Tropical Wetland Journal
Journal homepage: twj.ulm.ac.id | Published by Postgraduate Program - Lambung Mangkurat University | e-ISSN: 2654-279X

Original article
DOI 10.20527/twj.v5i2.72

The Breeding of Climbing Perch (*Anabas testudineus*) with Meristic Phylogenetic Hybridization Technique Sampled from Three Types of Swamp Ecosystems

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Received: 14 October 2019; Accepted: 25 November 2019; Published: 25 November 2019

ABSTRACT

This study provides a valuable information on the patterns of hybridization in producing Climbing perch's fry which having a superior character to beneficially supports fish farming. The research was carried out at Pokdakan Rawa Sejahtera Amuntai, South Kalimantan Province, Indonesia from June to December 2018. The hybridization procedures were applied to the selected broodstocks (total 135), which taken from three different types of habitats namely rain reservoirs, monotonous and tidal swamps. A complete random design was used as a research method with three treatments and nine repetitions. A comprehensive investigation was done to provide the best performance among the treatments. The results showed that the hybridized fish from monotonous swamp × tidal swamp was the most superior across the trials in term of fecundity (18,500 eggs), GSI (18.1%), hatching rate (89.5%), relative length growth (1,375%), relative weight growth (1,850%) and the percentage of birth female (84 %) with the lowest mortality rate (29%). For aquaculture practices, the use of the fry unisexual female would increase the fish growth 270% faster than the fry male.

Keywords: Climbing perch, female, hybridization, monotonous swamp.

1. Introduction

Climbing perch (*Anabas testudineus* Bloch 1792) is commercially important freshwater fish species and beneficially supports fish farming and domestic market particularly in Indonesia (Akbar et al., 2016), Malaysia (Zalinaet al., 2012), Philippines (Bernal et al., 2015), Thailand (Piwpong et al., 2016); Cambodia (Sverdrup, 2002), Lao PDR (Sokhenget al., 1999), and Viet Nam (Phuonget al., 2006), Bangladesh (Uddinet al., 2016) and India (Ziauddin et al 2016). It has high quality meat, easy breeding, disease resisting, good consumer acceptance, and very adaptable to adverse environmental conditions such as low dissolve oxygen (DO). It has a high nutrition value of 19.50% of protein and 2.27% of lipid (Ahmed et al., 2012). It is also rich in iron and copper that support haemoglobin synthesis (Sarmaet al., 2010) and has high quality poly-unsaturated fats and many essential amino acids (Kohinoor et al., 1991). It can be found in all freshwater bodies such as rivers, streams, swamps, ponds, lakes, canals, reservoirs, and estuaries (Sarkar et al., 2005; Rahman and Marimuthu, 2010), and can also be cultured at cages, tanks, nylon hapas and ponds (Hasanet al., 2010; Mondalet al., 2010; Kumar et al., 2013) with different culture strategies (Phuonget al., 2006; Chotipuntu and Avakul, 2010; Putra et al., 2016, Izmaniar et al., 2018). It is categorized by the International Union for Conservation of Nature and Natural Resources (ICUN) as avulnerable species. Destructive fishing practices and polluted habitats may potentially threat this species (Kalita and Deka, 2013; Hossainet al., 2015). Some of studies are dedicated to describe on fecundity (Marimuthu et al., 2009; Ziauddin et al., 2016), stocking density (Habib et al., 2015; Uddin et al., 2016); breeding biology (Singh et al., 2012; Hafijunnaharet al., 2016), boldness (Binoy, 2015), morphometric characteristic (Hossain et al., 2015), length-weight relationship and condition factor (Kumar et al., 2013; Rahman et al., 2015), genetic characteristics (Sekino and Hara, 2000; Jamsari et al., 2010);growth performance (Muchlisin et al., 2017); feeding and social behavior (Zworykin, 2018) or environmentally friendly fishing practices for this species (Irhamsyah et al., 2017; Ahmadi, 2018; Ahmadi et al., 2018).

