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Breeding and Embryonic Development of *Hampala macrolepidota* (Van Hasselt and Kuhl, 1823)

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ABSTRACT

The present study investigated the breeding and embryonic development of sebarau, *Hampala macrolepidota* from fertilization until early hatched. The matured eggs and sperm were obtained by induced breeding using a commercial hormone, ovaprim. Dosages given for female and male were 0.6 and 0.3 mL ovaprim kg^{-1} b.wt., respectively. Fertilized eggs were adhesive, spherical and sticky. Fecundity for females weighing 180-280 g ranged from 34,985-75,646 eggs/fish. Average diameter of the fertilized eggs ranged from 0.55±0.13-1.06±0.36 mm. Fertilization rates ranged from 18-28% after 24 h incubation. The observation on the embryonic development covers various stages from newly fertilized eggs, cell division, epiboly, somites until hatched.

Key words: Sebarau, *Hampala macrolepidota,* breeding, embryonic development

INTRODUCTION

National production from the aquaculture sector amounted to 581,048.41 metric tonnes with value MYR 2,798.74 million in 2010 which include production from seaweed, brackish pond and fresh water pond. This amount has increased compared to previous year (2009) that was 453,860.13 metric tonnes which worth MYR 2,268.74 million. Moreover, in the year 2010, aquaculture sector have contributed 28.84% to the nation's fish production and 1.3% to the gross domestic product of the country (Department of Fisheries Malaysia, 2010). This mean, communities in Malaysia have begun to accept freshwater fish as an alternative to marine fish. High demand for fish as food, recreational and ornamental have resulted in the over exploitation of certain species with the possibility of extinction. In Malaysia, choices of freshwater fishes are quite limited. Some indigenous fish species have good potential for aquaculture, it is due to the unavailability of seed supply has that limits its culture. Many of these fishes are not able to reproduce spontaneously in captivity. Therefore, in an effort to introduce these species, induced breeding technique was applied. Induced breeding using hormone has been used for almost 60 years is the only reliable method to increase seed production (Rottmann, 1991).

Hampala macrolepidota, locally known as sebarau (Kamaruddin *et al*., 2011) or jungle perch is a carnivorous fish (Setiadi *et al*., 1987). It can be found in Mekong and Chao Phraya basins, Malay Peninsula and Indonesia. The most prominent characteristic of this fish is the black bar running vertically down the side of the body between the dorsal and pelvic fins of adults (Binohlan, 2011). This fish is very sensitive to poor water quality and only can be found in clear

pristine rivers. Therefore, the distribution of this fish in natural river can be an indicator to the current water condition of a particular area. The cyprinid genus *Hampala* is represented by five Southeast Asian species: *H. macrolepidota* (Valenciennes), *H. ampalong* (Bleeker), *H. bimaculata* (Popta), *H. lopezi* (Herre) and *H. dispar* (Smith). These species are very closed in meristic and proportional characters and hence their discrimination has mostly been based on coloration except for *H. lopezi* it is endemic to Busuanga Island, Philippines. However, their colour patterns are also subjected to age and individual variations (Taki and Kawamoto, 1977; Doi, 1994). *H. macrolepidota* is a predatory species and dominant at all levels of depths in the lake (Zainudin, 2005). The limited availability of these fishes caused price hike. In the local market, sebarau can easily cost MYR80-120 kg^{-1}.

There are very few documented reports on *H. macrolepidota*. Artificial breeding using Human Chorionic Gonadotropin (HCG) and common carp pituitary was first carried out by Ambak *et al.* (1982), followed by study on food and feeding habits (Aizam and Ang, 1984). However, until today, sebarau is still not listed as one of the cultured species in Malaysia. It is a popular sportfish and found to be used in recreational fishing ponds in Malaysia. As an ornamental fish, it can cost RM5-7/fingerling. Until now, very little information is available on this fish. Thus, the objective of this study was to breed *H. macrolepidota* using a commercial hormone, ovaprim and then to observe its embryonic development until hatched.

