



INDUCTION OF SPAWNING AND NURSING PANGAS, *PANGASIANODON HYPOPHTHALMUS* (SAUVAGE, 1878) UNDER HATCHERY SYSTEM

B. K. Chakraborty

Department of Fisheries, Bangladesh & Bangladesh Agricultural University

*Corresponding author: bborty@gmail.com

Article Info:

Research article

Received

18.06.2021

Reviewed

17.07.2021

Accepted

27.07.2021

Abstract: Induced breeding of pangas, *Pangasianodon hypophthalmus* was carried out from May to August, 2020. The experiment was carried out in the month of May, June, July and August 2020 with PG and LHRH-A. Two hormonal sources were tested to evaluate their efficacy on ovulation, fertility and hatching rate of pangas under controlled condition. Double doses of PG (an initial dose of 2.0 mg/kg body weight and final dose of 8.0-10.0 mg/kg body weight) and single dose of Ovuline® (LHRH-A) (0.50 -0.60 ml/kg body weight) showed better results in case of female. In the month of June, PG and LHRH-A showed better results in ovulation, fertility and hatching rate. Males were administered with a single dose of LHRH-A (0.15 ml/kg body weight) and PG (2 mg/kg body weight) showed better results in spermiation. The hatchery operators may use both PG and LHRH-A for induction of spawning better performance of *P. hypophthalmus* breeding.

Keywords: Hatchling, LHRH-A, Ovulation, Pangas, PG.

INTRODUCTION

Fishes are exclusively aquatic vertebrates having fins, gills and streamlined body (Verma and Prakash, 2020). Fishes are the important components of food for humans, as they have high nutritive value. The fishes are rich in protein, lipid, vitamins, minerals, essential amino acids, fatty acids and so on, required for the normal growth, development and maintenance of body and prevents several nutritional deficiency diseases (Kumar *et al.*, 2020). Since fishes play a key role in human nutrition therefore they have extensively studied across the world from various points of view (Verma, 2017, 2019; Chakraborty *et al.*, 2019, 2021; Prakash and Verma, 2019, 2020;

Chakraborty, 2020; Efe and Bemigho, 2021).

The Asian striped catfish, *Pangasianodon hypophthalmus*, is recognized as a superior aquaculture species for tropical regions, as well as a major aquaculture product in world markets (Michael V. McGee, 2014). Catfishes of the family Pangasiidae are of great economic importance in India (Padiyar *et al.*, 2014). Striped catfish (*P. hypophthalmus*) is a large freshwater fish, commonly known as pangas in Bangladesh, belongs to the family Pangasiidae, under the order Siluriformes. According to Roberts and Vidthayanon (1991), the origin of *P. hypophthalmus* was from the Mekong river of Vietnam to the Chao Phraya River to Thailand and it was spread over other countries such as

Malaysia, Indonesia, Bangladesh and China. Pangas species is accepted as delicious food by a wide range of people. Because this species is a good source of protein and calorie of people in rural as well as urban areas. Pangas species have been cultured in earthen ponds and this shark catfish attained weights of 3 kg within 2 years of birth (Bardach *et al.*, 1972).

The information about reproductive behavior and embryonic development of pangas is available little. In the catfish world, there are some studies on the supply of food and culture techniques (Zulkafli and Zahari, 1989); growth, reproduction and breeding of Thai pangas (Manat *et al.*, 1990); taxonomical and behavioral difference of *P. sutchi* and *Pangasianodon gigas* (Wanpen, 1984); rearing of patin fry (*Pangasius pangasius*) in different salinity (Arifin, 1990) and early embryonic development of Thai pangas (Asiful, 2005). The aim of the study is to find out the early life cycle from oocyte activation to the beginning of the adult fry of pangas under hatchery conditions.

It has been introduced in Singapore, Philippines, Taiwan, Malaysia, China, Myanmar, Bangladesh, Nepal and India. 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). *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 catfishes, spawning strategies and breeding techniques but the literature on the early developmental stages of hybrid catfish is still limited. 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, two pairs of barbels and terminal mouth. There are six branched dorsal fin rays and the pelvic fins have 8-9 soft rays. The gill rakers are described as being normally developed, with small gill rakers being interspersed with larger ones.

