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Annals of Aquaculture and Research

Review Article

Seed Production an Urgent Need for Singhi (*Heteropneustes fossilis*) Farming – A Review

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Submitted: 10 February 2017

Accepted: 13 July 2017

Published: 17 July 2017

ISSN: 2379-0881

Keywords

- Induced breeding
- Fish reproduction
- Induced spawning
- Pituitary
- Artificial feed

Abstract

Among the freshwater air-breathing edible catfishes, the stinging catfish *Heteropneustes fossilis* commonly called singhi is very popular and high priced and preferred by consumers in South and South – East Asia. Catfish farmers are unable to practice singhi culture due to lack of seeds. Hence induced breeding is the only way to obtain quality seeds throughout the year. Ovaprim was successfully employed for induced spawning of commercially important edible fishes as well as ornamental fishes. CARE researchers recommended 0.5ml ovaprim/ Kg for native catfishes to produce seeds throughout the year. The objective of this paper is to review seed production of *Heteropneustes fossilis* by induced breeding, which could contribute an important insight in the sustainable culture of this species.

INTRODUCTION

Among freshwater edible fishes, murrels fetch high market demand followed by catfishes by consumers of South and South-east Asia but their culture systems are yet to be established in many Asian countries. Regarding freshwater air-breathing catfishes, the stinging catfish *Hetropneustes fossilis* commonly called singhi is very popular and high priced due to tender flesh, delicious taste, less fat, high digestibility and medicinal value and survival in oxygen depleted waters, tolerance to crowding stress and accepting pelleted feeds. However, their culture systems need considerable research and development [1-2]. *H.fossilis* and *H.microps* are the two known species of the genus *Heteropneustes* (Figure 1a and 1b) and among the two *H.fossilis* is widely distributed whereas the latter has a very restricted distribution.

Aquaculture of the stinging catfish *H.fossilis* is widely preferred by fish farmers. But, major sources of fry and fingerlings for culture systems are mainly from the capture fisheries [3], even though the Central Institue of Freshwater Aquaculture (CIFA) Bhubaneswar has succeeded in breeding and hatchery management of *H.fossilis* and *Clarias batrachus.* Moreover, monsoon failure often occurs in different parts of the country resulting poor spawning. In addition lack of gravid males and females due to anthropogenic activities also results in dwindling singhi populations in the wild [4,5]. At this juncture, to overcome these difficulties, induced breeding is the only way to obtain *H.fossilis* seeds throughout the year. Hence, based on our previous studies as well as from other reports, this review paper deals with seed production of *H.fossilis* by induced breeding.

Induced breeding

Fish reproduction is a periodic phenomenon and is controlled by environmental (exogenous) as well as internal (endogenous) regulatory mechanisms. The acts of breeding occur under optimal environmental conditions that are favorable to the survival of the young ones. Environmental stimuli are detected by sensory organs, relayed to brain, that triggers endogenous mechanism into action. Endogenous mechanism is mediated through cascade of various neurotransmitters and hormones secreted by tissues of brain-hypothalamus-pituitary-gonadal axis. The secretion of above axis is regulated through positive and negative feedback mechanisms involving specifically sensitive hormone receptors [6]. In fish, similar to all higher animals, hormones play a critical role in the reproductive process. The primary tissues involved in this hormonal cascade are the hypothalamus, pituitary gland, and gonads (Figure 2, 1).

History of induced breeding in *H.fossilis*

The first success of induced breeding in *H.fossilis* was achieved by Ramaswami and Sundararaj using homoplastic pituitary gland [7]. The All India coordinated Research Project on Air-breathing Fish Culture recommended a dose of 80-120 mg/ kg of female *H.fossilis*. Since then there is a growing interest in the seed production of this species for aquaculture [8-10]. During the early days, carp pituitary extract has been selected for induced breeding in obligatory air-breathing fishes. The ever increasing demand of donour pituitary and the cumbersome process obliged experts to test alternative hormones such as human chorionic gonadotropin (HCG; 10), luteinizing hormone releasing hormone [11,12], α 17 hydroxyl progesterone and ovaprim [10,13].