MATERIALS AND METHODS

Materials: The Department of Fisheries, Aquaculture Extension Center Perlok, Jerantut, Pahang, Malaysia has established a collection of *Hampala macrolepidota* broodstock caught from Pahang river. Therefore, the induced breeding was carried out at this center. Other materials used in this study were net, digital balance, clove oil, ovaprim (Syndel, USA), syringe, basins, bowl, towel, mud, tanks, rearing trays, dissecting microscope and petri dish.

Methods

Broodstock selection: *H. macrolepidota* broodstock were collected from pond by using net. Five females and ten males (1 F:2 M) were selected and used for this study.

Induced breeding: Body weight of each fish were measured by using a digital balance. Fishes were then anesthetized by using clove oil. Dosages were 0.6 and 0.3 mL ovaprim kg^{-1} female and male, respectively. Injections were given intramuscularly. This study was carried out at 4:00 p.m. on 9 February 2012.

Stripping: Fish were monitored for ovulation from time to time by applying a slight pressure on the abdomen. At 10:00 pm on the same day, eggs and milt were stripped from females and males, respectively. This stripping was carried out 6 h after the injection. Eggs stripped from females were weighed individually. Eggs samples were then collected for fecundity estimation.

Fertilization: Remaining eggs were then inseminated with sperm. Eggs were washed several times with mud to remove its adhesiveness. Eggs were then transferred onto trays placed in a flow through incubation tanks.

Fecundity and eggs diameter: Fecundity was estimated using 1 g of egg sample and then multiplied with total weight of the stripped eggs. Diameter of eggs was determined by taking randomly 30 samples of eggs from each female broodstock.

Embryonic development: The embryonic development was observed and photographed under a dissecting microscope. The observation on the embryonic stages was carried out until eggs hatched.

Water quality parameters: Four water quality parameters measured in this study were temperature, dissolved oxygen, pH and Total Dissolved Solids (TDS). The measurements were taken during the incubation period.

Statistical analysis: Data for eggs diameter were statistically analyzed by one-way ANOVA using SPSS 17.0 and Duncan Multiple Range Test (DMRT) used to determine the significant difference between the means.

RESULTS

H. macrolepidota with weight ranged from 180-280 g are able to produce 34,985-75,646 eggs/female. Mean egg diameter for *H. macrolepidota* injected with ovaprim is from 0.55-1.06 mm. There was no significant different ($p>0.05$) for the eggs diameter produced between the females. Newly fertilized eggs were spherical and adhesive. Fertilization rate ranged from 18-28%. Majority of the eggs hatched about 24 h after fertilization. Results for eggs fecundity, diameter and fertilization of *H. macrolepidota* are as shown in Table 1. Embryonic development of *H. macrolepidota* lasted for about 24 h. Description of the developmental stages is summarized in Table 2 and shown in Fig. 1. Four water quality parameters measured were temperature, pH,