MATERIALS AND METHODS

The experiment was conducted in the Satota hatchery, Trisal, Mymensingh. The induced

breeding experiments were conducted during May to August 2020. The mature male and female brood fishes were caught from the rearing pond with a seine net and they were placed in the separate breeding tank. For the purpose of breeding, the ripe fishes were selected based on physical and visual examination of the pectoral fin, abdomen and genital opening (Jhingran and Pullin, 1985). 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 a pinkish colour with egg. Intramuscular injection was done below the dorsal fin. Twenty four female fishes divided into eight groups were injected with PG extract (Fig. 1a) and Ovuline® (LHRH-A) (Fig. 1b) placed in separate spawning tanks in different times.



Fig. 1a: PG abstract Fig.1b: Ovuline® (LHRH-A)

The doses of PG extract in female at 1.5 to 2.0 mg/kg body weights were required for first injection. At the time of second injection, male fishes were injected with PG extract at 2.0 mg/kg body weight and female fishes were injected with PG extract at 8.0-10 mg/kg body weight. Again hormone Ovuline® (LHRH-A) at dose 0.5 ml/kg of spawner and 0.25 ml/kg male was administered to the selected brood. Three male and three female fishes were released in the separate tank. Breeding behavioral changes and spawning activities were observed up to ovulation time.

Eggs were fertilized by dry stripping method. Female fishes were stripped to collect eggs in an enamel tray or plastic bowl. Milt from the male fish was collected by applying slight pressure on male's abdomen. The sperm was diluted five times directly in a 0.9% NaCl solution at stripping. Stickiness of the eggs was removed by treatment with tannin 1%. The eggs and milt were mixed thoroughly in the plastic bowl with a soft and clean feather.

A few drops of water were added in the bowl and was stirred continuously for 5-6 minutes. The eggs were washed several times with freshwater and swollen eggs were transferred to different hatching jars under continuous water circulating system. The flow of water (600-800 ml/min) in the jar was regulated during the incubation period. The eggs hatched out within 22 to 25 hours at temperature range of 26 to 31°C. After 22 to 25 hours of fertilization, hatchlings were started to come out from the egg shell and hatching was completed within 2.0 to 4.0 hours. Unfertilized eggs and egg-shells were cleaned from the hatchling jar within an hour of hatching to protect larvae from fungal infection.

The fertilization rate and hatching rate were calculated by the following formula:

$$\text{Fertilization rate} = \frac{\text{Number of fertilized eggs}}{\text{Number of total eggs}} \times 100$$

(Okomoda *et al.*, 2017).

$$\text{Hatching rate} = \frac{\text{Number of hatchlings}}{\text{Number of fertilized eggs}} \times 100$$

An early developmental stage of Pangas, *P. hypophthalmus* was observed up to 72.0 to 74.0 hours starting from egg fertilization. The eggs collected randomly from the hatching jar. Boiled chicken egg yolk mixed with water and sieved through a glass nylon cloth. After hatching, the fine egg yolk emulsion was then spreaded in water to feed the hatchlings. Larvae from

different pair of parents were collected from hatching jars and released in the previously prepared different nursery ponds. The water temperature was recorded during experimental period.

Statistical analysis

The data were analyzed by one way ANOVA using MSTAT Software (version) followed by Duncan's Multiple Range Test to find out whether any significant difference existed among treatment means (Zar, 1984).

RESULTS AND DISCUSSION

In the present study, it was found that *P. hypophthalmus* was bred in the month of May to August where peak was in June and July months. Commencement of breeding season for *P. hypophthalmus* as observed in the present investigation agrees with the report of Sah *et al.* (2018) from Nepal. Breeding of *P. hypophthalmus* was performed at an ambient water temperature of 26.5 to 29.0°C. This range of temperature is suitable for breeding of most indigenous small fishes (Islam and Chowdhury, 1976). *P. hypophthalmus* seemed to have similar temperature requirement of Indian major carps. The information on the reproductive behavior of pangas is limited. In the catfish world, there are little studies on reproduction and breeding of Thai pangas (Manat *et al.*, 1990) and early embryonic development of Thai pangas (Asiful, 2005). Female and male brood fishes weighing between 3.80 kg and 4.8 kg and 4.40 to 5.5 kg respectively in good condition were selected for the induced breeding carried out during mid-May



Fig.2a: Collecting milt.