In South Kalimantan Province, climbing perch is locally called "*papuyu*" and contributes about 12% (8.31 tons) of total inland capture fisheries production (69.97 tons). The demand of climbing perch for consumption reaches 800 kg per day, where almost entirely sourced from the wild and only 10% produced from fish farming. Climbing perch is abundantly found in three different types of swamp areas, namely "monotonous swamp" located on Hulu Sungai Selatan District (452.704 ha), "rain reservoir swamp" in Banjar, Tanah Laut, and Pulau Laut Districts (169.094 ha), and "tidal swamp" in Barito Kuala, Tanah Laut, and Kota Baru Districts (372.637 ha). Climbing perch from swamp monotonous has the body colour of blue yellowish and a little dark, the size reaches about 25 cm length and 300 g weight, good taste and the growth is faster than those from rain reservoir swamp and tidal swamp. It is quite different from climbing perch of rain reservoir swamp, which has the body colour of yellow bluish, the size reaches 17 cm length and 150 g weight, the meat taste a little smell of mud and slower growth. While climbing perch of tidal swamp, it has body colour of green bluish, the size reaches 24 cm length and 300 g weight, the flavour of meat tasteless and growth faster than those of rain reservoir swamp (Slamat et al., 2011).

Recently aquaculture development of climbing perch on the controlled land is still much depended on the following factors such as the quality of seed, genetic, behaviour and its reproduction (Slamat, 2013). Climbing perch in habiting in different ecosystems will have different genetic diversity and the phenotype. The climbing perch of monotonous swamp have genetic diversity greater than that of rain reservoir swamp and tidal swamp. The genetic diversity of climbing perch can be seen from the diversity of meristic phenotype and of the DNA. It is already well-adapted for climbing perch sampled from different swamp ecosystems in South Kalimantan (Slamat et al., 2011). Analysis of genetic diversity is very important especially for endemic fish species to be used as brood stocks to produce superior seeds in aquaculture system. Griffit et al. (2000) suggested using the map of genetic diversity as a basic reference in performing the process of hybridization or breeding to look the pattern of gene dispersion that possibly forms organism body structure. Meanwhile, diversity of meristic phenotype is used to determine the level of the genetic diversity of fish. According to Frankham et al. (2002), the map of meristic diversity can be used as a tool in carrying out the process of breeding in animals and plants. The results of Identification and the characterizing of the meristic phenotype of climbing perch are divided into two groups of phylogenetic, i.e. the first group: monotonous and tidal swamps and the second group: rain reservoir swamp (Slamat et al., 2011). The characterizing of this meristic phenotype will be used as a basic of conducting hybridization research to provide high yield seeds. Increased productivity of climbing perch is being intensively done by both hatchery center and community hatchery units, but efforts to breeding them to produce superior seeds are still lacking. In line with population growth and economy improvement, it is predicted that market needs of climbing perch to meet public consumption for next 5 years ranging from 1.5 - 2 tons per day. For this reason, we carried out a series of laboratory and field experiments to make a breakthrough in generating superior seeds with rapid growth, marketable size (8-10 fish/kg), low mortality, high fecundity, tolerant of the adverse environment and more responsive to artificial feed. The result of this research will be the pioneer in breeding of swamp local fish species as part of the wetland excellent research of Lambung Mangkurat University.

2. Materials and Methods

This research was carried out from June to December 2018 at Pokdakan Rawa Sejahtera Amuntai, South Kalimantan Province, Indonesia. The brood stocks climbing perch were sampled from three different types of habitats namely rain reservoirs, monotonous and tidal swamps. The samples of broodstocks were transferred to Ichthyology Laboratory belongs to Faculty of Marine and Fisheries, Lambung Mangkurat University for further analysis. A complete random design was used as a research method with method with three treatments and nine repetitions. The hybridization process was undertaken by crossing bred of the brood stocks sampled. The tools and equipment used are a spawning pond, climbing perch brood stocks, spuit, hormone ovaprim, artificial feed, and fertilizer compost, microscope, camera, and digital balance. Data analysis was conducted on fecundity, gonadosomatic index (GSI), fertilization, hatching rates, mortality, the relative length growth, relative weight growth, food conversion ratio (FCR), mortality, and the percentage of male and female.