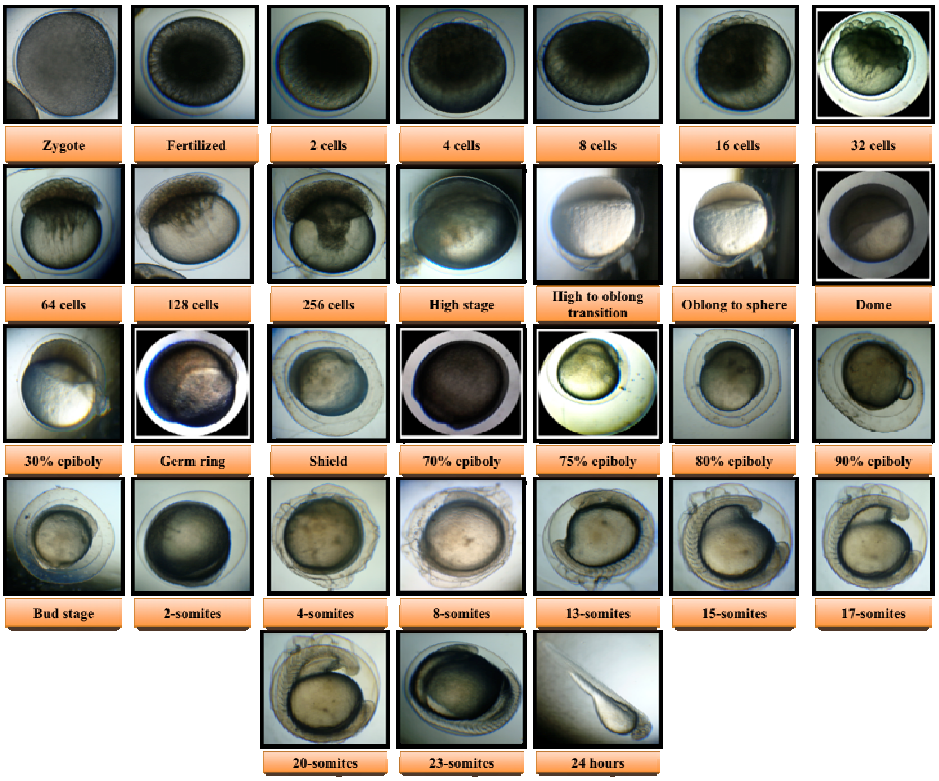

Fig. 1: Embryonic stages of *Hampala macrolepidota*

Table 1: Fecundity, fertilization rate and eggs diameter of *H. macrolepidota*

Weight of fish (g)	Fecundity (No. of stripped eggs/female)	Fertilization rate (%)	Mean of eggs diameter (mm)
280	49,100	28	1.06±0.36[a]
220	75,646	22	0.55±0.13[a]
220	52,100	22	0.69±0.20[a]
200	34,985	20	0.90±0.33[a]
180	51,506	18	0.82±0.28[a]

Mean with the same superscript letters are not significantly difference at p>0.05

Table 2: Description of the embryonic development of *H. macrolepidota* until hatching with each stage

	Times elapsed from fertilization*		
Stages	Hour	Minute	Observation
Zygote	0	4	Cytoplasm streams toward animal pole to form the blastodisc
Fertilized	0	7	Single cell at animal pole; with small perivitelline space
2 cells	0	10	Cleavage stage: first segmentation
4 cells	0	17	Cleavage stage: 2 times, 2 array of blastomeres
8 cells	0	37	Cleavage stage: 2 times, 4 array of blastomeres
16 cells	0	46	One pair of divisions, one on each side of 2nd cleavage line: 4 times, 4 array of blastomeres
32 cells	1	0	Cleavage stage: 4 times, 8 array of blastomeres
64 cells	1	8	3 regular tiers of blastomeres
128 cells	1	15	Cleavage planes irregular: 5 blastomere tiers
256 cells	1	26	7 blastomere tiers
High stage	1	38	More than 11 blastomere tiers; blastodisc flattening begins; YSL nuclei in two rows
High to oblong transition	1	58	Blastodisc flattening; multiple rows of YSL nuclei
Oblong to sphere	1	59	Spherical shape; flat border between blastodisc and yolk
Dome	2	14	Yolk cell bulging toward animal pole as epiboly begins
30% epiboly	2	26	Blastoderm seen as inverted cup of uniform thickness
Germ ring	2	39	Germ ring visible from animal pole; 50% epiboly
Shield	3	34	Embryonic shield visible from animal pole; 50% epiboly
70% epiboly	3	50	-
75% epiboly	4	17	Dorsal side distinctively thicker; epiblast, hypoblast, evacuation zone visible
80% epiboly	4	31	-
90% epiboly	5	15	Axis and neural plate; brain and notochord rudiments
Bud stage	5	18	Tail bud prominent; early polster; 100%-epiboly
2-somites	6	11	First somite furrow
4-somites	6	50	First somite furrow
8-somites	7	45	Polster prominent; optic vesicle, Kupffer's vesicle, neural keel
13-somites	10	15	Pronephros forms
15-somites	10	48	Otic placode, brain neuromeres
17-somites	11	23	Otic placode, brain neuromeres
20-somites	11	52	Lens, otic vesicle, hindbrain neuromeres
23-somites	13	24	Lens, otic vesicle, hindbrain neuromeres
24 h.	24	0	Hatching complete; early pigmentation, heartbeat

