-15) and chopped Chironomus. (days 16-25). Larval development was monitored until 35th day when all larvae were transferred to maneuvered cement-culturing tank (12 m × 6 m × 1.5 m size). In the laboratory, monitoring fish larvae were fed with live and frozen Chironomus foods, including earthworm, plankton and formulated feed. In tank culturing, additionally the young were fed two to three times a day initially at the rate of 5% of body weight. When they were able to eat whole worms, they were fed once daily. Survival from fry to fingerlings was recorded when cultured in laboratory conditions.

Young fish (Two to three month of Age)

After two months, the young fish were transferred to large pre-growing ponds (10 m × 7 m × 1.5 m) for another month when the young fishes were fed with a mixture of cooked egg yolk and proteinaceous flour (250 µ) at a daily rate of 7.5% of the biomass in the second month and 5% in the third month (Plate d-h). Feeding was carried out four times a daily. 1/3rd water was exchanged from the pond every fortnight.

Table 1. Experimental design regarding hormones used to induce breeding

Treatment	Hormone	Dosage used in the present study (% kg of fish)
1	Pituitary extract	Low dose – 1st injection 5 mg 2nd injection 10 mg
2		Medium dose– 1st injection 5 mg 2nd injection 10 mg
3		High dose – 1st injection 5 mg 2nd injection 10 mg
4	HCG	Low dose – 500 IU
5		Medium dose – 1000 IU
6		High dose – 1500 IU
7	Ovaprim	Low dose – 0.3 ml
8		Medium dose – 0.5 ml
9		High dose – 0.7 ml

HCG- Human Chorionic Gonadotropin hormone

Table 2. Induced spawning of *Neolissochilus tamiraparaniensis* using different hormones

Hormones	Mean female wt (g)	Mean male wt (g)	Dosage of hormone/kg	Spawning complete/partial	Remarks on hatching
Pituitary Extract (1st + 2nd injection) (Low)	520.00	390.00	5 + 10 mg	Partial	**
Pituitary Extract (1st + 2nd injection) (Medium)	553.33	416.67	5 + 20 mg	Complete	**
Pituitary Extract (1st + 2nd injection) (High)	503.33	419.16	5 + 30 mg	Complete	**
HCG (Low)	500.00	306.67	500 IU	Partial	**
HCG (Medium)	533.33	397.5	1000 IU	Complete	**
HCG (High)	506.66	374.16	1500 IU	Complete	**
Ovaprim (Low)	490.00	383.33	0.3 ml	Partial	**
Ovaprim (Medium)	520.00	426.16	0.5 ml	Complete	**
Ovaprim (High)	528.33	405.5	0.7 ml	Complete	**
Control (Saline)	507.00	410.33	0.4 ml	*	

*: No response **: Rate of hatching at 60% or above was regarded as normal

RESULTS

In the present study, three hormones (pituitary, HCG, ovaprim) were used for induced spawning of *N. tamiraparaniensis*. The results of induced spawning using pituitary extract were given in Table 1. In the higher dosages (5mg + 30 mg/kg), the latency period was 12.72 hr and the percent fertilization was slightly reduced than in lower dosages (52.33%) (Table 3.1). Statistical observation on latency period was significant ($p < 0.01$). However, increased fertilization rate and highest percent hatching (86.67 %) were observed in the middle doses followed by low and high dosages. Changes were observed in the diameter of ova for three samples (table 3.1). Changes in the diameter of ova were larger in higher dosage of pituitary (2.49 mm) and ovaprim (2.48 mm) injected fishes. The relationship between pituitary and HCG with egg diameter was statistically significant ($D < 0.05$) however, results were not significant between egg diameter and ovaprim (Table 3.1). Incubation period showed a significant difference ($P < 0.05$) among the three dosages. When compared with the effect of other hormones (HCG and ovaprim) used in the present study, high latency period (37.12 hr) and low fertilization 73% were observed in pituitary injected fish.

