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Induced breeding, egg and embryonic development of *Pangasianodon hypophthalmus* (Sauvage, 1878) under hatchery conditions of north Tamil Nadu (Chennai)

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Abstract
Induced breeding experiments were conducted during mid-August to September 2015 using GnRH based inducing agents (Synthetic hormones) viz., GONOPRO FH and WOVA FH. Of which, the successful release of gametes was observed in 5 experiments. Breeding hapa was used during the experiments. The inducing agents were tried in various in one/two split doses. Incubation time of 12.5-16.0 hours was given and breeders were stripped artificially. The fertilized egg has been adhesive and was treated with tannin. 60-80% of fertilization of eggs was achieved. The diameter of fertilized eggs increased further by 0.2 mm after fertilization. The eggs were allowed to develop in Jar Hatchery maintained with water flow. The egg development up to late C- cell embryo was attained.

Keywords: Induced breeding, *Pangasianodon hypophthalmus*, Pangas, Egg and embryonic development.

1. Introduction
The Asian striped catfish, *Pangasianodon hypophthalmus*, is recognized as a superior aquaculture species for tropical regions, as well as a major aquaculture product on world markets (Michael V. McGee.2014) [10]. Catfishes of the family Pangasiidae are of great economic importance in India (Paniyar *et al.* 2014) [14]. Striped catfish (*Pangasianodon hypophthalmus*) is a large freshwater fish. This fish has English names such as iridescent shark, pangasius catfish, striped catfish, sutchi catfish, freshwater catfish. It has the swim bladder which acts as accessory respiratory organ, although 13 pangasiid species were reported to belong to the local ichthyofauna (Roberts and Vidthayanon, 1991) [19], their biology and potential for aquaculture remain largely unknown. Culture of this species is growing day-by-day in India (Lakra and Singh, 2010; Singh and Lakra, 2012 Kumar *et al*., 2013) Vietnam (Phan *et al.*, 2009; Bui *et al*., 2010) [8, 21, 9, 14, 4], Bangladesh (Rahman *et al*., 2006; Ahmed and Hasan, 2007; Ahmed *et al*., 2013) [18, 2, 3]. And Indonesia (Griffith *et al*., 2010) [6]. *P. hypophthalmus* is native of Mekong river basin in Thailand, Cambodia and Vietnam (Michael V. McGee.2014) [10]. It has been introduced in Singapore, Philippines, Taiwan, Malaysia, China, Myanmar, Bangladesh, Nepal and India. In India, it was brought in West Bengal through Bangladesh during 1997 (Mukai, 2011) [12]. This species is being sold in more than 100 countries, mainly in European Unior, Russia, South-east Asia and USA in the form of fillets (Nguyen, 2009; Phuong and Onah, 2009; Phan *et al*., 2009) [13, 15, 16].
Initially, its culture was carried out in Andhra Pradesh and West Bengal in private sector but the Government of India permitted aquaculture of *P. hypophthalmus* in year 2010-2011. Young ones of the species are bottom feeder and carnivores while the fingerlings feed on snail, worm, insects, gastropods etc. Females of this attain maturity at the end of third year while male mature in two years (Phuong and Oanh, 2009; Griffith *et al*., 2010; Vidthayanon and Hogan, 2013; Anon, 2014) [16, 6, 22, 1]. *P. hypophthalmus* is highly fecund fish, seasonal spawner and breeds once in a year in flooded river. Although there are many literatures available on the reproductive behavior of different catfish, spawning strategies and breeding techniques, the literature on the early developmental stages of hybrid catfish is still limited. Recently, the striped catfish has been induced spawned in Advanced Research Farm Facility, Madhavaram, Chennai (13^0 09' 51.96^0 N and 80^0 14' 57. 78'E) a research centre of Fisheries College and Research Centre, Tamil Nadu Fisheries University, Chennai, Tamil Nadu by using

GnRH based inducing agent GONOPRO FH and WOVA FH (which are its full trade names). Yurembam *et al.* (2014) [23] reported the use of another synthetic hormone, Gonopro-FH (salmon-GnRH-a) marketed by Amrit Pharmaceuticals, Aurangabad, India as oral administration for induced breeding of giant zebra fish *Devario acquipinnatus*. An attempt was made to develop and standardize the egg and larval development stages for development of hatchery technique for mass production of the species for sustainable aquaculture.

Striped catfish is characterized by a laterally compressed body, a short dorsal with one or two spines, a well-developed adipose, a long anal fin, strong pectoral spines, and two pairs of barbells and has terminal mouth. There are six branched dorsal fin rays and the pelvic fins have 8-9 soft rays. The gill rakes are described as being normally developed, with small gill rakes being interspersed with larger ones.