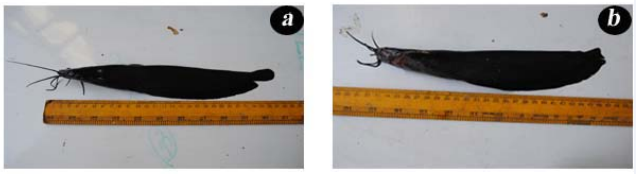

Figure - 1 The morphology difference between H. fossilis (a) and H. microps (b)

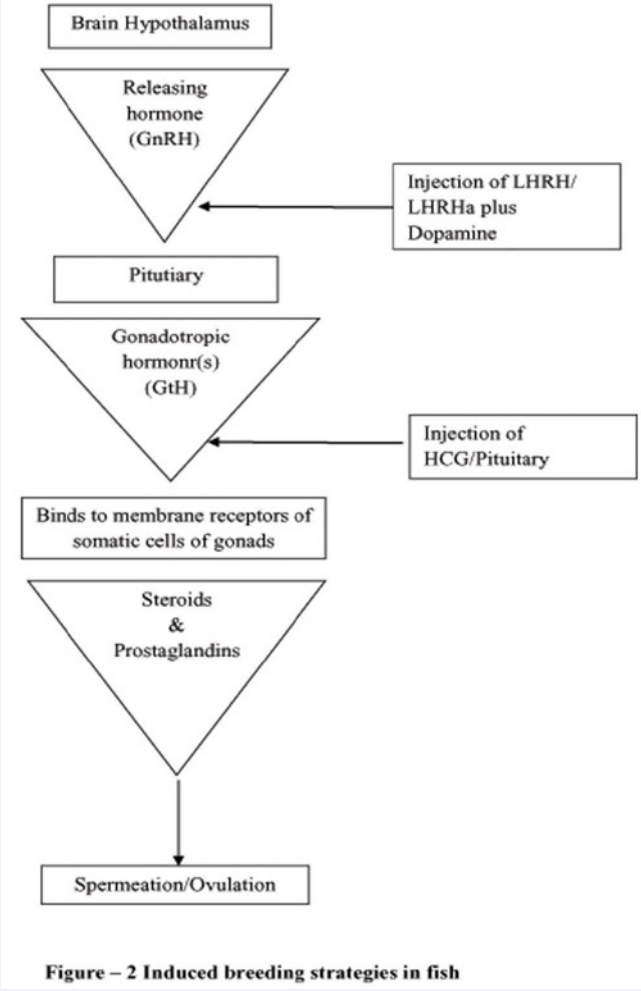

Figure – 2 Induced breeding strategies in fish

Broodstock management

Broodstock rearing: The broodstock for induced breeding experiment of *H.fossilis* have been purchased from fishermen at Melapalayam Fish Market (8.7044° N and 77.7137° E). The collected brooders were stocked in live gene bank of Center for Aquaculture Research and Extension (CARE) Aquafarm (Figure 3a). Gravid males and females (200-250g) were selected from the live gene bank and stocked in earthen ponds (6m x 4m x 1m) of CARE Aquafarm (Figure 3b) [14]. They were maintained under ambient photoperiod (12:12 hr) and temperature (27-29°C). A few aquatic plants viz: *Eichhornia crassipes* and *Hydrilla verticillata* were introduced into the pond to provide shelter. Nayak et al. [15], recommended stocking of male and female brooders in small ponds (100-200m^2) at a stocking density of 10-20 fish/m^3. According to them the maintenance of brood stock is also possible under laboratory conditions by rearing the fish in cemented cisterns. Saha et al. [16], stocked *H.fossilis* brooders in stocking ponds of 60 m^2 area at a density of 20000 fish/ha.

Broodtock nutrition: Significant advancements have been made in regard to developing brood diets to optimize successful reproduction. Brood fish nutrition not only influences reproductive performance but also egg and larval quality of fishes. The research team of CARE recommended chicken intestine (70% protein)/fish waste (56% protein) or any artificial feed with 60% protein suitable for *H.fossilis, Mystus gulio, Ompok malabaricus* and *Ompok bimaculatus* brooders for a maximum spawning of 6000-10000 eggs with 90% fertilization and hatching. The brooders were given artificial feed (10% fish meal + 10% soya meal + 80% wheat bran) at the rate of 12 to 15% body weight twice a day (16) *H.fossilis* brooders were fed with good quality feed (30% fish meal, 30%soynean meal, 30%wheat flour and 10% rice bran and vitamin premix) at the rate of 5% body weight [17]. Rahman et al. [3], fed the brood fishes of *H.fossilis* on supplementary diet formulated from fish meal (25%), rice bran (20%), wheat flour (20%), mustard oil cake (15%), molasses (4%) and vitamin premix (1%). The brooders were reared for four months with feeding at two times a day at the rate of 5-6% of body weight. In addition the ponds were treated with animal manure at 15 days interval at the rate of 1250 kg/ha. Furthermore inorganic fertilizers viz: urea and Triple Super Phosphate (TSP)

Figure 3

were applied at the rate of 50 kg/ha and 25kg/ha respectively.