*Eggs inseminated with sperm at 11:00 p.m. on February 9, 2012

Table 3: Water quality parameters during the eggs incubation period for *H. macrolepidota*

Parameter	Range
Temperature (°C)	24.29-24.90
pH	6.10-6.42
Dissolved oxygen (mg L^{-1})	7.57-7.77
Total dissolved solids (TDS) (g L^{-1})	0.165-0.169

Dissolved Oxygen (DO) and Total Dissolved Solids (TDS) with readings ranged from 24.29-24.90°C, 6.10-6.42, 7.57-7.77 mg L^{-1} and 0.165-0.169 g L^{-1}, respectively Table 3. Comparatively, these readings are within the acceptable ranges for fish culture.

DISCUSSION

Proper identification mature fishes to be used as broodstock is very crucial. Therefore knowing the life cycle of fishes is important (Hosseinzadeh *et al*., 2001). Matured females *H. macrolepidota* were identified by their rounded belhies while males slim and prominently colored. Previous study by Rosli (1987) mentioned that *H. macrolepidota* with body weight between 180-470 g, induced with pituitary extract and Human Chorionic Gonadotropin (HCG), produced to a maximum of 11,992 eggs/female. However, in this study at a much lower cost with the commercially available ovaprim, can produced higher fecundity (34,985-75,646 eggs/female). The use of anaesthesia reduced the stress during handling of fish thereby, producing better fecundity.

Zohar and Mylonas (2001) stated that ovaprim produced better results as compared to ovatide, another commercial hormone used in the induced breeding of fish. Several other factors may affect the fish fecundity, are age and size of the female (Thorpe *et al*., 1984), the strategy of life history (Morita and Takashima, 1998), temperature and food (Fleming and Gross, 1990). The fecundity of this *H. macrolepidota* is low when compared to others cyprinid. For example, *Labeo dero* fecundity is about 90,000 (Biswas *et al*., 1984). According to Rideout and Burton (2000), degeneration or malfunction of ovaries reduces the female fecundity and reproductive potential. Fecundity increases with body size maybe because of energy amount of the fish available for egg production and the body cavity accommodating the eggs increases with fish size (Jonsson and Jonsson, 1999).

Egg diameter for *H. macrolepidota* produced in this study was between 0.55-1.06 mm. Comparatively, these eggs are larger than 0.52473-0.52492 mm produced by Rosli (1987). As for the embryonic development it is very similar to the others cyprinids. In this study, majority of the eggs hatched 24 h after fertilization. The blastomeres are regular in shape and size and the morphology of this blastomere has been used as a predictive indicator of egg viability within a clutch in most species (Shields *et al*., 1997).

Different species of fish have different water quality requirements. In this study, the incubation period lasted for 24 h at water temperature of 24°C. Incubation period was only 16-18 h in *Heteropneustess fossilis* at a temperature of 26°C (Kohli and Vidyarthi, 1990). It is possible to manipulate the temperature to reduce the incubation period. However, care must be taken to ensure that the species studied are able to tolerate higher temperature. Water parameters measured through out this study were within acceptable range for fish culture.

CONCLUSION

It can be concluded that *H. macrolepidota* can be induced breed with a less costly and commercially available hormone, ovaprim. Fertilized *H. macrolepidota* eggs are able to hatch in just

24 h, therefore this information can be useful for the larval rearing of this species. The finding of this study can be used to establish and improve the fry production of this species so that in the near future, this species can be introduced as an aquaculture species in Malaysia.

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REFERENCES

Aizam, Z.A. and K.J. Ang, 1984. Fecundity of ikan sebarau, *Hampala macrolepidota* (Van Hasselt) and its relationship to fish length. Pertanika, 7: 95-96.

Ambak, M.A., A. Zainal Abidin and A.K.M. Mohsin, 1982. Induced breeding of Ikan Sebarau. Pertanika, 5: 117-118.

