International Journal of Innovative Studies in Aquatic Biology and Fisheries
Volume 5, Issue 2, 2019, PP 11-20
ISSN 2454-7662 (Print) & ISSN 2454-7670 (Online)
DOI: http://dx.doi.org/10.20431/2454-7670.0502003
www.arcjournals.org

Variation of Broodstock Sizes and Types of Aquatic Plantstoward Breeding and Survival of Newly Hatched Larvae of Climbing Perch(*Anabas testudineus*Bloch 1792)

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Abstract:*A series of laboratory experiments were conducted to find out the best performance of different broodstock sizes (14 and 16 cm TL) and type of water plants (Eichhorniacrassipesand Hydrillaverticillata)on the basis of breeding process and survival of newly hatched larvae of Climbing perch.The experimental design used was Factorial Randomized Completely Design (18 treatment units). A total of 54 individual broodstocks (sex ratio of 2M:1F) associated with aquatic plants and control were investigated. Overall, the best performance was found in treatment of A2B1 (broodstock 16 cm TL with E.crassipes) particularly in fecundity (37,440± 19,970 eggs) and fertilization rate (81.92 ± 10.33%).The ANOVA test showed that the treatment significantly affects the whole parametersobserved.Low survival in the present study was more attributable to inability of larvae to consume natural food at early days rather than water quality condition factor.In addition, Climbing perch broodstocks in present study grew negatively allometric (b = 2.63-2.68). During the research, water quality was in tolerance range for Climbing perch larvae rearing: temperature was 26-30ºC, pH 6 -7, DO 3.4- 4.0 mg L^{-1}, and Ammonia 0.001-0.043 mgL^{-1}.*

Keywords: *Climbing perch, broodstock size, fish breeding, larval survival,aquatic waters.*

1. INTRODUCTION

Like other countries (e.g. Malaysia, Philippines, Thailand, Viet Nam, India and Bangladesh), the presence of Climbing perch (*Anabas testudineus*) in Indonesia also plays important role in both fisheries and aquaculture [1] due to its high quality meat, easy breeding, disease resisting, good consumer acceptance, and very adaptable to adverse environmental conditions such as low dissolve oxygenand thermal change [2,3].It is rich in iron and copper that support haemoglobin synthesis [4] and has high quality poly-unsaturated fats and many essential amino acids [5]. It also provides 19.50% of protein and 2.27% of lipid [6], whilecarbohydrate to lipid ratio (CHO: L) of 2.29 performed the best for growth performance, feed and proteinutilization and whole body composition [7]. This species inhabiting all freshwater bodies such as swamps, rivers, wetlands, canals, and reservoirs [8,9,10], and can be cultured at cages, tanks, nylon hapas, ponds and brackish water [11,12,13] with different culture management systems[14,15,16]. It is categorized by theInternational Union for Conservation of Nature and Natural Resources (ICUN) as avulnerable species.Habitat modification, destructive fishing practices, and polluted habitats may potentially threat this species [17,18,19]. Somefundamental studies have been addressed to describe on breeding biology [2,20,21], fecundity [10,22,23], stocking density [12,24], boldness [25],seasonal gonad cycle [8], length-weight relationship and condition factor[9,26], morphometric characteristic [17],genetic characteristics [27,28], growth performance [16,29],feeding and social behavior [30],food habits [31], environmentally friendly fishing practices [1,32,33] or business prospect of this species[34].

In South Kalimantan Province, Climbing perch is locally called “*papuyu*”.This species is abundantly found in three different types of swamp areas, namely “monotonous swamp” located in Hulu Sungai Selatan District (452.704 ha), “rain reservoir swamp” in Banjar, Tanah Laut, and PulauLaut Districts (169.094 ha), and “tidal swamp” in Barito Kuala, Tanah Laut, and Kotabaru Districts (372.637 ha). It 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, which is almost entirely sourced from the wild and only 10% produced from fish farming. 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 the time being, the fish farmers are still being constrained by some fundamental factorssuch as slow growth, high mortality, low hatching rate, and high feedconversionratio. After all,the broodstocksselection factor with the suitable environmental conditions is essential to success in order to improve the quality of fish seed, reproduction and also its genetics. Moreover,the existence of aquatic plants (e.g. *Eichhorniacrassipes* and *Hydrillaverticillata*)in culture systemwas reported successfully in increasing survival rate,fertility and hatchability of Nile tilapia [35], gourami [36],comet goldfish [37], and pearl gourami [38]; while its implication to Climbing perchis still questionable. For these reasons, we carried out a series of laboratory experiments to find out the best performanceof different broodstock sizes and type of aquatic plants used on the basis of breeding process and survival of newly hatched larvae of Climbing perch.