Fig.2b: Collecting egg.



Fig.2c: Mixing sperm with eggs.

Pertinent data regarding the time of injection and ovulation, fertilization rate, time of hatching, hatching rate and temperature are furnished in

table 1. In the present experiment, injection of pituitary extract of 2 mg/kg body weight at first and of 8-10 mg/kg body weight of second

injection to the *P. hypophthalmus* showed better ovulation, fertility and hatchability success. In case of male, the amount of PG required to promote spermatogenesis found to be 2.0-2.5 mg PG/kg of body weight administered at the time of application of second injection to the females. Best spawning occurred during last June under dual hormonal regime at the PG dose of 2.0 and 8.0 mg/kg body weight in the case of female in the month of June.

In the month of May and August, administration of PG extract in female at a dose of 9.0 and 8.5 mg/kg body weight showed lower fertilization and hatching rate. Ovulation occurred after 8-10 hours of 2nd injection (Fig. 2a 2b and 2c) and hatchings occurred after 18 to 24 hours of fertilization. Under the same PG doses fertilization and hatching rates were found to be 90.440.84% and 78.331.76%, respectively with significance differences with other doses. Thus, the doses of PG have been optimized to 2.0 mg and 8.0-10.0 mg/kg body weight at first and second injection, respectively, for female of *P.*

hypophthalmus at an interval of 6 hours, which was more or less similar to breeding of *Labeo rohita* and *Cirrhinus cirrhosus* and *Cirrhinus reba* (Hossain, 2001).

Ovuline®(LHRH-A) was found to be very effective agent for induced spawning of *P. hypophthalmus*. In the month of June, best spawning occurred at the dose of 0.50 ml/kg body weight in case of female and 0.20 ml Ovaprim/kg body weight in case of male injected at the same time. In the month of May and August, with increase in the amount of hormone *i.e.* a dose of 0.60 ml/kg and 0.55 ml/kg body weight showed good fertilization and hatching rate. Ovulation occurred after 13.0-15.0 hours of hormonal injection (Fig. 2b and 2c) and hatchlings came out after 18 to 24 hours of fertilization. In July better spawning occurred at the dose of 0.52 ml/kg body weight in case of female and 0.23 ml Ovuline (LHRH-A)/kg body weight in case of male injected at the same time. Best fertilization and hatching rates were found to be at 88.140.84% and 72.310.92 %, respectively in the month of June.

Table 1: Effect of different doses of hormone on the spawning of *Pangasianodon hypophthalmus*.

Hormone	PG (Double dose)	Body weight		Doses of 1st injection (ml/kg or mg/kg)		Doses of 2nd injection ((ml/kg or mg/kg)		Ovulation period (hr)	Fertilization rate (%)	Hatching period (hr.)	Hatching rate (%)	Incubation temperature (°C)
		Male (g)	Female (g)	Male	Female	Male	Female					
PG (Double dose)	May	4.20 ±4.22	4.90 ±2.82	-	2.5	2.0	9.0	6-8	74.22 ^b ±2.22	18.0- 24.0	66.11 ^c ±0.86	26.2- 29.2
	June	4.80 ±3.50	4.80 ±5.24	-	2.0	2.0	8.5	6-7	90.44 ^a ±0.84	18.0- 22.0	78.33 ^a ±1.76	
	July	4.62 ±2.25	5.02 ±3.82	-	2.0	2.5	8.0	6-7	88.84 ^c ±1.22	18.0- 22.0	72.0 ^b ±1.33	
	August	4.40 ±2.25	5.40 ±3.82	-	2.4	2.5	8.5	6-8	72.84 ^c ±1.22	18.0- 24.0	70.0 ^b ±1.33	
Ovuline® (LHRH-A)	May	4.60 ±3.29	5.10 ±4.92	-	-	0.25	0.60	13-15	70.0 ^b ±2.22	18.0- 24.0	60.13 ^c ±1.06	26.2- 29.5
	June	3.80 ±2.20	4.40 ±4.92	-	-	0.20	0.50	13-14	88.14 ^a ±0.84	18.0- 21.0	72.31 ^a ±0.92	
	July	4.10 ±3.22	5.50 ±4.92	-	-	0.23	0.52	13-14	80.84 ^c ±1.22	18.0- 22.0	70.11 ^c ±0.98	
	August	4.80 ±4.25	5.50 ±3.82	-	-	0.22	0.55	13-15	70.33 ^c ±1.22	18.0- 24.0	68.11 ^c ±0.98	

