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Hormone induced spawning and feeding conditions for Betok fish (*Anabas testudineus*)

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Abstract

The objective of this study is to optimize the fertility and hatchability of eggs from the climbing perch (*Anabas testudineus*) by using an Ovaprim® and hCG hormonal treatment. The impact of the feeding method on the growth and survival of the fish larvae was also investigated. A completely randomized design of experiments with five treatments and three replications was used in the study. The optimum hormonal treatment was found to be an injection of 0.5 ml Ovaprim®/kg body weight yielding a fertility and hatchability rate of 74% and 75%, respectively. The best tested diet for the growth and survival of the fish larvae was Tubifex worms varying from 3 to 43 days after hatching. This yields an absolute weight value of 0.828 gram, an absolute growth of 2.9 cm, a daily weight growth rate of 0.170% and a survival rate of 88.9%.

Keywords: climbing perch, Ovaprim®, hCG, fish farming

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BACKGROUND

The climbing perch (*Anabas testudineus*), also known as “betok” in the Indonesian language, is one of 31 important fish species found along the waters of the Kampar River, one of the four largest rivers in the Riau province of Indonesia. Wild climbing perch is an important source of food for the locals. Local fishermen are rarely aware of management practices for fish stock and do not respect restrictions on the size of the caught fish. This can affect the sustainability of the climbing perch population in its natural environment. Developing fish farming technologies for this species could remediate the problem. However, little is known about optimum fish farming conditions for the climbing perch. To remediate this lack of knowledge, two critical aspects for the farming of this species were investigated.

The first aspect was the optimization of the production and hatching rate of eggs through hormonal treatment. Hormonal treatment stimulates the production of eggs and semen in the female and male parent fish, respectively. Two hormones were considered, namely Ovaprim® and Human Chorionic Gonadotropin (hCG). Ovaprim® has been successfully used for the spawning of fish. Every 1 ml of Ovaprim® contains 20 µg sGnRH-a (D-Arg6, Trp7, Leu8, Pro9-NET) - LHRH and 10 mg anti dopamine (Nandeesha et al. 1990, Harker 1992). HCG is also an effective hormone to replace the extract of goldfish pyrophism in stimulating ovulation in fish (Lam 1985). In addition, this hormone can also give a positive effect on the hatching of fish eggs in fish (Yanhar et al. 2009). After hatching of the eggs, the survival and growth rate of the larvae are the next most critical parameters to consider for optimizing the productivity of the fish farming process. The influence of the feeding material and feeding schedule on these two parameters were part of the second aspect of the fish farming process investigated in this article.

RESEARCH METHODS

This research was conducted from May to July 2017 in the Fish Breeding Laboratory (PPI), of the Faculty of Fisheries and Marine Sciences, at the University of Riau, in Indonesia. Adult fish were kept in a concrete tank partitioned into 1 x 1 x 1 m^3 sections while larvae and juveniles were kept in smaller size aquariums.

A first series of experiments was performed to determine the influence of the hormonal treatment on the fertility and hatching rate of the eggs. As indicated in **Table 1**, treatments consisting of injections with various concentrations of Ovaprim® or hCG were administered to female adult fish (TKG IV). The fifth level of the treatment consisted of a 0.95% NaCl physiological solution which served as the control.

The eggs obtained from the different populations were fertilized with semen obtained from the male fish. The efficiency of the treatment was evaluated by measuring the fertility and the hatchability of the

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Table 1. Hormonal treatments used in the present study

Treatment label	Injected solution/kg of body weight
P1	0.5 ml Ovaprim®
P2	0.7 ml Ovaprim®
P3	500 IU hCG
P4	1000 IU hCG
P5	1 ml 0.95% NaCl

Table 2. Diets used in the present study

Treatment label	Diet after hatching day
T1	Artemia sp from day 3 to 43
T2	Artemia sp from day 3 to 13, Tubifex sp from day 14 to 43
T3	Artemia sp from day 3 to 23, Tubifex sp from day 24 to 43
T4	Artemia sp from day 3 to 33, Tubifex sp from day 34 to 43
T5	Tubifex sp from day 3 to 43