Materials and Methods

The induced breeding experiments were conducted during mid-August to September 2015 as the maturation of brood fish was observed during this period. Matured brood stocks of *P. hypophthalmus* were selected based on their condition. Males oozing milt on slight pressing of abdomen was selected and female with distinct budging of abdomen with egg size of about 1 mm diameter was selected for present study. Intramuscular injection was done below the dorsal fin. Fully-matured brood stocks were induced using GnRH based hormones viz., GONOPRO FH and WOVA FH (at various dose ranges from 0.3ml/kg to 0.5 ml/kg female and from 0.3 ml/kg to 0.35 ml/kg for male of body weight) in single or double doses. GONOPRO FH and WOVA FH was used as inducing agent 7 and 3 nos. of induced breeding experiments respectively. Intra muscular mode of injection was used. Males were injected once at time of final injection to female. Female were given single or two split doses at 6 hours interval. After hormone injection, brood stocks were placed in the breeding hapa (2m×1m×1m) tied in the lined pond (13^0 09' 51.96^0 N and 80^0 14' 57. 78'E) and an incubation time of 13-15 hours after single/ end of second injection was given. The sex ratio female: male was in range between 1:1 to 1:3. The water temperature and other water quality parameters were maintained in optimum condition. Eggs were fertilized by dry stripping method. The sperm was diluted five times directly in a 0.9% NaCl solution at stripping, and were temporarily preserved at 4 °C until analysis. Stickiness of the eggs was removed by treatment with tannin 1%. Then, eggs were rinsed with clean fresh water to remove excess of sperm before transferring them into jar hatchery for incubation. Fertilized eggs were continuously observed under a light photo microscope to identify morphological changes and to measure length. Water flow rate maintained in the Jar Hatchery at the moderate level. The diameter of egg was measured by using microscope (CKS41, Olympus). About 50 nos. of fertilized egg samples were collected over different period of time in petridish to analyse the embryonic development under a dissecting microscope. The eggs containing uniform and round yolk sphere and smooth perivitelline space were considered for embryonic study. Number of eggs was kept in petridish and was checked at regular interval to record the timing of embryonic development for each stage. The developmental stage of the eggs were also captured under microscope with photographic attachment (CKS41, Olympus). A total of observations were made from the eggs collected from three breeding operations in different times in the season to record the maximum variability of developmental timing.

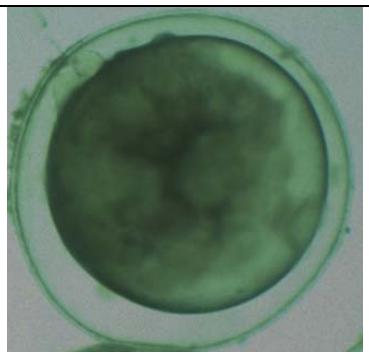
Results

Brood fish selection and Hormone Injection:

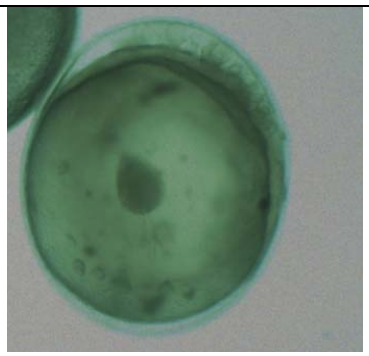
Females and male brood fish weighing between 2.3 kg to 3.5 kg and 1.48 kg to 2.6 kg respectively in good condition were selected for the induced breeding experiments carried out during mid-August to September 2015. Of which successful release of gametes was achieved in 5 nos. of experiments. The selected brood fishes were injected with hormones and the details are presented in Table 1 and 2. After the incubation time the successful release of gametes were achieved in 3/7 experiment (no. 2, 3 and 7) under GONOPRO FH inducement and in 2/3 experiments under WOVA FH inducement. Male yielded good quantity of milt in all experiments. The details of numbers of eggs released and percentage of fertilization are also given in Table 1 and 2.

Egg and Embryonic development

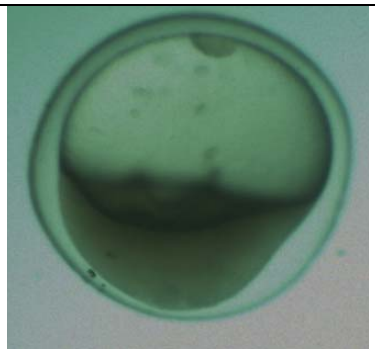
Individual diameter of ova before fertilization was in range between 1.0 to 1.15 mm**.** The events in embryonic development and their respective time of starting and completion *Pangasianodon hypophthalmus* are presented in Table 3 and Figure 1. The ovulated or fertilized eggs were round, adhesive in nature and looked transparent. The diameter of eggs after fertilization and water hardening increased further around 0.2 mm. The water temperature during the developmental stage was maintained between 26-29 ^{0}C. Eggs at various stage of embryonic development could be noticed at a given point of time. The developmental stage of eggs was achieved up to late egg C- embryonic stage and there was no further development.