Fecundity

Fecundity is one of the important factors of the biology and population dynamics of fish (Alam et al., 2012). Fecundity is the number of ovum incurred in one cycle of spawning. Fecundity analysis is done to predict the number of offspring to be released in a spawning season. Fecundity is calculated bythe following formula (Hartman and Conkle, 1960):

$$\text{GSI} = \frac{\text{the average number of eggs x weight of gonads}}{\text{Weight of sub samples}}$$

Gonadosomatic Index

Gonadosomatic index (GSI) is an alternative method to determine sexual maturity or reproductive activity (Wang et al., 2003). GSI is the percentage of gonad weight to the total weight of the fish. It is used as an indicator for gonadal development in fish. GSI is determined using the following formula (Hafijunnahar et al., 2016):

$$\text{GSI} = \frac{\text{Gonad weight}}{\text{Total body weight}} \times 100$$

Fertilization

Fertilization is the process in which an egg and a sperm cell come together to form a zygote or the embryo. Observation on the eggs was done after spawning. The process of the embryo formation to the stage of the morula was observed under a camera microscope to ensure whether or not the ovum has been fertilized. After the morula phase, the observation is made every two-hour up to the hatchingphase. The fertilization rates of eggs were determined by randomly sampling 100 eggs in a petri dish. Fertilized eggs with an intact nucleus were counted to determine the percent fertilization. The fertilization rate was calculated by using the formula (Amornsakun, 2017):

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

Hatching Rate

Hatching rate is computed by using the following formula (Amornsakun, 2017):

$$\text{Hatching rate} = \frac{\text{Number of hatched eggs}}{\text{Total number of eggs}} \times 100$$

Survival Rate

Fish survival rate is calculated using the following equation (Yousuf et al., 2016):

$$\text{Survival rate} = \frac{\text{Number of survived fish}}{\text{The initial number of fish}} \times 100$$

Length Growth

The length growth of fish is the increase of the body length during cultivation process.

Weight Growth

The weight growth of fish is the increase of the bodyweight during the cultivation process.

Food Conversion Ratio (FPR)

Feed conversion ratio (FCR) is the amount of feed needed to produce one kilogram of meat. FCR indicating that the energy obtained from the feed for its metabolic activities. The feed conversion ratio was calculated by using the following formula (Asuwaju et al., 2014):

$$\text{Food Conversion Ratio} = \frac{\text{Diet fed (g)}}{\text{Weight gain (g)}} \times 100$$

Percentage of Male and Female

To differentiate between male and female, it can be easily visually done by observation of physical condition of the fish. Male has a small body, slender, and keep balancing its body in the water. While female has a long and large body, fattish, and brighter colors.

The relationship between the total fecundity and the body length is expressed as Y = a + bX. Where, Y is the fecundity, X is the total length, a is the values of intercept, and b is regression coefficient.

3. Results and Discussion

The overall measurement results on the size of broodstocks and the hybridized Climbing perch taken from three different types of swamp habitats were described in Table 1-2.

Table 1. The size of broodstocks Climbing perch used for hybridization treatments (mean±SD).

Broodstock Size	Monotonous swamp		Tidal swamp		Rain reservoir swamp	
	Male	Female	Male	Female	Male	Female
Number of fish sample	30	15	30	15	30	15
Total length (cm)	12±0,5	17.00±0.57	12±0,5	17.07±0.70	12±0,5	16.93±0.46
Body weight (g)	35±0,5	79.13±8.25	35±0,4	80.67±7.04	13±0,5	77.67±6.51

Table 2. Descriptive parameters of the hybridized Climbing perch from three different types ofswamp ecosystems.

Parameter Test	Hybridized Climbing perch		
	Monotonous ×tidal swamps	Monotonous × rain reservoir swamps	Tidal × rain reservoir swamps
Fecundity (eggs)	18,500	17,110	16,220
Gonadosomatic index (%)	18.1	17.7	17.5
Fertilization rate (%)	90.5	90.2	81.0
Hatching rate (%)	89.5	85.12	70.7
Relative length growth (%)	1,375	1,175	1,192
Relative weight growth (%)	1,850	1,550	1,450
Food Coversion Ratio (Protein 40%)	1.75	1.79	1.90
Percentage of male and female	84 ♀	78 ♀	71 ♀
Mortality (%)	29	33	30