Table 3. 1. Summary of statistical data on Induced spawning of *Neolissochilus tamiraparaniensis* using various hormones (Mean ± SD values)

Hormone dosage	Latency period (hr)					Number of Egg					Egg diameter (mm)				
	Pituitary	HCG	Ovaprim	F	D	Pituitary	HCG	Ovaprim	F	D	Pituitary	HCG	Ovaprim	F	D
Low	37.12 ±0.99	28.30 ±0.13	23.96 ±0.99	200.91*	3.39	1165.67 ±47.89	1106.67 ±219.67	2083.00 ±55.51	50.29*	226.64	2.14 ±0.08	1.23 ±0.08	2.14 ±0.09	118.23*	0.14
Medium	28.09 ±0.04	23.73 ±0.32	12.72 ±0.29	1024.57*	0.42	3284.67 ±226.05	2955.33 ±38.55	3076.67 ±51.96	4.54[NS]	229.63	2.45 ±0.02	2.27 ±0.03	2.43 ±0.00	95.69*	0.03
High	12.72 ±0.32	22.18 ±0.03	12.15 ±0.03	1676.91*	0.32	3603.67 ±39.68	3278.67 ±152.96	3322.67 ±257.39	8.07[NS]	295.59	2.49 ±0.02	2.31 ±0.00	2.48 ±0.00	252.27*	0.02
F	1253.72*	778.80*	371.47*			287.48*	168.99*	53.82*			46.52*	488.67*	34.62*		
D	1.02	0.33	1.01			229.46	264.67	262.67			0.08	0.08	0.09		

Mean ± SD; *p < 0.01; **p < 0.05; NS - p > 0.05; D < 0.05

Table 3. 2. Summary of statistical data on Induced spawning of *Neolissochilus tamiraparaniensis* using various hormones (Mean ± SD values)

Hormone dosage	Fertilization rate (%)					Incubation period (hr)					Hatching percentage				
	Pituitary	HCG	Ovaprim	F	D	Pituitary	HCG	Ovaprim	F	D	Pituitary	HCG	Ovaprim	F	D
Low	54.67 ±4.04	63.67 ±3.79	65.67 ±2.52	8.35**	5.95	46.89 ±1.41	48.09 ±2.69	43.12 ±1.04	5.90**	3.14	67.67 ±3.06	61.67 ±1.53	75.00 ±2.00	25.62*	3.87
Medium	73.00 ±5.29	77.67 ±2.31	76.00 ±2.65	1.25**	6.22	44.33 ±0.02	42.16 ±0.03	43.15 ±0.02	6759.92 *	0.04	86.67 ±4.51	74.00 ±1.00	79.00 ±2.00	14.46*	4.93
High	52.33 ±9.02	74.33 ±4.16	76.33 ±2.08	15.49*	9.93	44.25 ±0.02	42.11 ±0.03	43.20 ±0.20	241.34*	0.20	73.33 ±1.53	63.33 ±3.51	83.00 ±2.65	80.17*	4.56
F	9.18**	13.01*	18.74*			10.19**	14.75*	0.02**			26.76*	25.72*	9.6**		
D	10.97	5.95	4.11			1.37	2.63	1.04			5.54	3.87	3.79		

Mean ± SD; *p < 0.01; **p < 0.05; NS - p > 0.05; D < 0.05

Table 3. 3. Summary of statistical data on Induced spawning of *Neolissochilus tamiraparaniensis* using various hormones (Mean ± SD values)

Hormone dosage	Survival at first feeding (%)					Survival at fry stage (%)					Survival at fingerlings stage (%)				
	Pituitary	HCG	Ovaprim	F	D	Pituitary	HCG	Ovaprim	F	D	Pituitary	HCG	Ovaprim	F	D
Low	61.33 ±9.07	54.67 ±2.08	62.00 ± 1.00	3.06**	9.16	58.00 ± 1.73	53.00 ± 3.00	72.67 ±3.79	35.71**	5.02	63.33 ±4.16	55.33 ±1.53	86.33 ±2.31	11.64*	4.89
Medium	74.33 ±3.06	65.67 ±4.16	67.67 ±2.08	5.98**	5.45	68.00 ±3.61	67.33 ±3.51	76.00 ± 2.00	33.05**	5.30	84.67 ±3.06	81.00 ± 1.00	74.67 ±4.04	47.64*	5.05
High	64.67 ±3.79	71.67 ±2.08	73.00 ± 1.73	8.32**	4.56	75.00 ±3.00	58.33 ±3.06	76.00 ± 1.73	41.55*	4.52	83.33 ±4.16	64.00 ± 2.65	76.00 ± 2.00	74.02*	5.21
F	11.32*	25.73*	32.68*			61.48*	15.40*	1.56**			29.21*	12.03*	9.62**		
D	10.08	4.99	2.83			4.99	5.42	4.52			6.49	3.15	4.96		