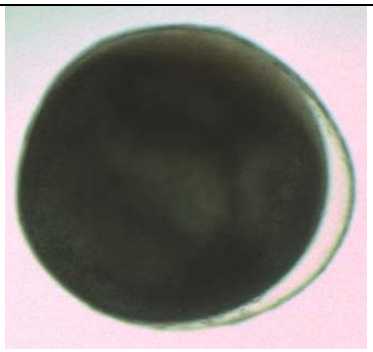
a. Cell division stage



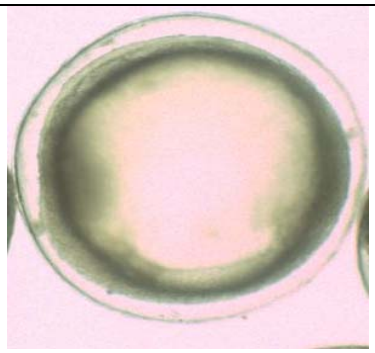
b. Cell division stage



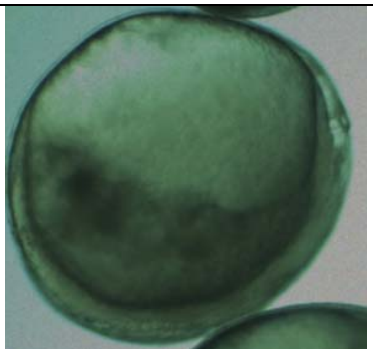
c. Morula



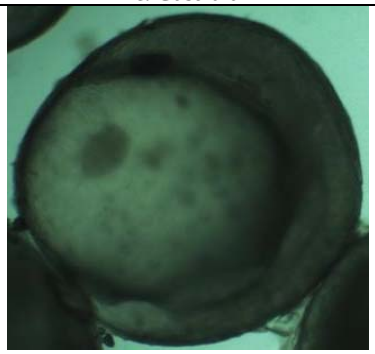
d. blastula



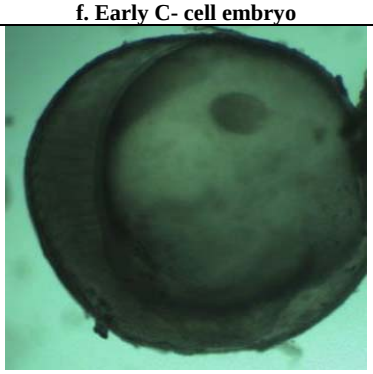
e. Gastrula



f. Early C- cell embryo



g. Late C- cell embryo



h. Late C- cell embryo

Table 1: Induced breeding experiment with GONOPRO FH

Exp. No.	Sex	Length (Cm)	Weight (Kg)	Volume Of GONOPRO FH Used (Ml) 1st Dose	2nd Dose	Incu -Bation Time (In Hours)	No. Of Eggs Released (In Lakhs)	% Of Fertili -Zation
1	Female	70	2.611	1.30	-----	15	Nil	Nil
	Male	58	1.651	0.50	-----			
	Male	63	2.078	0.60	-----			
2	Female	68	2.970	0.90	0.90	16	7.8	60%
	Male	54	1.480	----	0.45			
3	Female	67	2.310	0.70	0.70	12.5	1.6	70%
	Male	61	2.200	----	0.66			
	Male	37	2.500	----	0.75			
4	Female	70	2.980	0.90	0.90	12.5	Nil	Nil
	Male	67	2.600	----	0.78			
	Male	60	2.450	----	0.73			
5	Female	70	2.512	0.75	0.75	15	Nil	Nil
	Male	61	1.982	----	0.60			
	Male	70	2.480	----	0.75			
6	Female	65	3.518	1.05	1.05	15	Nil	Nil
	Male	60	2.051	----	0.60			
	Male	62	1.985	----	0.60			
7	Female	71	3.020	0.90	1.5	13	5.9	70%
	Male	61	1.990	----	0.7			
	Male	61	2.010	----	0.7			

