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Effect of different broodstock diets on breeding performance of the critically endangered peninsular carp *Hypselobarbus pulchellus* (Day, 1870)

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Abstract

Effect of different broodstock diets on breeding performance of the critically endangered peninsular carp *Hypselobarbus pulchellus* (Day, 1870) was evaluated. Male and female fish were reared in earthen ponds and fed one of the following diets *viz.* (i) Control-Fishmeal based feed (crude protein, CP 35%); (ii) Control feed supplemented at 2% with a commercial product used for lipid regulation in poultry (LC feed) and (iii) Specially formulated diet (CP 35%) with horse gram and black gram in addition to fishmeal (PB feed). Breeding trials were first conducted in 2019. No significant difference in fecundity was recorded among fish from different groups. The experiment was repeated in 2020, where, the Control feed was fed to fish maintained in a pond with aquatic weed *Vallisneria spiralis* (C+V treatment). Highest relative fecundity and spawn to fry survival was obtained with C+V treatment, there being no significant difference among other breeding performance parameters between treatments. The study indicated that the presence of submerged aquatic plants in the culture environment improves the breeding performance of *H. pulchellus*.

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Introduction

Hypselobarbus pulchellus (Day, 1870), endemic to the peninsular rivers of India, mainly Krishna, Godavari, Tungabhadra, Sita and Tunga and once a major fishery of Tungabhadra Reservoir, has presently declined to the status of a 'Critically Endangered' fish species (Devi and Ali, 2011). *H. pulchellus* is a bentho-pelagic species which inhabits the deeper parts of large streams and rivers along the base of Ghats. Under riverine conditions this fish is reported to attain a maximum size of 780 mm in length and up to 8 kg in weight (David and Rahman, 1975). *H. pulchellus* is considered to be the only indigenous fish consuming aquatic weeds and submerged grasses (David and Rahman,1975; 1982) and could be used in controlling aquatic vegetation in reservoirs, tanks and irrigation canals. Though herbivorous, *H. pulchellus* is known to change its feeding habits depending up on the availability of food. This fish, which is capable of attaining 8 kg (David and Rahman, 1975), could become a welcome addition to pond culture practices of India, especially for composite fish culture. The need for diversification of farmed fish species has resulted in renewed interest in the breeding, propagation and culture of *H. pulchellus* in recent years. Barlaya *et al.* (2021) emphasised the importance of this species in the context of diversification as well as for alleviating its critically endangered status. Sridhar *et al.* (2014) were successful in induced breeding of this species for the first time. In natural waters, breeding of *H. pulchellus* is reported to commence soon after monsoon from September which continues until April with a peak in September and January (Gangadhar *et al.*, 2020).

Nutrition is known to have a profound effect on the reproductive performance of fish (Gunasekera *et al.*, 1996; Mokoginata *et al.*, 1998). Reduced fecundity can be caused by the influence of a nutrient imbalance on the brain-pituitary-gonad axis of the endocrine system or by the restriction in the availability of a biochemical component for egg formation (Izquierdo *et al.*, 2001). Several studies have confirmed that reproductive performance, egg quality, production of hatchlings and their viability are influenced by nutrients (Watanabe and Kiron, 1995; Christiansen and Torrissen, 1997; Gunasekera and Lam, 1997; Choubert *et al.*, 1998; Mokoginata *et al.*, 1998; Siddiqui *et al.*, 1998; Manissery *et al.*, 2001; Keshavanath *et al.*, 2006). It is opined that diets for broodstock should be tailor-made to ensure good egg quality as different fish species have different dietary requirements (Brooks *et al.*, 1997; Izquierdo *et al.*, 2001). This is particularly true for batch spawners like *H. pulchellus*, which are known to enter a starvation period after gonadal development and rely on bodily stores for the massive quantity of energy and specific nutrients for ovarian maturation and spawning (Vijayagopal, 2016). Therefore, it is essential to provide specialised nutrition to the broodfish for build-up of bodily reserves. Earlier studies (Sridhar, 2017) reported on visceral fat deposition and its negative effect on breeding performance in *H. pulchellus* maintained in a confined pond environment and fed on formulated diets. It is hypothesised that commercially available lipid regulators may be beneficial in dissipating the fat deposits.

No information is available on suitable broodstock diet for *H. pulchellus*. Hence the present study was conducted to evaluate the effect of different dietary treatments on the spawning performance of *H. pulchellus*.