The impact of brood diet on reproductive performance has been shown for several freshwater species. Lane et al. [18], evaluated the effect of dietary lipid and fatty acids on reproductive success. Four diets containing 45% crude protein and 15% lipid and graded levels of menhaden oil (0, 25, 75 and 100%) or corn oil were tested and reported that higher egg hatchability was associated with higher level of n-3 (Highly Unsaturated Fatty Acids) HUFAs. Diets with 10% fish oil increased spawning success, fecundity, individual egg weight, eggs/spawn, total egg lipid concentration, hatching success and fry survival compared to the diet with 4% fish oil [19]. The formulated diets with higher level of arachidonic acid (1.8%) resulted in signification higher fertilization rate and hatching rate [19].

Sexual dimorphism: Externally sexes can be accurately distinguished only during breeding season, when secondary sexual characteristics become prominent. The best morphological character indicative of a ripe *H.fossilis* brood female is bulging vent [14] and a well round abdomen, the fullness of which extends posteriorly to the pelvic fins. The males look lean with pale vent and a papilla like structure with a pointed tip (Figure 3c). In a mature female, the genital papilla remains in the form of a raised prominent structure, round and blunt with a slit like opening in the middle (Figure 3d). Sexual maturity is usually attained at the end of the first year [2].

Injection of hormones and seed production: Induced spawning of *H.fossilis* was successfully carried out using ovaprim and HCG injected intramuscularly into the dorsolateral region of both males and females in ratio of 2:1 (Figure 3e) in a single dose and released into cement tanks [13]. Eggs were collected from each breeding tank and introduced into plastic troughs (Figure 3f) and the percentage of fertilization and hatching were estimated [10]. The post-larvae (Figure 3g) were fed with plankton initially for one week and after that macerated chicken liver, macerated yellow yolk and fish waste powder were supplied till they reach fry stage (Figure 3h) [20]. In this study, spawning was complete in the medium (0.3 ml ovaprim and 2000 IU HCG) and high (0.5 ml ovaprim and 3000 IU HCG) doses. But no spawning was observed in low doses of both hormones indicating the low doses of these hormones were not sufficient to induce spawning. Zonneweld et al. [21], recommended a dose of 3000 IU HCG to induce spawning in *Clarias batrachus. H.fossilis* spawned after a latency period of 18-24 hr as a function of both hormones. No doubt the dose of the hormone definitely plays a major role in deciding ovulation. Fertilization rate as a function of ovaprim was slightly less (70-75%) when compared to HCG (70-78%) where as hatching rate was more (60-75%) as a function of HCG when compared to ovarprim (50-60%) injected test fish. Similarly, the survival rate of hatchlings was also higher for HCG (50-60%) injected *H.fossilis* than those injected with ovaprim (10-30%; Table 1).

Rahman et al. [3], studied induced breeding of *H.fossilis* using different hormones. Ovulation rates were higher in the ovaprim treated individuals (90%) compared to that of pituitary administered *H.fossilis* (78.7%; Table 2). The latency period was significantly shorter in ovaprim treated fish (10 hr) compared to pituitary (15 hr) and HCG (15 hr) treated individuals in contrast to observations made by Kohli and Goswami [22] and Haniffa and Sridhar [10] who reported longer latency period as a function of ovarprim. Fertilization rates were higher in eggs of the ovaprim treated *H.fossilis* compared to that of pituitary administered fish (70%) and HCG treated fish (75%; Table 2). Begum et al. [23], reported highest rate of fertilization (98%) in *H.fossilis* injected with pituitary whereas Rahman et al., [3]. reported higher hatching rates (77-91%) for eggs of ovaprim treated *H.fossilis* compared to that of pituitary (70-73%) and HGC (67%) administered fishes (Table 2).