Binohlan, C.B., 2011. *Hampala macrolepidota* Kuhl and Van Hasselt, 1823 Hampala barb. Stockholm, Sweden. http://www.fishbase.org/summary/Hampala-macrolepidota.html

Biswas, S.P., S.A.K. Nasar and K. Chatterjee, 1984. Inter and intraspecific comparisons of some aspects of the reproductive biology of the two carps, *Labeo pangusia* and *Labeo dero*. Arch. Biol., 95: 11-27.

Department of Fisheries Malaysia, 2010. Perangkaan Perikanan Tahunan 2010. http://www.dof.gov.my/html/themes/moa_dof/documents/jld1_ 2010.pdf

Doi, A., 1994. A new cyprinid fish, *Hampala salweenensis*, from the Mae Pai River system, Salween basin, Thailand. Jap. J. Ichthyol., 40: 405-412.

Fleming, I.A. and M.R. Gross, 1990. Latitudinal clines: A trade-off between egg number and size in Pacific salmon. Ecology, 71: 1-11.

Hosseinzadeh, H., M. Soltani and F. Dadvar, 2001. Reproductive biology of *Sillago sihama* in Persian Gulf. Iran. Sci. Fish. J., 10: 37-55.

Jonsson, N. and B. Jonsson, 1999. Trade-off between egg mass and egg number in brown trout. J. Fish Biol., 55: 767-783.

Kamaruddin, I.S., A.S. Mustafa-Kamal, A. Christianus, S.K. Daud, S.M.N. Amin and L. Yu-Abit, 2011. Length-weight relationship and condition factor of three dominant species from the lake Tasik Kenyir, Terengganu, Malaysia. J. Fish. Aquatic Sci., 6: 852-856.

Kohli, M.S.P. and S. Vidyarthi, 1990. Induced breeding embryonic and larval development in *Heteropneustes fossilis* (Bloch) in the agroclimatic conditions of Maharastra. J. Indian Fish. Assoc., 20: 15-19.

Morita, K. and Y. Takashima, 1998. Effect of female size on fecundity and egg size in white-spotted char: Comparison between sea-run and resident forms. J. Fish Biol., 53: 1140-1142.

Rideout, R.M. and M.P.M. Burton, 2000. Peculiarities in ovarian structure leading to multiple-year delays in oogenesis and possible senescence in Atlantic cod (*Gadus morhua*). Can. J. Zool., 78: 1840-1844.

Rosli, A.Z., 1987. Pembiakan aruhan ikan sebarau Hampala macrolepidota (Van Hasselt) dengan menggunakan ekstrak pituitari, human chorionic gonadotropin dan kombinasi antara kedua-duanya. Laporan Projek FFPSS 1987.

Rottmann, R.W., 1991. Introduction to Hormone-Induced Spawning of Fish. Southern Regional Aquaculture Center, USA., Pages: 3.

Setiadi, K.E., W.S. Rabegnatar and A. Hardjamulia, 1987. Some biological aspects of the predominant fish species in the jatiluhur reservoir, West Java, Indonesia. Proceedings of the Workshop on Reservoir Fishery Management and Development in Asia, November 23-28, 1987, Kathmandu, Nepal, pp: 98-104.

Shields, R.J., N.P. Brown and N.R. Bromage, 1997. Blastomere morphology as a predictive measure of fish egg viability. Aquaculture, 155: 1-12.

Taki, Y. and A. Kawamoto, 1977. Differentiation of the Cyprinids, *Hampala macrolepidota* and *H. dispar*. Jap. J. Icthyol., 24: 61-65.

Thorpe, J.E., M.S. Miles and D.S. Keay, 1984. Developmental rate, fecundity and egg size in Atlantic salmon, *Salmo salar* L. Aquaculture, 43: 289-305.

Zainudin, M.R.Y., 2005. Assessment of fish community distribution and composition in the Perak River in order to determine biological indicators for freshwater health. M.Sc. Thesis, Universiti Sains, Malaysia.

Zohar, Y. and C.C. Mylonas, 2001. Endocrine manipulations of spawning in cultured fish: From hormones to genes. Aquacult., 197: 99-136.