The status of the hybridized fish was varying based on the characteristic of the crossbred brood stocks came from. The highest fecundity (18,500 eggs) produced by the broodstocks of monotonous swamp × tidal swamp, followed by monotonous swamp × rain reservoir swamp (17,110 eggs) and tidal swamp × rain reservoir swamp (16,220 eggs). This indicates that there was an aptness of breeding pairs between brood stocks of different habitats in performing a process of reproduction. The aptness of this breeding pair would optimize the function of hormone to produce new individuals which are superior to their brood stocks. The fecundity in the present study was considerably lower than the fecundity of climbing perch taken from the Layar Tengah River near Sungai Petani, Kedah, Malaysia (Marimuthu et al., 2009) or from Rupali Fish Hatchery, Bangladesh (Hafijunnahar et al., 2016). The success level of a spawning fish can be assessed from the percentage of fecundity and of juveniles to become adult fish (Alam et al., 2012). According to Slamat (2013), fecundity can be used to estimate the number of offspring to be born in a spawning season, brood stock productivity and the age class of the fish.

Observation on Gonadosomatic index (GSI) was under taken to comprehend the readiness of brood stocks to spawn. The highest GSI produced by the brood stocks of swamp monotonous × tidal swamp (18.1%), followed by monotonous swamp × rain reservoir swamp (17.7%) and tidal swamp × rain reservoir swamp (17.5%). The values of GSI in this study were relatively higher than GSI of climbing perch sampled from the river Layar Tengah near Sungai Petani, Kedah, Malaysia (Marimuthu et al., 2009) or from Rupali Fish Hatchery, Bangladesh (Hafijunnahar et al., 2016). The crossbred climbing perch of swamp monotonous and tidal swamp in the present study was closely related to their environmental conditions that more stable, food source in great abundance and greater gonad size (Slamat, 2012). The fertilization and the egg hatchability can be used as indicator to find out the optimal spermatozoa in fertilizing ovum generated by brood stocks from different swamp habitats. The crossbred brood stocks of swamp monotonous and tidal swamp generated the highest rank in fertilization rate (90.5%) and hatching rate (89.5%), followed monotonous swamp × rain reservoir swamp (FR=90.2%, HR=85.12%) and tidal swamp × rain reservoir swamp (FR=81.0%, HR=70.7%). The fertilization rate obtained in the present study was similar to induced breeding of climbing perch in laboratory, Siliguri, India (Sarkar et al., 2015). The higher percentage of fertilization and hatching rates the better the quality of eggs and sperm produced by the fish. Variation in the value of fertilization and hatching rates may be attributed to brood stocks health, GSI, environmental conditions, brood stocks origin, age and size of brood stocks and nutrients supply during pregnancy. Observation on the relative length and weight growth was intended to see accumulation of the hybridization treatment that influence the growth of fish tested.

Observation on fish larvae was made started from 0 to 60 days. The length and weight growth is a performance indicator to ensure the success of hybridization practices.

The results also clearly showed that the hybridized fish from monotonous swamp × tidal swamp was found to be the dominant characteristic in the length growth (1,375%) and weight growth (1,850%). The functional growth dominance of climbing perch inhabited monotonous swamp is more based on their performance characteristic such as a greater genetic diversity, more adaptable to new environment, great feeding response, tamer and relatively resistant to the negative effects of environment change. Slamat (2013) strongly recommended selecting the brood stocks of monotonous swamp and tidal swamp with the body size of ≥ 300 gr per individual in order to produce offspring greater than their brood stocks. The idea is in line with the previous study reported by Wahyudewantoro and Haryono (2013). Apparently the hybridized fish from monotonous swamp × rain reservoir swamp had a similar character with tidal swamp × rain reservoir swamp especially feeding response resulted in slow growth.