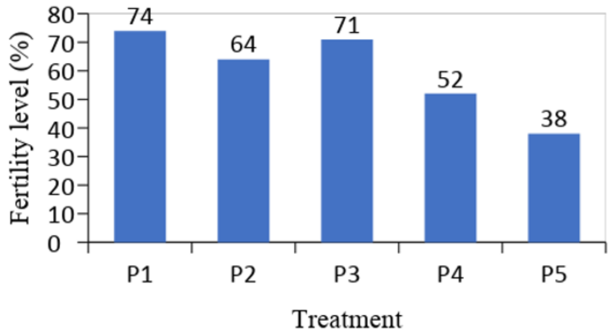

Fig. 1. Measured fertility rates

produced eggs. Measurements were performed following a completely randomized design with 3 replications for each treatment. The response model is described as:

$$Y_{ij} = \lceil + |_i + \Sigma_{ij} \quad (1)$$

where Y_{ij} is the fertility or hatchability of the individual receiving the i-th treatment (i=1 to 5) and the j-th test, $\lceil$ is the general average, $|_i$ is the effect of i-th treatment and Σ_{ij} is he error term.

The hatched larvae from the best treatment were kept for 40 days. Subsequently, the larvae were fed with the various diets described in **Table 2**. The number of larvae for each treatment was 500. As before, a completely randomized design with 3 replications was used. The measured test parameters were the absolute weight growth, the absolute longevity growth, the daily growth rate of weight and the livelihood of the fish.

RESULTS AND DISCUSSION

Fertility Rate

The measurements of the fertility rate were given in error: reference source not found. The highest fertility rate obtained from the treatment of P1 with 74% followed by P3 with 71%, P2 with 64% and P4 with 52%. The control treatments lead to a fertility rate of 38%. Further analysis using Newman Keuls Study test showed that P5 was significantly different ($P < 0.05$) to P4, P2, P3 and P1.

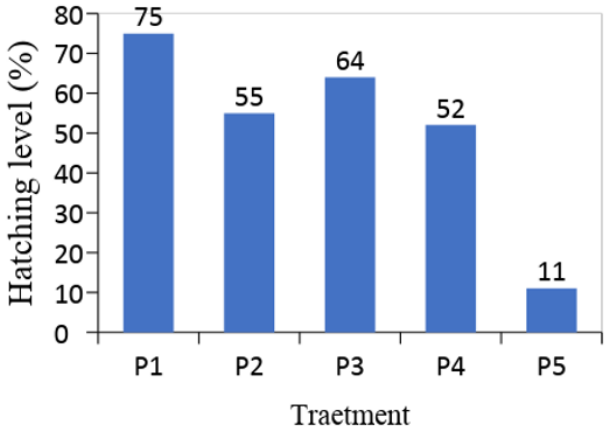

Fig. 2. Measured hatching rates

The ovaprim concentration corresponding to the treatment in P1 optimizes the ovulation and the quality of the eggs, which in turn leads to the best fertility value. Literature results on the influence of hormonal treatments on the fertility of fish include the work of Ramdhani (2011) using sGnRH-a + domperidon on comet fish and obtaining a fertility rate of 60,5% and belida fish (Notopterus notopterus) carried out by ovaprim injection by Yulindra et al. (2017) obtained the highest fertilization value of 44.15%

In the work of Arfah et al. (2006) with carps, the fertility rate is 4.30%. In addition to the characteristics of the eggs, the quality of the sperm from the male fish also has a strong influence on the fertility rate. Therefore, a superior sperm donor was been selected. Environmental factors such as temperature, dissolved oxygen, pH, salinity, and light intensity also greatly affect the success of the hatching. However, these environmental factors were controlled during this research.

Hatching Rate (HR %)

The measurements of the hatching rate were given in error: reference source not found. The highest hatching value obtained in treatment P1 was equal to 75% followed by P3, which was equal to 64%, P2 at 55%, P4 at 52% and P5 at 11 %. Further test results using Newman Keuls Study showed that the P5 treatment was significantly different ($P < 0.05$) to P2, P4, P3 and P1.