Mean ± SD; *p < 0.01; **p < 0.05; NS - p > 0.05; D < 0.05

Results of induced spawning in *N. tamiraparaniensis* by administering HCG were presented in Table 3.1. In the higher dosage (1500 IU/kg) injected fishes spawning was 3278.67 for *N. tamiraparaniensis* and 74.33% of fertilization was obtained. In the medium and high dosages there was statistical difference in the latency period (P<0.01) (Table 3.1) and no statistical difference in the fertilization (medium vs. high) rate was obtained. Incubation period was almost equal for medium Vs high doses confirmed by Q test and the results were statistically significant (P<0.01). In the present study, the increased egg diameter (2.31 mm) was observed in higher dose. The rate of hatching in the fish injected with different doses was significant (P<0.01).

The ovulation of latency period was minimum (12.15 hr) in high dose. The maximum egg number and fertilization rate (65-76 %) were achieved in ovaprim injected fishes. Egg diameter was statistically (P<0.01) significant among the three doses (Table 3), but incubation period and survival at hatching rate showed (P<0.05) significant difference among the three doses (Table 3). Survival at first feeding was high (73%) with high dose of ovaprim. Standard analysis showed that the survival at first breeding of fish injected with ovaprim differed significantly (P<0.01) with other hormones (Table 3.3). The survival of the fingerlings was normal in the entire dose administered. The hatching percentage was highest (83%) in fishes treated with high dose ovaprim. AVOVA showed that there was a significant difference between treatment (hormone wise) and difference between any two treatments (dosage wise) was performed by Q test (Table 3.3).