Materials and methods

Broodstock rearing

The study was conducted over a period of two years during 2019 and 2020, the duration of the experiments being 6 months each year. Earthen ponds of 100 m^2 were initially manured with cow dung @ 4 t ha^{-1}, 10 days prior to stocking of brood fish. Ten numbers each of male and female fish of 1.5-2.0 years of age (F1 generation, weight ranging from 800-1200 g) were stocked at 1:1 ratio in the 1st week of June 2019. A water depth ranging from 1.3-1.5 m was maintained throughout the rearing period. Fish in duplicate tanks were fed one of the three experimental diets in trays at 3% of body weight, once daily in the morning hours.

Experimental diets

The experimental diets used were: (i) Fishmeal based control feed (Control) (crude protein 35%), (ii) Control feed supplemented at 2% level with a commercial product commonly used for lipid regulation in poultry industry having a combination of lecithin, emulsifiers and lipase promoting factors (LC feed, 35% crude protein) and (iii) Formulated pulses based diet incorporating horse gram and black gram in addition to fishmeal (PB feed, 34.39% crude protein). The experiment was repeated in 2020, with an additional treatment, where, the Control feed was fed to fish maintained in ponds having the aquatic plant *Vallisneria spiralis* (C+V treatment). None of the other experimental ponds had any aquatic plants, including *V. spiralis*. Ten numbers each of male and female fish of 1.5-2.0 years of age (F1 generation, weight ranging from 900-1500 g) were stocked at 1:1 ratio in the 1st week of June 2020. Other experimental set-up and study parameters were the same as that of the first year trials. Nonetheless, a few additional parameters like hatching rate (percentage of hatching), spawn to fry survival and fry recovery from total eggs (%) were also recorded during this study.

The ingredient proportion of the formulated diets is given in Table 1. Different broodstock diets were prepared as per Barlaya *et al.* (2021). All the ingredients were sieved through a fine meshed screen (0.5 mm). The required quantity of the ingredients except vitamin-mineral mixture and lipid regulator were mixed with hot water to make a dough. The dough was allowed to cool, vitamin-mineral mixture (also commercial lipid regulator in the case of LC feed) added, mixed thoroughly and then pressed through a hand pelletiser to get uniform sized pellets (3 mm). The pellets were sun-dried and packed in air-tight bags.

Table 1. Ingredient proportion (%) of formulated diets

Ingredient	Control	LC feed	PB feed
Fish meal	22	22	15
Groundnut cake	30	30	18
Rice bran	38	36	18
Ragi (Finger millet)	8	8	-
Horse gram	-	-	13.5
Black gram	-	-	13.5
Rice flour	-	-	10.5
Maize flour	-	-	9.5
Vitamin and mineral mixture[1]	2	2	2
Lipid regulator[2]	-	2	-

[1]Each kg contains: Calcium-25.5%, Phosphorus-12.75%, Magnesium-6000 mg, Sulphur-0.72%, Sodium-5.9 mg, Potassium-100 mg, Copper-1200 mg, Cobalt-150 mg, Zinc-9600 mg, Iron-1500 mg, Iodine-325 mg, Selenium-10 mg, Manganese-1500 mg, Vitamin A-7,00,000 IU, Vitamin D3-70,000 IU, Vitamin E-250 mg, Nicotinamide-1000 mg, DL-methionine-1929 mg, L-lysine-4400 mg, *Lactobacillus* sp. 1.5 × 10^{11} CFU, *Saccharomyces cerevisiae*-30,000 million CFU

[2]Each kg contains: Emulsifiers-50000 mg, Lecithin-100000 mg, Moisture-1%, Sterols-3%, Free carbohydrates-5%, Other phosphatides-5.5%, Phosphatidylethanolamine-14%, Phosphatidyl choline-20%, Inositol phosphatides-20.5% and Soybean oil-34%.