The feed conversion ratio (FCR) Is a helpful way of describing efficiency in terms of how much feed is required to produce 1 kg of fish. FCR is the basis of all feed formulations because feeds are compounded to support profitable and healthy growth of the fish. Feed conversion ratios are important calculations for the fish farmers to determine if feeds are being used as efficiently as possible (Goddard, 1996). The feed consumed by the fish should have enough protein to support its growth. In the present study, the fish were fed with pellet containing protein up to 40%. There was a variation in the value of FCR among the hybridized fish tested. The highest percentage of FCR was found in the hybridized fish fr om tidal swamp × rain reservoir swamp (1.90%), followed by monotonous swamp × rain reservoir swamp (1.79%) and the lowest FCR was provided by monotonous swamp × tidal swamp (1.75%). Environmental conditions that conducive, proper nutrition and source of guaranteed seeds are needed to produce healthy fish. The health indicator of the fish can be seen from a perfect physical condition, normal behavior, great feeding response and optimal growth. Generally the hybridized fish in the present study was considered fit and healthy, and tends to be better than its brood stocks. One of the success of hybridization indicated by the increase percentage of male-female which is greater than its brood stocks. The hybridized fish from monotonous swamp × tidal swamp showed the highest percentage of female (84%), followed by monotonous swamp × rain reservoir swamp (78%) and tidal swamp × rain reservoir swamp (71%). If spawned normally, population derived from the same habitat resulted in the ratio of male to female is 50 : 50, and it tends higher percentage of male spawned as compared to female due to some factors such as high temperature, high intensity of sunlight and non-selective brood stocks. By selecting the broodstocks properly, we can produce the unisex female up to 84%. Moreover, the hybridized fish from swamp monotonous and tidal swamp was observed to have the lowest mortality rate among others.

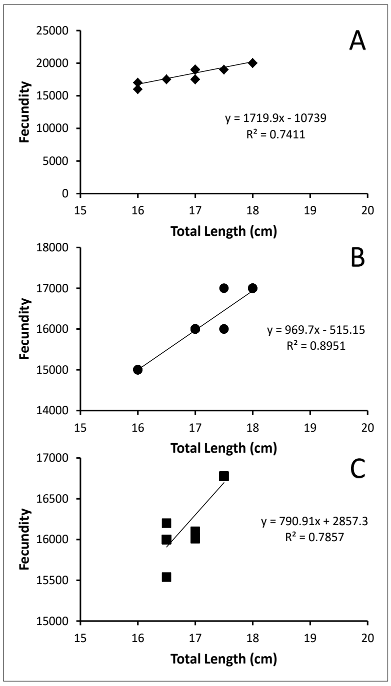

Figure 1. The relationship of fecundity and total length of Climbing perch from monotonous swamp (A), tidal swamp (B) and rain reservoir swamp (C)

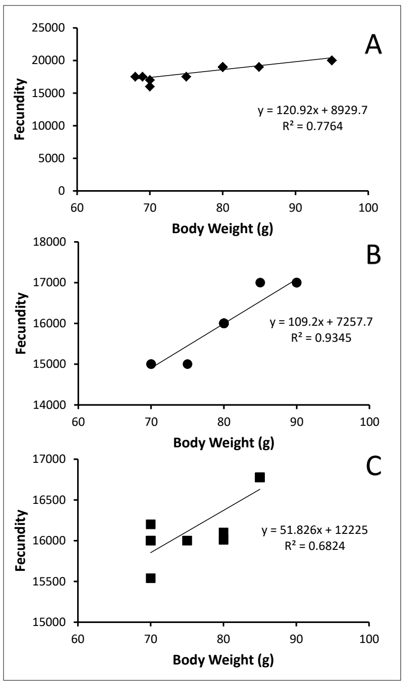

Figure 2. The relationship of fecundity and body weight of Climbing perch from monotonous swamp (A), tidal swamp (B) and rain reservoir swamp (C)

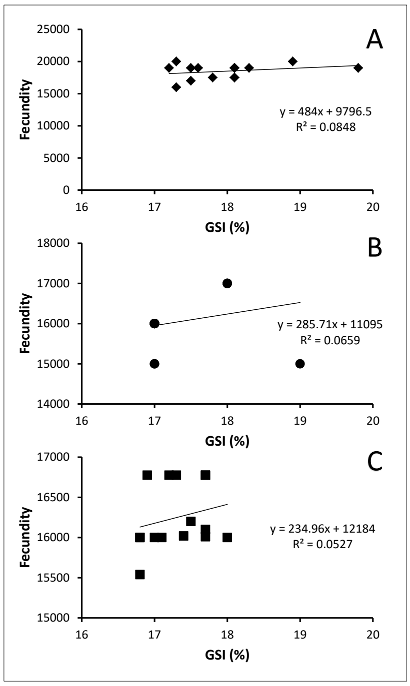

Figure 3. The relationship of fecundity and GSI of Climbing perch sampled from monotonous swamp (A), tidal swamp (B) and rain reservoir swamp (C)