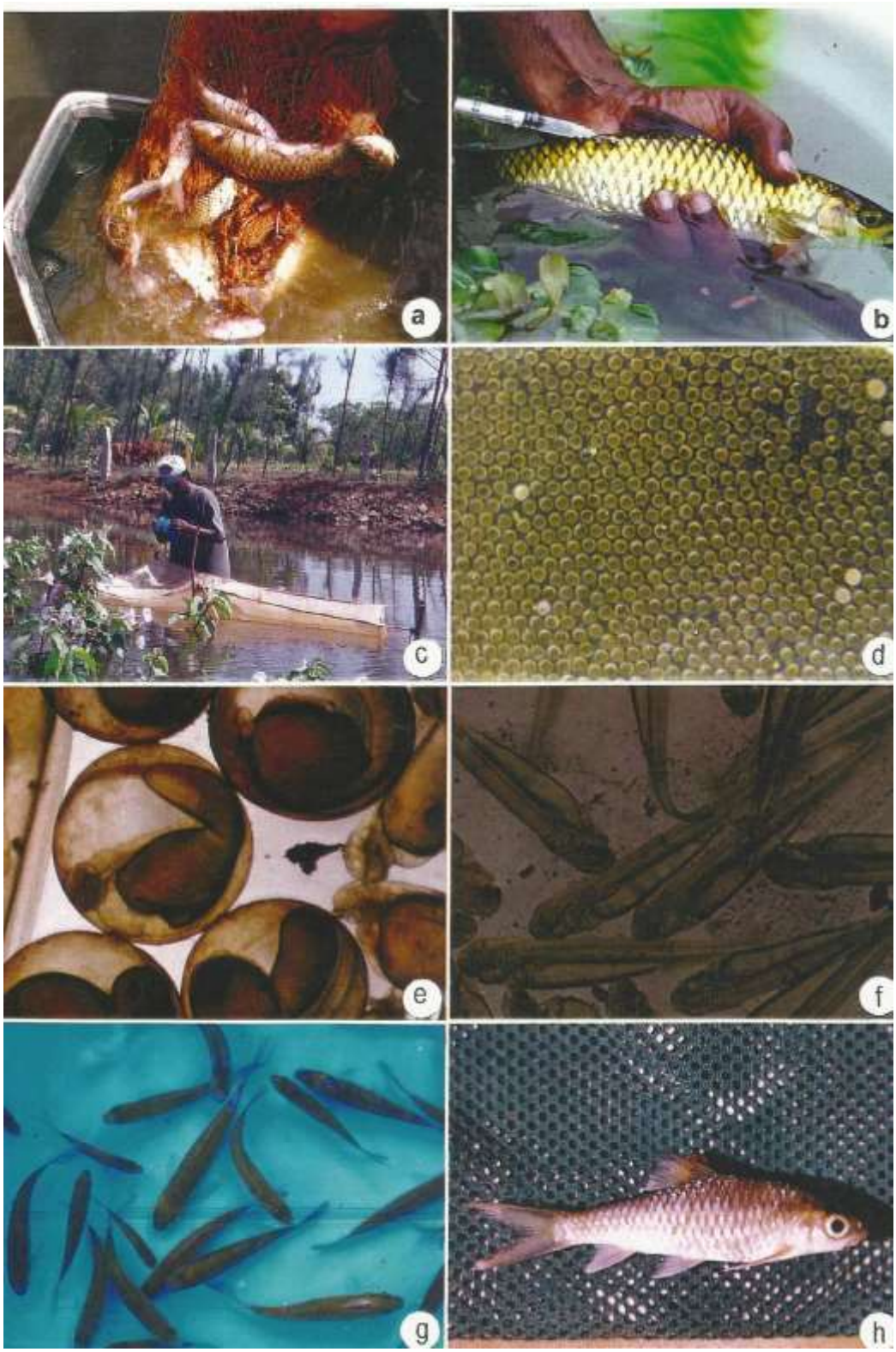

Plate 1: a - Collection of Brood Fishes; b - Administering intra-muscular injection; c - Experimental hapa; d - Collection of egg; e - Developed embryo just before hatching (enlarged view); f - Collection of hatchlings; g - Collection of two month old fingerlings; h - Three month old Cultured fish.

DISCUSSION

Captive breeding and reintroduction-techniques are hence increasingly popular in the recovery of endangered species in different countries (Tear *et. al* 1993). The latency period is directly proportional to the dosage of hormone. Greater the hormone injected, lesser in the latency period. In the study on *N. tamiraparaniensis* latency period ranged from 37 hr is in low dose. Similar results are also observed with the experiments conducted with carp in the local hatchery by Brzuska and Adamak (1997) in which the time between pituitary hormone application and ovulation was 16-20 hr. In the present study, it has been observed that the time taken for ovulation in dosage dependent for *N. tamiraparaniensis*, *Ompak bimaculatus* and *O. malabaricus* (Vijayakumar 2010). In the present data it seems that injection of HCG in *N. tamiraparaniensis* shows not much variation in the latency period. Higher latency period of 22 hr has been observed in the fish, injected with HCG at a range of water temperature 22-31ºC. Similar results have also reported for grey mullet *Mugil liza* (Alvarez- Lajonchere et.al., 1988). Higher latency period of 18 hr has been observed in *Ompak. pabda*, injected with carp pituitary extract on various factors such as temperature, pH, and hardness. Hatching percentage with carp pituitary extract was significantly inferior to ovaprim induction (Nihar Ranjan Chattopadhyay, 2018). After the injection of ovaprim, spawning commenced within 12.72 hr in *N. tamiraparaniensis* in medium dose treatment and it is equivalent to higher doses of pituitary compared to all other hormones in the present study, ovaprim induced fish latency period found to be short responses in *N. tamiraparaniensis.*

The ovaprim has been found effective in inducing ovulation in *O. bimaculatus* (Sridhar *et.al*., 1998) and in *O. malabaricus* (Vijayakumar, 2002). Fertilization rates were found to be higher in ovaprim treated fishes (82.48%) compared to that of pituitary hormone and HCG treated fishes (Md. Mosaddequr Rahman *et.al*., 2013). In the present study, doses between 0.3 0.5 ml/kg body weight of ovaprim treated fish has a egg diameter of 1.23 mm. In *N. tamiraparaniensis*, the pituitary (medium dose) treated group shows the best mean value (74%) than other two doses (low and high) of hormones. It may be a suitable dose for *N. tamiraparaniensis*. From this it is clear that survivability has been enhanced not only by environmental factors but also by hormonal action. The survival range at first feeding and fry in fish with ovaprim (higher dose) treated group is found be a higher than pituitary, but in fry and fingerlings stage pituitary shows the higher performance (above 83%) than other hormones. From this finding it is suggested that pituitary extract may be suitable for *N. tamiraparaniensis*.

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