Figures with different superscripts in the same column varied significantly (P < 0.01).

Ovuline® (LHRH-A) at 0.50 ml/kg body weight gave rise to complete ovulation in the stipulated time (13-14 hours) which was very much similar to carp breeding (Nandeesa *et al.*, 1990). The effective doses of Ovuline (LHRH-A) for induction of spawning have been optimized to 0.50-0.60 ml/kg body weight at single doses of injection for female of *P. hypophthalmus*, which was more or less similar to breeding of *Catla catla*, *Labeo rohita* and *Cirrhinus cirrhosus* (0.40-0.50 ml, 0.30-0.40 ml and 0.25-0.30 ml/kg body weight), respectively (Peter *et al.*, 1988). A slightly increased amount of Ovuline (LHRH-A) as required in case of *P. hypophthalmus* seemed to be related with the species specificity phenomenon.

Khan and Mukhopadhyay (1975) noted that the success of induced breeding depends largely on proper selection of brood fishes, which has proved very true in the present experiment. Accomplishment of successful spawning depends on selection of suitable recipient fish at the proper stage of ovarian development and creation of congenial spawning conditions (Nash and Shehadash, 1980) which is very accurate in the present experiment. 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). 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 incubation in the hatchery. Many teleosts under Siluriformes show adhesive nature of the eggs (Puvaneswari *et al.*, 2009; Sarma *et al.*, 2012).

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.

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. After hatching 71-74 hours, yolk sacs were totally absorbed and the hatchlings were found to perform horizontal movement with sign of commencement of first feeding. Chicken egg yolk emulsion was fed for 300,000 hatchlings/one egg/day to meet up the dietary requirement. The purpose of this was to start the alimentary canal functioning before transferring them in the nurseries (Price, 1989). The water temperature was the main key factor for ovulation and hatchling. Temperature was recorded 26.2-29.6 °C during experimental period.

CONCLUSION

It is evident from the findings of the present study that both PG extract and Ovuline® (LHRH-A) are equally effective in induction of spawning in *P. hypophthalmus* under controlled hatchery condition. The hatchery operators may use any of the two sources of reproductive hormones as per their choice. But considering the ease of hormone administration, cost and easy availability, pituitary gland (PG) seems to be advantageous over Ovuline® (LHRH-A) for artificial propagation of *P. hypophthalmus*.

ACKNOWLEDGEMENTS

The author acknowledge to Satota hatchery, Trisal, Mymensingh for financial help and other research facilities.

REFERENCES

1. **Anon** (2014). Iridescent Shark (*Pangasianodon hypophthalmus*). 1-4. Wikipedia - The Free Encyclopedia.
2. **Arifin Z.** (1990). Rearing of patin fry (*Pangasius pangasius* HB) in different salinities. *Bull. Penelitian Perikanan Barat (Indonesia)*. 9 (1): 43-51.
3. **Asiful I.** (2005). Embryonic and early development of Thai pangas (*Pangasius sutchi* Fowler, 1937). *Dev. growth Differ.* 47:1-6.
4. **Bardach J. E., Ryther J. H. and Melarney W. O.** (1972). Aquaculture-the Farming and