Low cost breeding and hatching techniques: Research team of CARE has succeeded in induced breeding of *H.fossilis* using cement aquaria with mud/sand beds. But the hatching rate was poor due to low oxygen level and high turbidity of water. Hence Vijaykumar et al. [9], fabricated low cost breeding and hatching devise for catfish farming. The breeding chamber contains a removable earthen tile to allow fish to hide and it was covered with a net to prevent them from jumping out during breeding. The hatchery consisted of a cylindrical glass tube (8 cm dia and 30 cm height) of 1 litre capacity (Figure 4 a,b). The tube had one opening at the bottom and two openings at the top, one for water outlet (5 mm dia) and the other to introduce eggs (15 mm dia). The inlet opening (5 mm dia) at the base of the glass tube was connected to a flexible tube (5mm dia) for water flow (Figure 4c).

The hormone injected brooders were released into the breeding chamber containing 20 l of well-water. The fish spawned 10-14 hours after injection and each female laid 8000-12000 eggs (Figure 4d). The submerged eggs were collected from the floor of the chamber and transferred directly to the hatchery. The hatchery unit (cylindrical glass tube) was filled with well-water and was placed inside a plastic trough of 25 l capacity. The plastic trough had an outlet covered by a fine net. The collected eggs were introduced into the hatching unit through the large opening at the top and the opening was plugged and water was pumped through the inlet. On an average, 30000 eggs were incubated at a time. For the first 8 hours, water flow was maintained at 0.5l/min to keep the eggs rotating inside the hatchery jar. The hatched larvae were collected from the plastic trough of the hatchery unit and transferred to separate plastic troughs of 50 l capacity

Figure 4

Table 1: Induced breeding in *H.fossilis* using different hormones by CARE research team.

Hormone	Dose	Latency Period (hr)	Fertilization (%)	Egg Output	Hatching (%)	Survival of Hatchlings (%)	Reference
Ovaprim	0.5ml/Kg			80			Vijaykumar et.al (9)
TPE	4mg/kg 6mg/kg		42.7 72.8	6368 ± 205 10177 ± 300	72.8 82.6		Arockiyaraj et.al (24)
FPE	4mg/kg 6mg/kg		45.3 86.2	7051 ± 212 10218 ± 327	73.1 88.6		
Ovaprim	0.5ml/kg 0.7ml/kg		91.2 93.6	121.126 ± 436 10215 ± 188	91.3 95.5		
H.f PE	4mg/kg 6mg/kg		38.6 74.7	3215 ± 170 8138 ± 213	7.7 75.2		
ovatide	0.5ml/kg	10-14	65-93				Marimuthu et.al (29)
Ovaprim	0.3ml/kg 0.5ml/kg 0.7ml/kg	18-24 18-24 18-24	70.0 75.0 70.0	258 ± 85 1052 ± 220 6692 ± 790	50.5 60.0 50.0	10 30 15	Haniffa and Sridhar (32)
HCG	IU 1000 IU 2000 IU 3000	18-24 18-24 18-24	78.0 75.0 70.0	6336 ± 800 18376 ± 1020 82922 ± 5432	75.0 60.5 60.0	60 50 55	

TPE – Total Pituitary extract
FPE – Frog Pituitary extract
H.f. PE – *Heteropneustes fossilis* Pituitary extract

Table 2: Induced breeding in *H.fossilis* attempted by different researchers.

Hormone	Dose	Latency Period (hr)	Fertilization rate (%)	Hatching rate (%)	Survival of Hatchlings (%)	Reference
ovaprim	0.6-0.8ml/kg			96.3		Nayak et al. (12)
Ovaprim Ovatide wova-FH	0.5ml/kg 0.5ml/kg 0.5ml/kg		92.33 96.0 87.33	94.87 90.33 77.33		Karl Marx and Chakrabarty (33)
Pituitary Ovaprim HCG	2mg 6ml/kg 01-0.3	15 10 15	69.23 86.67 75.33	72.72 76.92 66.58		Rahman et al. (3)

Table 3: Ingredients of semimoist feed formulated by CARE research team.

Ingredients	Semimoist feed
Soy Flour (%)	25
Tapioca Flour (%)	10
Wheat Flour (%)	10
Rice Flour (%)	20
Rice Bran (%)	10.7
Fish Oil (%)	2.3
Vitamin / Mineral Mix (%)	2
Anchovy (%)	10
Jawala (%)	10

covered with bamboo mesh to minimize light. Four days after hatching, by which time yolk absorption had taken place; larvae were fed with boiled egg white. A one liter plastic bottle was used to incubate 25000 to 40000 eggs with an average of 80% hatching rate. Many bottles can be connected serially to meet the demands of a small scale aquaculture unit [9].