Analyses of experimental diets and water quality

Analysis of the feed for proximate composition was performed in triplicate for each feed sample as per the method of AOAC (1995) and Barlaya *et al.* (2021). Thermo gravimetric method of drying the finely ground sample at 100°C in an oven (PSM Scientific Instruments, India) till constant weight was performed to estimate the moisture content. Ash content was determined by incinerating the sample in a muffle furnace (PSM Scientific Instruments, India) at 600°C for 6 h. Nitrogen content was estimated by micro-Kjeldahl method and multiplied with the factor 6.25 to calculate the crude protein content. Fat was determined by solvent extraction with petroleum ether (boiling point 40-60°C). Crude fibre in the feed was estimated by acid digestion (1.25%), followed by alkali digestion (1.25%). Carbohydrate content was calculated as nitrogen free extract (NFE) by the difference method of Hastings (1976). The energy content of the feed was calculated using the values of 22.6 kJ g^{-1} for protein, 38.9 kJ g^{-1} for lipid and 17.2 kJg^{-1} for carbohydrate as NFE (Mayes, 1990). Water quality parameters of ponds (APHA, 2005) and general fish conditions were monitored on monthly and fortnightly basis, respectively. The biomass of fish recorded during fortnightly sampling was used for calculating the feed quantity.

Breeding trials

A series of breeding trials were conducted between the last week of September and first week of November, selecting mature fish from different treatment ponds. The fish were successfully bred using a commercial hormonal formulation consisting of salmon gonadotropin releasing hormone analogue and Domperidone administered @ 0.5 ml kg^{-1} body weight, twice to females and males on consecutive days. The injected brooders were released into the breeding pool of a Chinese hatchery and were allowed to remain there with circulating water and over-head shower running throughout. After 20 h of the second hormone administration, the fish was anaesthetised for stripping by submersing in 25 ppm solution of clove oil. Dry method of stripping was followed. Immediately after fertilisation, the eggs were transferred to the egg holding trays of a specially designed hatchery (Gangadhar *et al*., 2020). The hatchlings emerged between 48-72 h at 22-24˚C water temperature. When complete yolk absorption took place by 4-5 days post-hatching, the larvae were shifted to glass aquaria maintained at 26˚C with constant aeration, @1000 nos. m^{-3} of water volume and were fed filtered zooplankton for a period of 5 days, followed by a combination of filtered zooplankton and finely ground pelleted feed containing 35% crude protein (Table 1) as additional supplementary feed for another 10 days. The dead larvae, if any, along with unconsumed feed and faecal matter at the tank bottom were removed daily before feeding. Water removed from the tank during cleaning was replenished with freshwater. After rearing for 15 days in glass aquaria, the fry were shifted to soil based, manured rearing tanks for further rearing.

Relative fecundity was calculated based on the number of eggs obtained per kg body weight of female fish. Total number of eggs released, number of unfertilised and unhatched eggs, spawn that hatched out, but died and final number of fry obtained from each breeding were also estimated.

Statistical analysis

The proximate composition, water quality and breeding performance parameters evaluated were subjected to statistical analysis separately by one way ANOVA ($p<0.05$), followed by Duncan's multiple range test (Duncan, 1955).

Results and discussion

Proximate composition of different diets used is given in Table 2. All the three formulated diets were isonitrogenous and isocaloric. Results of water quality analysis conducted at monthly intervals showed no significant difference between the treatment ponds indicating negligible effect of different diets on water quality (Table 3 and 4). The water used for hatchery operations had a total alkalinity of 121.08±2.17 mg l^{-1}, hardness of 112±3.08 mg l^{-1} and pH of 8.09±0.74.

The number of fish bred successfully and the fecundity data from the breeding trials conducted during 2019 are given in Table 5. The number of successfully bred fish ranged from 8 in PB diet to 10 in LC treatment. The weight of successfully bred females ranged from 0.95 to 1.16 kg. Fecundity showed no difference ($p>0.05$) among fish fed the different diets.

The breeding performance indicators of the trials conducted during 2020 are given in Table 6. No difference ($p>0.05$) in the mean weight of females bred, hatching rate and fry recovery was noticed between the groups. However, relative fecundity was significantly lower ($p≤0.05$) in the group fed

Table 2. Proximate composition (mean ±SD; n=3) of experimental feeds

Proximate composition	Control	LC feed	PB Feed
Moisture	$5.87±0.09^{c}$	$4.04±0.05^{a}$	$4.72±0.06^{b}$
Crude protein	$35.23±0.71^{a}$	$35.48±0.25^{a}$	$34.39±0.56^{a}$
Fat	$11.24±0.40^{b}$	$11.27±0.50^{b}$	$6.42±0.49^{a}$
Ash	$8.56±0.03^{a}$	$9.42±0.07^{a}$	$9.87±0.25^{a}$
Crude fibre	$10.17±0.12^{b}$	$12.00±0.09^{c}$	$6.98±0.03^{a}$
NFE	28.89^{a}	27.80^{a}	37.61^{b}
Gross energy (Kj g^{-1})	17.30^{a}	17.18^{a}	16.73^{a}