- Husbandry of Fresh Water and Marine Organisms. Wiley Inter Science, New York.
5. **Chakraborty B. K.** (2020). Rearing and Nursing of Thai Pangas, *Pangasianodon hypophthalmus* (Sauvage, 1978) with different feeds. *International Journal of Biological Innovations*. 2 (2):277-286. <https://doi.org/10.46505/IJBI.2020.2224>
 6. **Chakraborty B.K., Shahroz M.H., Bhuiyan A.B., Bhattacharjee S. and Chatteraj S.** (2019). Status of Indian major carps spawns in the Halda River along with marketing and economic condition of the Fishers and related collectors. *International Journal of Biological Innovations*. 1 (2):40-50. <https://doi.org/10.46505/IJBI.2019.1202>
 7. **Chakraborty B.K., Bhattacharjee S. and Muniya S.** (2021). A Study on Aquatic Biodiversity of Shuthi-Shaiduli River of Bangladesh. *International Journal of Biological Innovations*. 3 (1): 58-67. <https://doi.org/10.46505/IJBI.2021.3104>
 8. **Efe O. and Bemigho I. R.** (2021). Fish Fauna Composition, Abundance and Distribution of Forcados River Estuary. *International Journal of Biological Innovations*. 3 (1): 139-147. <https://doi.org/10.46505/IJBI.2021.3113>
 9. **Griffith D. P., Van Khanh and Trong T. Q.** (2010). Cultured Aquatic Species Information Programme: *Pangasius hypophthalmus* (Sauvage, 1878). Fisheries and Aquaculture Department, FAO, Rome.
 10. **Hossain Q. Z.** (2001). Induced breeding of the fish *Cirrhinus reba* by pituitary gland extract and survival of spawn in nursery ponds. *J. Asiat. Soc. Bangladesh Sci.* 27: 205-213.
 11. **Islam Z. and Chowdhury A. Q.** (1976). Induced spawning of major carp commercial production of fry in the fish seed farm. *Bangladesh J. Zool.* 4: 51-61.
 12. **Jhingran V.G. and Pullin R. S. V.** (1985). A hatchery manual for the common carp, Chinese and Indian majors carps. ICLARM studies and reviews, III: 191p.
 13. **Khan H. A. and Mukhopadhyay S. K.** (1975). Production of stocking material of some air breathing fishes by hypo-physation. *J. of Inland Fish. Soc. India.* 7: 156-161.
 14. **Kumar A., Bajpeyee A. K. and Yadav C.B.** (2020). Effects of Dietary vitamin-C on Biochemical and Morphometric parameters of *Labeo rohita*. *International Journal of Biological Innovations*. 2 (2): 174-177. <https://doi.org/10.46505/IJBI.2020.2214>.
 15. **Manat C., Sraphong W. and Sanga L.** (1990). Breeding of Chao Phya Giant catfish, *Pangasius sanitwongsei*. *Thai-Fish Gazette*. 43 (2): 95-97.
 16. **Michael V. McGee** (2014). Pangasius Culture in Western Hemisphere Strong Market Support could Aid Expansion in Region. *Global Aquaculture Alliance*. November/December 2014:73-75.
 17. **Mookerjee H. K., Mazumder S. R.** (1950). Some aspects of the life history of *Clarias batrachus* (Linn). *Proc. Zool. Sci. Bengal*. 3(1):71-79.
 18. **Nash C. E. and Shehadesh Z. H.** (1980). Review of breeding and Propagation Techniques for Grey Mullet, *Mugil Cephalus* L. ICLARM Studies 3. ICLARM, Manila, Phillipines. 87p.
 19. **Nandeesh M. C., Keshavanath P., Varghese T. J., Shetty H. P. C. and Rao G. K.** (1990). Alternate inducing agents for carp breeding: Progress in Research. *Proc. Workshop carp seed production technology*. Sept. 2-4. 1988; Spl. Publ. 2 Asian Fish Soc., India branch. Mangalore, India: 12-15.
 20. **V. T., Chukoh I.V., Hussain A., Amornsakun T. and Sheriff S.M.** (2017). Performance and characteristics of the progenies from the reciprocal crosses of *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822). *Aquaculture*. 489:96-104. [10.1016/j.aquaculture.2018.02.011](https://doi.org/10.1016/j.aquaculture.2018.02.011).
 21. **Padiyar A. P., Ravibabu G., Rao G. K. and Murthy C. K.** (2014). Pangasius market of A. P: An analysis. *Fishing Chimes*. 33:72-74.
 22. **Peter R. E., Lin H. R. and Krack G.V.** (1988). Induced ovulation and spawning of cultured fresh water fish in China: Advances in