Chotipuntu and Avakul (2010) state that the hatching of fish eggs occurs 24 hours after spawning. Effendi (1997) states that the fertilized eggs subsequently develop into embryos and eventually hatch into larvae while unfertilized eggs will die and rot. The development of embryos into hatching larvae is highly dependent on fish species and water temperatures. Until a certain limit that can be tolerated by the eggs, higher water temperature leads to faster hatching time. In addition, the dissolved oxygen content also affects the hatching of eggs, because oxygen can affect the number of monistic elements in the embryo (Novianto 2004).

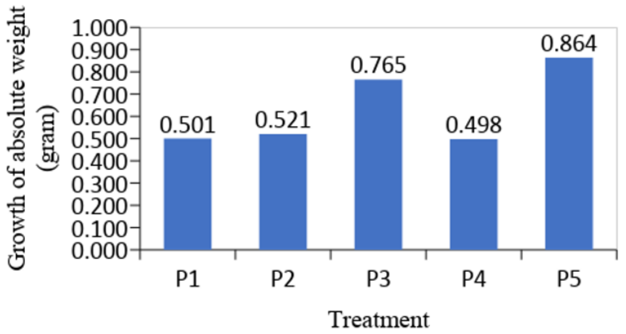

Fig. 3. Measured absolute weight gain 40 days after hatching

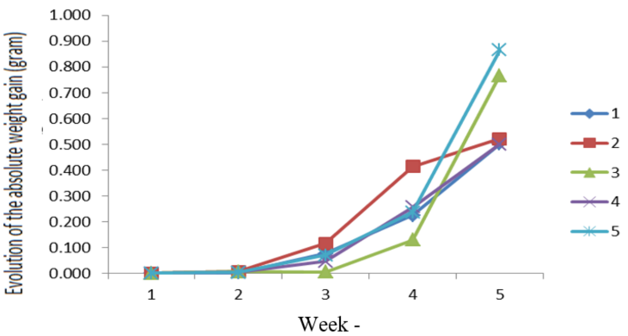

Fig. 4. Evolution of the absolute weight gain during the study period

During the development of embryo, it uses energy from the yolks and oils so that the greater is the diameter of the egg, the better is the embryo development. As the embryo develops, the yolk will be consumed to develop into the embryo's organs. The embryo's organs continue growing into larvae that end up breaking and separating from the egg shell. Sarkar et al. (2015) states that fish eggs will hatch between 19 and 22 hours after fertilization. The highest hatching value obtained from this study was greater than the highest hatching value of betok fish (Anabas testudineus Bloch) which was given LHRH hormone which was 65.33% (Zalina et al. 2012).

Absolute Weight Gain

The gain in absolute weight 40 days after hatching was given in error: reference source not found for the different feeding treatments of **Table 2**. The evolution of the absolute growth during the period of the study is given in error: Reference source not found.

The highest absolute weight gain is obtained for the T5 treatment with 0.864 g followed by T3 with 0.765 g, T2 with 0.521 g, T1 with 0,501 g and 4 of 0,498 gram. ANOVA tests indicate that the treatments have no significant effect (P>0.05) on absolute weight gain. Furthermore, from the observation of the weekly weight gain in Error: Reference source not found, it can be seen that there is no significant differences between treatment. It is believed that the highest growth rate was obtained with the treatment of T5 because the Tubifex sp. worm is the most favoured natural diet of the "betok" fish and because of its high protein content (Subandiyah et al. 2003), which is 57% according to Priyadi (2010). Furthermore, *Mohseni et al*. (2012) stated that the nutritional quality of feed is one of the main parameters in determining the high growth rate in fish.