Figure 1A shows the relationship between fecundity (F) and total length (TL) of Climbing perch females from monotonous swamp, which was indicated by the equation : F = 1719.9TL– 10739. The coefficient of determination (R^2) obtained was 0.7411 indicating that more than 74% of variability of the fecundity is explained by the length. The coefficient of correlation(r) was 0.8609, found to be higher than 0.5, showing the fecundity-length relationship is positively correlated. For females taken from tidal swamp, the relationship between fecundity and total length was given in the formula: F = 969.7TL– 515.15 (Figure 1B). The coefficient of determination (R^2) obtained was 0.8951 indicating that more than 89% of variability of the fecundity is explained by the length. The coefficient of correlation was highly positive (r = 0.9461). A linear relationship was also observed between fecundity and total length of Climbing perch sampled from rain reservoir swamp (Figure 1C). Such relationship was expressed as: F = 790.91TL + 2857.3 (R^2 = 0.7857), indicating that more than 78% of variability of the fecundity is explained by the length. The coefficient of correlation was found to be positive (r = 0.8864).

The relationship between fecundity (F) and body weight (W) of Climbing perch females collected from monotonous swamp was shown in Figure 2A, and was expressed as F = 120.92W + 8929.7 (R^2 = 0.7764), indicating that more than 77% of variability of the fecundity is explained by the weight. The coefficient of correlation obtained was highly positive (r = 0.8811). The minimum and maximum numbers of eggs were 16,000 and 19,995 spawned by females weighted 70 g and 95 g respectively. For females sampled from tidal swamp, the relationship between fecundity and body weight was expressed in the formula: F = 109.2W + 7257.7 (Figure 2B). The R^2 value obtained was 0.9345, indicating that more than 93% of variability of the fecundity is explained by weight. The coefficient of correlation(r) was 0.9667, indicating that the fecundity-weight relationship is positively correlated. The minimum and maximum numbers of eggs were 16,000 and 17,000 for corresponding body weight of fish is 70 g and 90 g respectively. A linear relationship was also observed in females sampled from rain reservoir swamp (Figure 2C). Such relationship was expressed as: F = 51.826W + 12225 (R^2 = 0.6824), indicating that more than 68% of variability of the fecundity was explained by the weight. The correlation coefficient was found to be positive (r = 0.8261). The minimum and maximum numbers of eggs were 15,540 and 16,776 generated by females with 70 g and 95 g weight respectively.

Figure 3A shows the relationship between fecundity (F) and GSI (%) of Climbing perch females from monotonous swamp. The equation of curve on the graph was F = 484GSI + 9796.5 (R^2 = 0.0848). GSI varied between 17.20 and 19.80% (17.98±0.68%). For females taken from tidal swamp, the relationship was expressed as: F = 285.71GSI + 11095 (Figure 3B). GSI falls between 17.00 and 19.00% (17.40±0.63%). A linear relationship was also observed in females sampled from rain reservoir swamp (Figure 3C). The fecundity-GSI relationship was shown in equation: F = 234.96GSI + 12184 (R^2 = 0.0527). GSI ranged from 16.80 and 18.00% (17.31 ±0.40%). The variation in fish fecundity is not only due to fish length and weight but also influenced by age, nutritional diet, environmental condition in the water bodies and food availability (Sivashanthini et al., 2008; Ghafari and Jamili, 2010; Lawson, 2011). A linear and positive relationship in the present study correspond well with the previous studies (Marimuthu et al., 2009, Zalina et al., 2012; Islam et al., 2012; Hafijunnahar et al., 2016). After all, the use of the fry unisexual female would increase the fish growth 270% faster than the fry male.

4. Conclusions

The hybridized Climbing perch from monotonous swamp × tidal swamp was considered the most superior among other treatment approaches for this species. The use of fry-unisexual female is strongly recommended for the success of aquaculture operations.

Acknowledgments

We would like to thank the Heads of Water Quality Laboratory and Ichthyology Laboratory of FPK-ULM who have provided facilities and supported us during research periods. Our gratitude goes to the reviewers for significantly improving the contents of manuscript to a publishable level.

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