Values bearing different superscripts in the same row differ significantly ($p<0.05$)

Table 3. Water quality parameters (mean, n=6) recorded in the culture ponds during 2019

Parameters	Control	LC feed	PB feed
Temperature (°C)	24.68±1.45[a]	24.72±1.45[a]	24.65±1.45[a]
pH	8.55±0.19[a]	8.32±0.24[a]	8.32±0.30[a]
Dissolved oxygen (mg l^{-1})	7.20±2.01[a]	7.13±2.34[a]	6.53±2.63[a]
Total alkalinity (mg l^{-1})	308.47±72.11[a]	273.88±74.96[a]	304.33± 55.76[a]
Hardness (mg l^{-1})	172.67±35.36[a]	206.33±23.56[a]	187.33±21.82[a]
Ammonia (µg l^{-1})	83.86±15.06[a]	62.76±19.47[a]	54.62±23.39[a]
Nitrite (µg l^{-1})	381.47±122.43[a]	364.31±270.10[a]	301.52±209.83[a]
Nitrate (µg l^{-1})	906.80±306.42[a]	1563.72±782.80[a]	1191.18±545.92[a]
Phosphate (µg l^{-1})	54.50±12.03[a]	59.45±21.58[a]	44.87±22.37[a]

Values bearing same superscripts in the same row do not differ significantly (p>0.05)

Table 4. Water quality parameters (mean, n=6) recorded in the culture ponds during 2020

Parameters	Control	C+V	LC feed	PB feed
Temperature (°C)	25.2 ± 1.01[a]	25.08 ± 1.01[a]	25.18 ± 0.99[a]	25.21 ± 0.92[a]
pH	8.53 ± 0.50[a]	8.54 ± 0.43[a]	8.43 ± 0.33[a]	8.33 ± 0.24[a]
Dissolved oxygen (mg l^{-1})	5.20 ± 2.51[a]	5.70 ± 2.43[a]	6.97 ± 2.41[a]	6.85 ± 3.51[a]
Total alkalinity (mg l^{-1})	238.72 ± 55.63[a]	269.39 ± 66.97[a]	281.77 ± 58.98[a]	284.92 ± 63.19[a]
Hardness (mg l^{-1})	201.00 ± 34.05[a]	216.53 ± 33.35[a]	207.14 ± 31.13[a]	209.71 ± 26.21[a]
Ammonia (µg l^{-1})	76.55 ± 34.0[a]	82.77 ± 10.04[a]	48.68 ± 14.77[a]	53.33 ± 25.76[a]
Nitrite (µg l^{-1})	327.73 ± 101.62[a]	281.99 ± 91.05[a]	279.05 ± 109.08[a]	256.91 ± 88.54[a]
Nitrate (µg l^{-1})	1006.43 ± 522.64[a]	1073.52 ± 586.59[a]	919.12 ± 413.73[a]	954.83 ± 366.65[a]
Phosphate (µg l^{-1})	172.13 ± 103.35[a]	155.29 ± 88.90[a]	139.53 ± 82.20[a]	122.21 ± 42.08[a]

Values bearing same superscripts in the same row do not differ significantly (p>0.05)

Table 5. Breeding performance of *H. pulchellus* fed different broodstock diets during 2019

Treatment	No. of females successfully bred	Wt. of female (kg, mean±SD)	Relative fecundity (No. of eggs kg $female^{-1}$)
Control	9	1.16±0.13[a]	3689±1626[a]
LC feed	10	0.95±0.18[a]	3541±1754[a]
PB feed	8	1.08±0.11[a]	3345±1847[a]

Figures in the same column with same superscript do not differ significantly (p> 0.05).