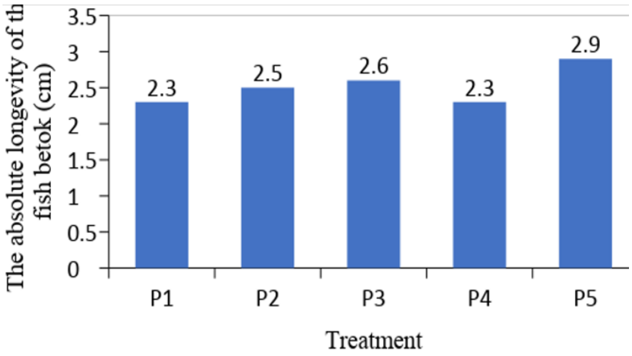

Fig. 5. Measured absolute length increase 40 days after hatching

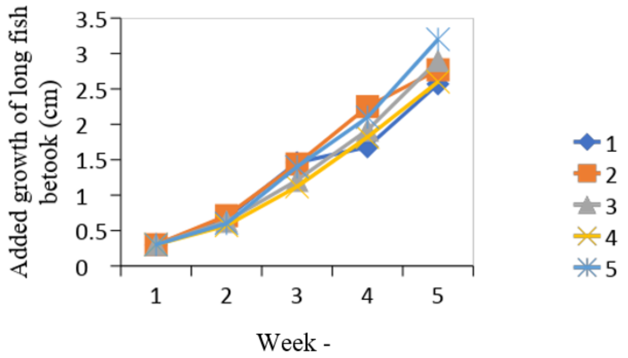

Fig. 6. Evolution of the increase in absolute length during the study period

Absolute Length Increase

The absolute length increase of the larvae 40 days after hatching is given in error: reference source not found, while the weekly increase is given in error: reference source not found. The highest increase in length is obtained with 2.9 cm for the treatment T5, followed by P3 with 2.6 cm, P2 with 2.5 cm, P1 and P4 with 2.3 cm. ANOVA indicates that the treatments have no significant effect (P> 0.05) on the absolute increase in length.

The high value of the absolute increase in length obtained with the T5 treatment is directly related to the absolute weight gain, which is a maximum for treatment with T5. In addition, the high value of absolute longevity growth in this treatment is in accordance with the high protein content of Tubifex sp. as well as having a suitable size for the mouth opening of fish larvae during maintenance. Unlike the case with artemia, the water fleas were smaller in size, so with the growing size of fish the artemia and water fleas were not optimal to be given to the fish anymore.

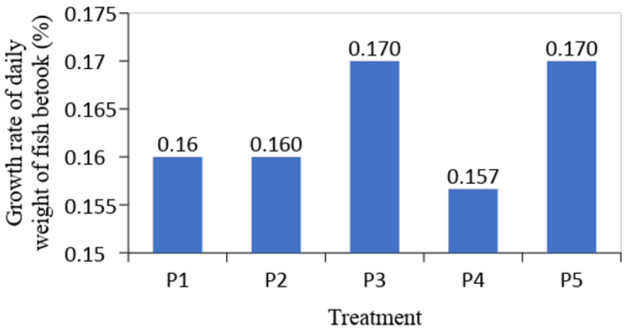

Fig. 7. Histogram growth rate of daily fish weight of each of the treatments during the study

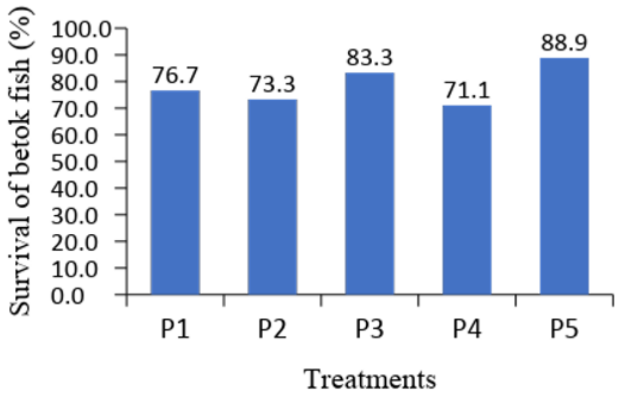

Fig. 8. Histogram of the survival of fish larvae from each treatment during the study

Growth Rate of Daily Weight

The results of the observations on growth rate of the daily weight of the fish from each of the treatments can be seen in **Fig. 7**. The highest daily weight gain value obtained were for treatments P3 and P5 each of 0.170%, followed by P2 and P1 respectively of 0.160% and P4 by 0.157%. The results of the analysis of variance (Anava) in any given treatment had no significant effect (P> 0.05) on the growth rate of daily weight.