To reduce the cost of hatchery production of seeds of *H.fossilis,* Arockiaraj et al., [23]. used pituitary extracts of the common Indian toad, *Bujo melanostictus* and the tree frog *Hyla arborea* and compared its efficacy with ovaprim and homoplastic catfish pituitary extracts, *H.fossilis* injected with ovaprim laid the highest number of eggs (121,126) followed by those administered frog pituitary (10,218) and toad pituitary (10,177) and *H.fossilis* pituitary (3215). Regarding fertilization rate, ovaprim secured the top rank (93.6%) followed by [24], frog pituitary (86.2%), *H.fossilis* pituitary (74.7%) and toad pituitary (72.8%) extracts (Table 1). Ovaprim injected individuals showed the best results of 95.5% hatching of eggs followed by frog pituitary (88.6%), toad pituitary (82.6%) and *H.fossilis* pituitary (75.2%) extracts (Table 1). The rates of egg output, fertilization and hatching in this study are higher than those reported by Mollah and Tan [25] for *Clarias macrocephalus.* The potency of amphibian pituitary extracts as inducing agents for catfish spawning offers additional advantages because of the widespread availability of toads and frogs and limited economic utilization.

Artificial breeding: Artificial breeding of *Heteropneustes fossilis* (Bloch) was attempted in the hatchery of the Center for Aquaculture Research and Extension. Sexually matured fish weighing from 200 to 300 g were stocked in rectangular cement tanks (3x1x1m) and the fish were fed the finely chopped and cleaned chicken viscera *ad libitum*. Water quality parameters of

pH: 6.6-7.5, dissolved oxygen: 5-6 mg/l, temperature: 28°C, and photoperiod: 12:12 (light: dark) were recorded.

For this experiment, three male and three female fish were selected based on the external morphological features [14,26]. Both the female and male fish were artificially induced by intra-muscular injection of 0.4ml of ovaprim/kg body weight. Hormone injected male and female fish were then released separately into cement tanks (3x3x1 m) containing dechlorinated tap water. Aquatic macrophytes like *Eichhornia crassipes* (Mart.) and *Hydrilla verticillata* were provided in the breeding tank for hiding purposes.

Approximately 10 to 11 h after the administration of ovaprim, the females were checked for their ovulatory response. The release of eggs through the genital pore following gentle pressure on the abdomen was considered as commencement of ovulation. Eggs from ovulated females were stripped into rectangular plastic fertilization trays. Following ovulation, the testes were removed from the male fish and sperm was pressed into a sterile dry petridish. Stripped eggs were allowed to fertilize with the diluted sperm suspension. After 2 min of gentle stirring, the fertilized eggs were washed several times with freshwater to remove excess milt. The fertilized eggs were immediately transferred to three glass aquaria (45x30x30 cm) for incubation. The eggs were then examined under a microscope ten to fifteen minutes after gametes-mixing, the blastodisc formation was observed as an indication of successful fertilization. Two hours after insemination, the unfertilized eggs remained translucent. The unfertilized eggs were removed carefully from the incubation tank.

Latency period showed variations not only due to hormone type but also for the same hormone as well as for the same dose (Tables 1 and 2). As Gheyas et al. [27], suggested a group of factors is likely to influence biological experiments particularly those involving hormones there by resulting in deviations in the observed latency periods [3]. Tables 1 and 2 show that fertilization rate as a function of ovaprim is always more (75-94%). Most of our previous studies also indicated that the rate of fertilization is always higher due to ovaprim treatment. Such deviations in fertilization rate could be not only due to type of the hormone and dose of the hormone but also due to maturity of the brood fish. In addition, seasonal variations in environmental factors (rainfall, temperature, light etc) and water quality parameters (DO, pH and hardness) also play a major role [28]. In terms of hatching rate also ovaprim yielded better results (60-96%) No doubt, ovaprim treated brooders always showed better performance when compared to *H.fossilis* treated by other hormones including ovatide (Table 1) [29]. Korzelecka-Orkisz et al. [30], selected three females (total length: 17-25cm) and four males (total length: 10-18cm) and gametes were collected from the spawners injected with 5mg carp pituitaries. Ovulation occurred after 12 h of injection.

The sperm collected from the genital papilla using a syringe was diluted with 200m Nacl and was used to fertilize the eggs and the eggs were incubated at 23±0.2°C in soft as well as hard water. Observations on the hatchlings developing in water of different hardness showed the number of hatchlings with swollen yolk sacs and deformed abdomens to increase with decreasing hardness.