Table 6. Breeding performance of *H. pulchellus* fed different broodstock diets during 2020

Treatment	No. of females successfully bred	Wt. of female (kg, mean±SD)	Relative fecundity (No. of eggs kg $female^{-1}$)	Hatching rate (%)	Spawn to fry survival (%)	Fry recovery from total eggs (%)
Control	9	1.16±0.19[a]	5351.59±919.09[ab]	66.65±15.89[a]	73.96±8.72[ab]	43.61±10.73[a]
C+V	8	1.18±0.20[a]	6950.60±2879.65[b]	64.47±11.21[a]	84.81±6.18[b]	46.68±9.91[a]
LC feed	9	1.35±0.21[a]	6370.36±1951.77[ab]	67.69±11.40[a]	69.10±7.13[a]	39.26±4.73[a]
PB feed	6	1.36±0.14[a]	3251.72±943.65[a]	52.36±9.71[a]	76.86±1.24[ab]	40.32±8.12[a]

Figures in the same column with same superscript do not differ significantly (p>0.05)

PB diet, compared to C + V treatment which showed the highest relative fecundity and spawn to fry survival.

H. pulchellus is reported to spawn four times a year in reservoir, tanks and rivers (Sridhar *et al.*, 2014). Hence, the number of ova released at each spawning (relative fecundity) is less in *H. pulchellus* compared to other carps, which breed once a year. Sridhar *et al.* (2014) recorded an absolute fecundity (calculated based on samples obtained from the ovaries) ranging from 19,284 to 41,970 eggs kg^{-1} in wild collected specimens of this species. However, the relative fecundity recorded in pond raised *H. pulchellus* in the present study was lower than the values reported by Sridhar *et al.* (2014). The difference in fecundity values obtained between these two studies for *H. pulchellus* is attributable to the fact that Sridhar *et al.* (2014) estimated absolute fecundity which includes the total number of oocytes including mature and immature ones extrapolated to total weight of the ovary, as compared to relative fecundity which is based on the count of released eggs (mostly mature) calculated per kg of female. Lower relative fecundity in pond-raised Deccan mahseer (*Tor khudree* Sykes, 1839), as compared to wild specimens was also reported by Kulkarni (2000) and Keshavanath *et al.* (2006).

The values of reproductive performance indicators like relative fecundity, spawn to fry survival and fry recovery were higher with the fish reared in ponds with aquatic plants compared to those reared in the ponds without plants and provided with the same feed. *H. pulchellus* is considered to be the only indigenous fish consuming aquatic weeds and submerged grasses both in the juvenile and adult stages (David and Rahman, 1975; 1982). Due to its reported consumption of aquatic weeds like *Chara, Hydrilla, Vallisneria*, grass, water hyacinth (*Eichhornia* sp.) roots and filamentous algae, this fish is called indigenous grass carp (Hora, 1955; Hickling, 1962). Accordingly, it is presumed that under natural conditions this fish feeds mainly on aquatic vegetation, attains sexual maturity and breeds. By simulating its natural habitat through the provision of *V. spiralis* in the ponds, the fish could most probably get the additional nutrients required for its gonadal development and better breeding performance leading to higher relative fecundity, spawn to fry survival and fry recovery observed in this experimental group.

Among different diets, the Control and LC feed were similar in major nutrient content except for the supplement in the latter. The commercial supplement used in LC feed was a combination of lecithin, emulsifiers and lipase promoting factors (LPF). While lecithin helps in emulsification and digestion of fats, LPFs activate the lipase enzyme system and help in hydrolysis of triglycerides into glycerol and fatty acids for optimal utilisation in the tissues. Emulsifiers help to break the fat particles for better digestion and utilisation. We used this supplement in LC feed in view of the earlier report by Sridhar (2017) on visceral fat deposition and its negative effect on breeding performance in *H. pulchellus* fed formulated diets and maintained in a confined pond environment. Though a comparison of visceral fat in fishes from different groups was not undertaken in the present study, the almost similar breeding performance of *H. pulchellus* in all the groups indicated no effect of the supplement on the reproductive performance of the fish.

The PB feed with horse gram, black gram, rice flour, maize flour and 15% fishmeal performed almost equally as that of the Control diet with 22% fishmeal. Legume incorporated diets have been reported to produce better results with other carp species too. In an earlier study on catla (*Catla catla* Hamilton, 1822), Manissery *et al.* (2004) recorded higher fecundity, fertilisation and hatching rate with a formulated broodstock diet having ingredients similar to that used in the present study, as compared to an isonitrogenous diet containing only rice bran and groundnut cake. In another study, also by the same group (Krishnamurthy *et al.*, 1995) on common carp (*Cyprinus carpio* Linnaeus, 1758), hatching rate and egg weight were higher in legume incorporated diet fed group. They opined that feeding a diet containing protein from more than one ingredient source results in better performance than that from a single source. Studies by Chattopadhyay *et al*. (2011) in catla corroborated these findings.