- application of GNRH analogues and dopamine antagonists. *Aquaculture*. 74: 1-10.
23. **Phuong N. T. and Oanh D. T. H.** (2009). Striped catfish (*Pangasianodon hypophthalmus*) aquaculture in Vietnam: an unprecedented development within a decade. In: Success stories in Asian Aquaculture (De Silva, S.S. and F.B. Davy, eds.). NACA & IDRC, Bangkok and Ottawa. 133-149.
 24. **Prakash S. and Verma A.K.** (2019). Acute toxicity and Behavioural responses in Arsenic exposed *Mystus vittatus* (Bloch). *International Journal on Agricultural Sciences*. 10 (1): 1-3.
 25. **Prakash S. and Verma A.K.** (2020). Toxicity of arsenic on Organic Reserves of Liver of *Mystus vittatus* (Bloch). *Journal of Experimental Zoology, India*. 23 (2): 1799-1802.
 26. **Price C. R.** (1989). A Guide to Carp Culture in Bangladesh. Institute of Aquaculture, University of Stirling, Scotland, UK. 3: 11-23.
 27. **Puvanewari S., Marimuthu K., Karuppasamy R. and Haniffa M. A.** (2009). Early embryonic and larval development of Indian catfish, *Heteropneustes fossilis*. *Eur Asia. J Bio Sci*. 3:84-96.
 28. **Roberts T. R. and Vidthayanon C.** (1991). Systematic revision of the Asian catfish family Pangasiidae, with biological observations and descriptions of three new species. *Proc. Acad. Nat. Sci. Philadelphia*. 143:97-144.
 29. **Sah U., Wagle S. K., Mehta S. N. and Mukhiya Y. K.** (2018). Preliminary observations on breeding and fry rearing of pangas (*Pangasius hypophthalmus*) in eastern terai region of Nepal. *International Journal of Fisheries and Aquatic Research*. 3(3):13-16.
 30. **Sarma D., Das J., Dutta A. and Goswami U. C.** (2012). Early embryonic and larval development of *Ompok pabo* with notes on its nursery rearing. *Euro. J. Exp. Bio*. 2: 253-260.
 31. **Verma Ashok K.** (2017). A study on ichtho-diversity of Muntjibpur pond of Allahabad (U.P.) India. *Flora and Fauna*. 23 (1): 220-224.
 32. **Verma A.K.** (2019). Study of Fish Distribution in Balapur Pond of Prayagraj (U.P.). *International Journal on Biological Sciences*. 10(1): 7-10.
 33. **Verma A.K. and Prakash S.** (2020). Status of Animal Phyla in different Kingdom Systems of Biological Classification. *International Journal of Biological Innovations*. 2 (2): 149-154. <https://doi.org/10.46505/IJBI.2020.2211>.
 34. **Vidthayanon C. and Hogan Z.** (2013). *Pangasianodon hypophthalmus*: The IUCN Red List of Threatened Species. IUCN, Gland, Switzerland.
 35. **Wanpen M.** (1984). Taxonomical and behavioral difference of PlaBuk, *Pangasian dongigas* Chevry and Pla Swai. *Pangasius sutchi* Fowler fingerling. Department of Fish Bangkok, Thailand. 12.
 36. **Zar J. H.** (1984). *Biostatistics*. Prentice-Hall, Inc. Englewood Cliffs, New Jersey, USA, 718p.
 37. **Zulkafli A. R. and Zahari A.** (1989). The supply of food and culture techniques of *Pangasius sutchi* Fowler in floating net cages. Fisheries Research Seminar, IPP, Penang, Malaysia, 78-85p.