Post – larval rearing: Saha et al. [16], transferred the four days old hatchlings of *H.fossilis* to polythene covered trays (30.48cmX60.96cmX15.24cm) until the larval period was completed without feeding. After completion of the larval period, the post-larvae were transferred in trays at 10-20 post-larvae/tray. The post-larvae were fed on powdered milk (100g) egg (one), boiled potato (100g) and raw fish muscle with or without skin (100g) in paste from at 10% body weight twice per day. Successful rearing of post-larvae up to the stage suitable for stocking in nursery ponds remains as the major challenge for the expansion of culture practice of *H.fossilis* at commercial level. Hence suitable feed is the basic requirement for the growth and survival of fish larvae. Though most of the fish larvae relish best on planktonic fauna in the young age, they need nutritionally balanced food in bulk quantity at later stages of their life [31], demonstrated that the larvae of 21-day old showed highest survival (55.00%) and moderate SGR (12.96) in comparison to 14-day (30.21%, 17.38%) and 7-day (14.06%, 8.56%) larvae. The reason being that the 7-day larvae being small in size needed exclusive or more of plankton (*Artemia nauplii*) in comparison to formulated diet which was not available to the satiation level in the larval rearing tanks. They also demonstrated that the fry of *C. batrachus* may be fed with planktonic fauna up to 14-days and thereafter supplementation with formulated diet can greatly enhance growth as well as survival of larvae. The transition from one type of food to another can be a challenge for larviculture. The period of transition often referred to as a period of co-feeding or weaning may be between two types of live feed or from a live feed to an inert formulated diet [19]. Moreover how to reduce the use of live feeds by weaning the larvae to formulated diets earlier in the life history has been an issue. Significant improvements in formulated diets for larval fish have occurred in regard to feed size, palatability and nutritive quality. Artemia are a widely used live feed for many fish larvae and can be a significant part of the cost of fry production.

SUMMARY AND RECOMMENDATION

Freshwater aquaculture makes an important contribution to the national economy of India, as well as contributing to improved livelihoods and nutrition of rural people [34-38]. Among freshwater fish species, murrels (snakehead) and catfish are favored species in South and South East Asia and are amongst the most economically significant species. Worldwide there are about 2,500 catfish species belonging to 30 families and most of which are freshwater. In India catfishes form a significant component of capture fisheries. Indian fish farmers often prefer the exotic catfish viz: the African catfish (*Clarias gariepinus*) and the Thai catfish *Pangasius sutchi* due to availability of seeds, wider feeding spectrum, cheap dietary requirements, fast growth and short culture period. These exotic catfish pose a heavy threat to native fish biodiversity and hence the Government of India put a ban on them, although farmers are still producing them due to favorable short term profits. Hence it has become imperative to promote native catfish culture among fish farmers as an alternative to exotic fish culture for income generation and ultimately to conserve native fish biodiversity. A rectangular pond of 6mX4X1m is suitable for brood stock rearing as well as for netting operations. A minimum depth of 1m is recommended since *H.fossilis* is air-breathing. In the present situation, induced

breeding is inevitable to produce seeds undertake catfish farming. CARE research team recommended 0.5ml of ovarprim/kg for catfishes to produce seeds to throughout the year by induced breeding. Previous researchers also successfully employed ovaprim for induced spawning of commercially important edible fishes as well as ornamental and threatened fishes (12, 36 – 40 and 33). One of the major constraints in culture of *H.fossilis* is rearing of post-larvae, since they succumb heavily due to various reasons CARE research tea recommended live feed in the early stage and formulated feed in the last stage of post larvae (Table 3).

ACKNOWLEDGEMENT

The authors express their sincere gratitude to Department of Biotechnology, New Delhi, for proving financial assistance to Dr. M.A. Haniffa, for a major project on Backyard Fish culture for Rural populations of Tirunelveli, Tuticorin and Kanyakumari Districts of Tamilnadu (BT/PR15422/SPD/24/548/2011). Thanks are due to Management of St. Xavier's College Palayamkottai to establish Center for Aquaculture Research and Extension (CARE) in the college premises exclusively for murrel and catfish research.

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Cite this article

Haniffa MA, Jafar SS, Bhat AA (2017) Seed Production an Urgent Need for Singhi (Heteropneustes Fossilis) Farming – A Review. Ann Aquac Res 4(3): 1038.