The performance of *H. pulchellus* in terms of relative fecundity and hatching rate was marginally lower with the PB diet having 6.42% fat compared to the other two diets having 11.2% fat. Studies have shown that dietary lipid level can also influence the fecundity in fish (Duray *et al.*, 1994; Izquierdo *et al.*, 2001). Yeasmin *et al.* (2018) observed better breeding performance of common carp on a diet with 11% lipid compared to the one with 8% lipid.

Based on the above findings, it is reasonable to state that the presence of submerged aquatic plants in the culture environment improves the breeding performance of *H. pulchellus*. However, further studies on the effect of different levels of dietary protein and lipid on the reproductive indicators and breeding performance of *H. pulchellus* can give more insight into formulating the best broodstock diet for this species.

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References

AOAC 1990. *Official methods of analysis*, 16th edn. Association of Official Analytical Chemists, Washington DC, USA, pp. 32-42.

APHA 2005. *Standard methods for the examination of water and wastewater*. American Public Health Association, Washington DC, USA, 1268 p.

Barlaya, G., Narasimhan, S., Basumatary, P., Huchchappa, R. C., Kumar, A. and Kannur, H. 2021. Effect of stocking density on the growth and survival of the critically endangered peninsular carp *Hypselobarbus pulchellus* (Day,

1870) in fingerling rearing. *Aquac. Res*., 52: 2901-2906. https://doi.org/10.1111/are.15110

Brooks, S., Tyler, C. R. and Sumpter, J. P. 1997. Eggs quality in fish: What makes a good egg? *Rev. Fish Biol. Fish*., 7: 387-416. https://doi.org/10.1023/A:1018400130692

Chattopadhyay, D. N., Verma, J. P., Radheshyam, Behera, B., Sarkar, S. and Mukhopadyay, P. K. 2011. On-farm evaluation of formulated feeds on the breeding performance of *Catla catla* reared in rural ponds. *Aqua. Nutr*., 17: e30-e38.

Choubert, G., Blanc, J. M. and Poisson, H. 1998. Effects of dietary keto-carotenoids (canthaxanthin and astaxanthin) on the reproductive performance of female rainbow trout *Onchorhynchus mykiss* (Walbaum). *Aqua. Nutr*., 4: 249-254.

Christiansen, R. and Torrisen, O. J. 1997. Effects of dietary astaxanthin supplementation on fertilization and egg survival in Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 153(1-2): 51-62. https://doi.org/10.1016/S0044-8486(97)00016-1

David, A. and Rahman, M. F. 1975. Studies on some aspects of feeding and breeding of *Puntius pulchellus* (Day) and its utility in culturable waters. *J. Inland Fish. Soc. India,* 7: 225-38.

David, A. and Rahman, M. F. 1982. Experimental observations on the feeding of *Puntius pulchellus* (Day) and utility of the species possible eradication of aquatic plants. *Mysore J. Agric. Sci.*, 16: 85-95.

Devi, K. R. and Ali, A. 2011. *Hypselobarbus pulchellus*. *IUCN 2012. IUCN Red List of Threatened Species. Version 2020.1.* www.iucnredlist.org

Duncan, D. B. 1955. Multiple range and multiple F tests. *Biometrics*, 11(1): 1-42.

Duray, M., Kohno, H. and Pascual, F. 1994. The effect of lipid enriched broodstock diets on spawning and on egg and larval quality of hatchery-bred rabbit fish *Siganus guttatus*. *The Philippine Sci.,* 31: 42-57.

Gangadhar, B., Raghavendra, C. H., Ananda Kumar, B. S. and Hemaprasanth, K. 2020. Induced breeding of farm-bred and pond-raised critically endangered peninsular carp, *Hypselobarbus pulchellus*. *Aquac. Asia*, 24(4): 10-13.

Gunasekera, R. M. and Lam, T. J. 1997. Influence of dietary protein level on ovarian recrudescence in Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, 149: 57-69. https://doi.org/10.1016/S0044-8486(96)01441-X

Gunasekara, R. M., Shim, K. F. and Lam, T. J. 1996. Effect of dietary protein level on spawning performance and amino acid composition of eggs of Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, 146:121-134. https://doi.org/10.1016/S0044-8486(96)01365-8

Hickling, C. F. 1962. *Fish culture*. Faber and Faber, London, UK, 295 p.