The high value of growth rate of daily weight obtained in the treatment of P5 is due to Tubifex sp (**Fig. 7**). Which has the ability to survive a long time in a container so that maintenance can be better utilized by the fish to eat. Furthermore, artemia are small in size so that affects the overall size of the fish. The results of this study are similar to the results of research conducted by (Mahmood et al. 2004), where the highest daily growth rate of fish larvae also used the treatment of Tubifex sp. The average daily growth obtained from this study is greater than the average daily growth of the fish larva (Anabas testudineus) given + Aqua-Mos basalt feed which is 0.086 (Singh et al. 2018).

Larvae Survival

The measurement of the survival rate for the fish larvae from each treatment can be seen in **Fig. 8**. The highest survival value was obtained in T5 treatment 88.9%, followed by T3 83.3%, T1 equal to 76.7%, T2 equal to 73.3% and P4 by 71.1%. ANOVA indicates that the considered treatments did not have a real effect (P> 0.05) on the survival of the larvae of the "betok" fish.

The high survival rate of the larvae with the T5 treatment is related to the previous observations that the treatment T5 also leads to the highest absolute weight gain, absolute increase in length and daily weight gain. The survival rate obtained in this research (71.1 to 88.9%) as shown in **Fig. 8** was higher than in previous research by (Tampubolon et al. 2015) with 46.6 - 81%, Amin et al. (2015) who obtained a survival rate of 48.89-51.22%, Widiyati et al. (2018) who obtained the highest value of a survival fish betel (Anabas testudineus) 67.78 with maintenance at different bersalinitas water and by Bahera (2013) with the highest survival rate obtained 68.75%.

Several factors that influenced the occurrence of mortality are characteristics of the test individuals such as age and ability to adjust to the environment, but also external factors such as competition for getting food, population density, fish diseases, and other biological properties related to their life cycle (Nykolsky in Nusirhan 2009). Effendie (2002) states that natural food given in normal amounts can increase the larval life of farmed fish.

CONCLUSION

The best hormonal treatment to optimize the fertility and hatching rate of "betok" fish eggs was an injection of 0.5 ml of ovaprim / kg of body weight. This treatment leads to a fertility rate of 74% and an egg hatching rate of 75%. The best diet for the growth of the fish larvae consists of Tubifex sp from 3 to 43 days after hatching. This diet resulted in an absolute weight gain of 0.828 gram, an absolute increase in length of 2.9 cm, a daily weight gain of 0.170% and a survival rate of 88.9%.

REFERENCES

Amin MR, Rahman MM, Aktar S, Alam MB, Alam MA (2015) Induces Breeding of Vietnam's Koi (Anabas testudineus) Using Pituitary Gland (PG) And Ovaprim At Tasty Fish And Feeds Industries Limited. Melendh, Jamalpur. Asian Journal of Medical and Biological Research 1(3): 607-611. https://doi.org/10.3329/ajmbr.v1i3.26484

Arfah H, Maftucha L, Carman O (2006) Artificial Spawning on Gurame Fish (Osphronemus gouramy Lac.) With Ovaprim Injections. Department of Periran Cultivation, Faculty of Fisheries and Marine Sciences. Bogor Agricultural Institute.

Bahera S (2013) Survivability of Anabas testudineus larvae In Different Feed,Stocking Density And Water Depth. Journal of Bio Innovation 2(1): 1-7.

Chotipuntu P, Avakul P (2010). Aquaculture Potential of Climbing Perch, Anabas Testudineus, in Brackish Water. Walailak Journal Science and Technology 7(1).

Effendie MI (1997) Method of Fisheries Biology. Yayasan Dewi Sri. Bogor. 15-21. 122 p.

Effendie MI (2002) Fisheries Biology 2nd Edition (Revised Edition): Yogyakarta. Yayasan Pustaka Nusatama.

Harker K (1992) Breeding of hood by ovaprim in India. News Aquaculture 2(3).

Lam TJ (1985) Induced spawning in fish. In C. S. Lee and I. C. Liao (Eds). Reproduction and culture at Milkfish the Oceanic Institute, Hawaii.