Table 2: Induced breeding experiment with WOVA FH

Exp. No.	Sex	Length (cm)	Weight (kg)	Volume of WOVA FH Used (ml) 1st dose	2nd dose	Incu -bation time (in hours)	No. of eggs released (in lakhs)	Percentage of fertilization
1	Female	70	2.978	0.90	1.5	13	0.8	70%
	Male	60	1.982	----	0.7			
	Male	61	2.015	----	0.7			
2	Female	71	2.752	0.55	0.8	14	Nil	Nil
	Male	36	1.670	----	0.5			
3	Female	71	2.985	----	1.5	14	2.5	80%
	Male	37	1.702	----	0.5			
	Male	38	1.612	----	0.5			

Table 3: Events in egg and embryonic development in Pangasianodon *hypophthalmus*

Sl. No.	Developmental stage	Time of starting after fertilization	Time of completion post fertilization (in hours)
1	Cell division stage (2,4,16 & 64 division)	0 hours	3 hours
2	Morula	2 hours	6 hours
3	Blastula	6 hours	10 hours
4	Gastrula	9 hours	12 hours
5	Early C- cell embryo	11 hours	12 hours
6	Late C-cell embryo	12 hours	----

Discussions

Selection of best brood stock in good condition is a prime factor for success of breeding programme. In the present study brood stock with good quality of gametes were selected. The natural breeding and also the induced breeding season of Pangas was reported from May to July in different parts of the world such as Vietnam, Bangladesh, and Thailand etc. and in the present study mid-August to September in the 2015 was found to be the optimum breeding season. This may be due the agro climatic condition prevailing in North Tamilnadu (Chennai). Generally an incubation time of 12 to 15 hours is provided for successful hatching of eggs and in the present study a minimum of 12.5 and a maximum of 16 hours were required for final ovulation and release of gametes especially by female. This may be due to the inducing agent used, dosages given and the agro climatic variation occurring in the region over period of time. The eggs released during stripping of female varied between 0.8 lakhs to 7.8 lakhs per female, this may be due to fact that though the species spawns once in a season, it releases egg batch wise and the release of egg in each batch might have caused the variation. Of the two induced breeding agents used WOVA FH has comparatively given good results compared to the GONOPRO FH in the current study.

Mature unfertilized eggs of Thai pangas are elastic and spherical in shape, whereas mature fertilized eggs are circular and adhesive in nature. The egg capsule and yolk sphere are greenish or yellowish brown in color. Fish from the same catfish family (e.g. *Clarias batrachus*) have a greenish egg capsule (Mookerjee and Mazumder 1950) [11]. The ovulated eggs of *P. hypophthalmus* further increased around 0.2 mm in size after incubation of fertilized eggs in hatchery, which might be due to hydration of the eggs. The fertilized eggs were strongly adhesive and found in clutch among the eggs during egg incubation in the hatchery. Many teleost under siluriformes show adhesive nature of the eggs (Puvaneswari *et al*., 2009; Sarma *et al*., 2012) [17, 20]. The egg membrane got separated giving birth to the uniform perivitelline space. The yolk sphere pushed towards the vegetable pole as the embryonic development proceeded. This could be due to providing more space for the divisional activities of blastomeres at the animal pole. The clarity of blastomeres as in 2-4 cell stage was gradually reduced as the cleavage proceeded for 64 cell stage onwards. The identity of blastomeres was completely lost at morula and blastula stage. This loss of boundaries between the blastomeres might be due to repeated division and overlapping of cell resulting small and compact blastomeres at the animal pole as well as increased cellularity. The time variation or similarity at the same stage of development between these two close species is acceptable as also observed in the developmental stages between *O. bimaculatus* and *O. pabo* (Sarma *et al*., 2012; Chakrabarty *et al*., 2008). [20, 5] These variations are mostly related to species variability and due to water temperature. Islam (2005) [7] had reported the inverse relation of hatching in Thai pangas with temperature fluctuation. The embryonic developmental stage in the experiments attained up to egg to late C- cell stage and this may provide a basis for further studies on its ontogeny and further to develop key management during pangas hatchery seed production in Indian conditions.