Hora, S. L. 1955. *Handbook on fish culture in the Indo-Pacific region*, Food and Agriculture Organisation of the United Nations, Rome, Italy, 204 p.

Izquierdo, M. S., Ferna´ndez-Palacios, H. and Tacon, A. G. J. 2001. Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, 197(1-4): 25-42. https://doi.org/10.1016/S0044-8486(01)00581-6

Keshavanath, P., Gangadhar, B. and Basavaraj, N. 2006. Induced breeding of pond raised mahseer, *Tor khudree* using carp pituitary and ovaprim. *Asian Fish. Sci.,* 19: 411-422.

Kulkarni, C. V. 2000. Artificial propagation of *Tor khudree* (Sykes) and *Tor tor* (Ham.). In: Singh, H. R. and Lakra,W. S. (Eds.), *Coldwater aquaculture and fisheries*, Narendra Publishing House, New Delhi, India. pp. 203-218.

Krishnamurthy, D., Manissery, J. K., Gangadhara, B. and Nandeesha, M .C. 1995. Effect of different diets on the breeding performance of common carp. In: *Abstracts of National Workshop on Aquaculture Nutrition*, ICAR-Central Institute of Fisheries Education, Mumbai, India, 29-30 December, 1995, p. 15.

Manissery, J. K., Krishnamurthy, D., Gangadhara, B. and Nandeesha, M. C.2001. Effect of varied level of dietary protein on breeding performance of common carp, *Cyprinus carpio*. *Asian Fish. Sci.*, 14: 317-332.

Manissery, J. K., Venkateshmurthy, K., Gangadhara, B. and Nandeesha, M. C. 2004. Breeding performance of catla, *Catla catla* (Ham.) fed with different formulated diets. *Fishery Technol*., 41(1): 1-4.

Mayes, P. A. 1990. Nutrition. In: Murray, R. K, Graner, D. K, Mayes, P. A. and Rodwell, V. W. (Eds.), *Harper's biochemistry*, 22nd edn. Prentice Hall International Inc., New York, USA, pp. 571-579.

Mokoginata, I., Takeuchi, T., Moeljohardjo, D. S., Sumawidjaja, K. and Fardiaz, D. 1998.The effects of different ratios of n-6/n-3 fatty acids in broodstock diets on egg quality of catfish, *Clarias batrachus*. *Asian Fish. Sci*., 11: 157-168.

Siddiqui, A. Q., Al-Hafedh, Y. S. and Ali, S. A. 1998. Effect of dietary protein level on reproductive performance of Nile tilapia. *Aqua. Res*., 29: 349-358. https://doi.org/10.1046/j.1365-2109.1998.00206.x

Sridhar, N., Raghunath, M. R., Hemaprasanth, K. P., Raghavendra, C. H. and Eknath, A. E. 2014. Induced breeding of threatened Indian medium carp *Puntius pulchellus*. *Indian J. Anim. Sci.*, 84 (12): 1334-1340.

Sridhar, N. 2017. Bengaluru scientists revive endangered fish species. *Deccan Herald, January 02*. https://www.deccanherald.com/content/589452/bengaluru-scientists-revive-endangered-fish.html. (Accessed 12 March 2021).

Vijayagopal, P. 2016. Nutrition, feeds and feed technology for mariculture in India. In: Joseph, I. and Ignatius, B. (Eds.), *Course manual, Winter school on Technological advances in mariculture for production enhancement and sustainability*. ICAR-Central Marine Fisheries Research Institute, Kochi, India, pp. 244-248.

Watanabe, T. and Kiron, V. 1995. Broodstock management and nutritional approaches for quality offsprings in the Red Seabream. In: Bromage, N. R. and Roberts, R. J. (Eds.), *Broodstock management and egg and larval quality*. Cambridge University Press, Cambridge, UK, 424 p.

Yeasmin, M. Y., Rahman, H., Rahman, A., Al-Asif, A., Farid, A. and Billah, M. 2018. Influence of feeding administration of broodstock on breeding performance of common carp (*Cyprinus carpio* Linnaeus, 1758). *J. Aquac. Eng. Fish. Res*., 4(3): 127-137.