Mahmood SU, Ali MS, Anwar-Ul-Haque M (2004) Effect of Different Feed on Larval / Fry Rearing of Climbing Perch, Anabas testudineus (Bloch), in Bangladesh: II. Growth and Survival. Pakistan J. Zool. 36(1): 13-19.

Mohseni M, Pourkazemi M, Hassani S, Okorie O, Min T, Bai S (2012) Effects of Different Three Live Foods on Growth Performance and Survival Rates in Beluga (Huso huso) Larvae. Iranian Journal of Fisheries Sciences (1): 118-131.

Nandeesha MC, Rao KG, Jayanna R, Parker NC, Varghese TJ, Keshavanath P, Shetty HPC (1990) Induced spawning of Indian Major Carps through single Application of ovaprim. In: Hirano, R. and I. Hanyu (Eds). The Second Asian Fisheries Forum, Asian Fisheries Society, Manila, Philippines.

Novianto E (2004) Evaluation of Ovaprim-C Injections with Different Doses to the Sumatran Fish (Puntius tetrazona). Department of Aquaculture, Faculty of Fisheries and Marine Sciences. Bogor Agricultural Institute.

Nursihan TSE (2009) The Influence of Different Paste Feedstuffs on Growth and Life of Fish Cell Larva (Ompok hypophthalmus). Essay. Faculty of Fisheries and Marine Sciences University of Riau. Pekanbaru. 50 items (unpublished).

Priyadi A, Kusrini E, Megawati T (2010) Treatment of Various Natural Foods to Increase Growth and Larvae Upside Down Catfish (Synodontis nigriventris). Proceedings of the Aquaculture Technology Innovation Forum. 749-754.

Ramdhani F (2011) Effectiveness of Spawn prim as accelerator of ovulation in comet fish (Carasius auratus). Department of Cultivation Waters, Faculty of Fisheries and Marine Sciences. Bogor Agricultural Institute.

Sarkar S, Rai BK, Bhutia D, Singh S, Pal J (2015) Study on the breeding performance and developmental stages of climbing perch, Anabas testudineus (Bloch, 1792) in the laboratory (Siliguri, India). International Journal of Fisheries and Aquatic Studies 2(6): 198-201.

Singh AA, Monsang SJ, Nanda S (2018) Effect Of Aqua-Most On Growth Performance And Survivability Of Climbing Perch (Anabas testudineus) During Larval Rearing. Journal of entomology and zoology studies 6(5): 83-90.

Subandiyah, Satyani S, Aliyah D (2003) The Influence of Natural Feed Substitution (Tubifex) and Artificial against the Growth of Red Tomorrow Fish (Mastacembelus erythrotaenia Bleeker, 1850). Journal of Indonesian Anthropology 3(2): 67-72.

Tampubolon EH, Nuraini, Sukendi (2015) The Influence of Different Natural Feeding Against Growth and Life of Fish Betva Larvae (Anabas Testudinieus). Faculty of Fisheries and Marine Sciences University of Riau. Pekanbaru.

Widiyawati A, Asyiah BN, Prihadi TH, Puspaningsih D (2018) Rearing Technology For Climbing Fish (Anabas testudineus) On Different Salinity. 2nd Scientific Communication in Fisheries and Marine Sciences (SCiFiMaS 2018). Vol. 47. https://doi.org/10.1051/e3sconf/20184702003

Yanhar, Nuraini, Yurisman (2009) Effect of Different HCG Dosage on Ovulation and Fishing Fish Hatching (Helostoma temmincki C. V). Faculty of Fisheries and Marine Sciences University of Riau. Pekanbaru. 37 pages.

Yulindra A, Lumbantoruan RP, Zulkifli, Sukendi (2017) Effect of Granting Ovaprim with Different Dosage to Ovulation and Eggs of Knife Fish (Notopterus notopterus). International Journal of Ocean and Oceanography 11(2).

Zalina I, Saad CR, Christianus A, Harmin SA (2012) Induced Breeding and Embryonic Development of Climbing Perch (Anabas Testudineus, Bloch). Journal of Fisheries and Aquatic Science 7(5): 291-306. https://doi.org/10.3923/jfas.2012.291.306