References

1. Anon. Iridescent Shark (Pangasianodon hypothalamus). 1-4. Wikipedia - The Free Encyclopedia, 2014.
2. Ahmad N, Hasan MR. Sustainable livelihoods of pangus farming in rural Bangladesh. Aquaculture Asia Magazine, 2007; 12(4):5-10.
3. Ahmed GU, Chakma A, Shamsuddin M, Minar MH, Islam T, Majumdar MZ. Growth performance of Thai pangus (Pangasianodon hypothalamus) using prepared and commercial feed. Int. J Life Sci. Biotechnol. Pharma Res. 2013; 2(3):92-102.
4. Bui TM, Phan LT, Ingram BA, Nguyen TTT, Gooley GJ, Nguyen HV *et al*. Seed production practices of striped catfish, Pangasainodon hypophthalmus in the Mekong Delta region, Vietnam. Aquaculture, 2010; 306:92-100.
5. Chakrabarty NM, Chakrabarty PP, Mondal SC, Sarangi N. Embryonicdevelopment of pabda (Ompok pabda) with notes on its farming. Fishing Chimes 2008; 28:55-59.
6. Griffith DP, van Khanh, Trong TQ. Cultured Aquatic Species Information Programme: Pangasius hypothalamus (Sauvage, 1878). Fisheries & Aquaculture Department,

FAO, Rome, 2010.
7. Islam A. Embryonic and larval development of Thai Pangas (Pangasius sutchi Fowler, 1937). Dev. Growth Differ, 2005; 47:1-6.
8. Lakra WS, Singh AK. Risk analysis and sustainability of Pangasianodon hypothalamus culture in India. Aquaculture Asia Magazine, 2010; 15(1):34-37.
9. Kumar KK, Prasad P, Raman RP, Kumar S, Purushothaman CS. Association of Enterobacter cloacae in the mortality of Pangasianodon hypophthalmus(Sauvage, 1878) reared in culture pond in Bhimavaram, Andhra Pradesh, India. Indian J Fish. 2013; 60(3):147-149.
10. Michael V, McGee. Pangasius Culture In Western Hemisphere Strong Market Support Could Aid Expansion In Region. Global Aquaculture Advocate, November/December 2014:73-75.
11. Mookerjee HK, Mazumder SR. Some aspects of the life history of Clariusbatrachus Linn. Proc. Zool. Sci. Bengal 1950; 3(2):119-153.
12. Mukai Y. High survival rates of sutchi catfish, Pangasianodon hypothalamus, larvae reared under dark conditions. J Fish. Aquat. Sci, 2011; 6:285-290.
13. Nguyen TTT. Patterns of use and exchange of genetic resources of the striped catfish, Pangasainodon hypophthalmus (Sauvage, 1878). Reviews in Aquaculture, 2009; 1:224-231.
14. Padiyar AP, Ravibabu G, Rao GK, Murthy CK. Pangasius market of A. P: An analysis. Fishing Chimes, 2014; 33:72-74.
15. Phan LTT, Bui M, Nguyen TTT, Gooley GJ, Ingram B A, Nguyen H V, *et al*. Current status of farming practices of striped catfish, Pangasainodon hypophthalmus in the Mekong Delta, Vietnam. Aquaculture, 2009; 296:227-236.
16. Phuong NT, Oanh DTH. Striped catfish (Pangasianodon hypothalamus) aquaculture in Vietnam: an unprecedented development within a decade. In: Successstories in Asian Aquaculture (De Silva, S.S. and F.B. Davy, eds.). NACA & IDRC, Bangkok and Ottawa. 2009, 133-149.
17. Puvaneswari S, Marimuthu K, Karuppasamy R, Haniffa M A. Earlyembryonic and larval development of Indian catfish, Heteropneustes fossilis. EurAsia. J Bio Sci. 2009; 3:84-96.
18. Rahman MM, Islam MS, Haldar GC, Tanaka M. Cage culture of sutchi catfish, Pangasius sutchi (Flower, 1937): effects of stocking density on growth, survival, yield and farm profitability. Aquaculture Research, 2006; 37:33-39.
19. Roberts TR, Vidthayanon C. Systematic revision of the Asian catfish family Pangasiidae, with biological observations and descriptions of three new species. Proc. Acad. Nat. Sci. Philadelphia, 1991; 143:97-144.
20. Sarma D, Das J, Dutta A, Goswami UC. Early embryonic and larval development of Ompok pabo with notes on its nursery rearing. Euro. J Exp. Bio, 2012; 2:253-260.
21. Singh AK, Lakra WS. Culture of Pangasianodon hypothalamus into India: impacts and present scenario. Pak. J. Biol. Sci. 2012; 15:19-26.
22. Vidthayanon C, Hogan Z. Pangasianodon hypothalamus: The IUCN Red List of Threatened Species. IUCN, Gland, Switzerland, 2013.
23. Yurembam Motilan, Yumnam Bedajit, Waikam Vishwanath, Induced breeding of giant zebra fish, Devario acquipinnatus (Mc Clelland, 1839) by oral delivery of synthetic gonadotropin releasing hormone analogue (Salmon-GnRH-a) Gonopro-FH. Int. J Scientific Res. 2014; 3(1):502-503.