2. MATERIALS AND METHODS

2.1.Study Sites

This research was conductedin the Wet Laboratory belongs to Faculty of Marine and Fisheries, LambungMangkurat University from January to March 2019.The broodstocks of Climbing perch were obtained from local community hatchery unit (UPR Barabai, South Kalimantan Province) and were transferred to the Wet Laboratory as experimental animals.

2.2.Experimental Design and Procedure

The experimental design used was Factorial Randomized Completely Design.The first factor (A) was the variation of broodstocks sizes i.e. A1 = 14 cm TL and A2 = 16 cm TL (total length). The second factor (B) was types of aquatic plants i.e. B1 = water hyacinth (*E. crassipes*),B2= algae (*H. verticillata*), and B3 = without aquatic plants (control);thus there were six treatment combinations with three repetitions, resulted in 18 experimental treatment units.A total of 18 aquariums (60×40×30 cm) containing the broodstocks associated with aquatic plants and also the control were used.Each aquarium was first sterilized with Potassium Permanganate (KMnO 4) of 20 mg L^{-1} for 1 hour, and then washed off with clean water,dried,filled with water to 20 cm height and runaeration accordingly.A total of 54 broodstockscomprising 36 males (67%) and 18 females (33%) with the sex ratio of 2:1 were investigated (Table 1).For small individuals, the mean sizes of males were 12.75 ± 0.56 cm TL (12.20 - 13.30 cm) and 35.30 ± 5.31g weight (31.70 -42.60 g), while for females were 14.21 ± 0.08cm TL (14.10 -14.30 cm) and 48.31 ± 0.81g weight (47.90 -49.60 g).For large individuals, the mean sizes of males were 15.20 ± 0.08cm TL (15.10 - 15.30 cm) and 59.37 ± 0.10g weight (59.20 - 59.50 g), while for females were 16.21 ± 0.08cm TL (16.10 - 16.30 cm) and 68.32 ± 0.80g weight (67.40 - 69.20 g). The length-weight regression equations were also presented. During nighttime, both females and males were injected with the ovaprim hormone with doses of 0.2 mL and 0.1 mL per 100g of body weights, respectively. Females to spawn after the injection process of 5-8 hours. The breeding process ends marked with the eggs floating on the water surface at early morning and the broodstocks were taken out from the aquarium.Before this, we made*Daphnia*sp. culture by using the fermentedstraw and banana stems as growth media for a week.The larvae were daily feed with *Daphnia*sp. as much about 1,134 individuals per tank.

Table1.*The descriptive broodstock sizes of Climbing perchused for breeding process*

Broodstocksizes	Small individual		Large individual	
	Male	Female	Male	Female
Number of sample	18	9	18	9
Total length (cm)	12.74 ± 0.56	14.21 ± 0.08	15.20 ± 0.08	16.21 ± 0.08
Range (cm)	12.20 - 13.30	14.10 - 14.30	15.10 - 15.30	16.10 -16.30
Weight (g)	35.30 ± 5.31	48.31 ± 0.81	59.37 ± 0.10	68.32 ± 0.80
Range (g)	31.70 - 42.60	47.90 - 49.60	59.20 - 59.50	67.40 - 69.20
Length-weight regression equations	y = 0.0554x + 1.4821	y = 1.8417x - 0.4388	y = 0.1284x + 1.6219	y = 1.0548x + 0.5584
Coefficient determination (R^2)	0.0003	0.0003	0.1713	0.1900

2.3.Fecundity

Fecundity is one of the important factors of the biology and population dynamics of fish[39]. 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 was measured with volumetric calculation [40]:

$$R = \frac{n1+n2+n3}{3} \qquad V = \frac{Va}{n} \qquad F = V \times R$$

Where: n is sample volume, nais number of eggs taken for sample, R isthe average eggs taken from sample volume, V is water volume divided by sample volume, Vais volume of water, and Fis number of eggs.

2.4.Fertilizationand Hatching Rates

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.A total of 500 eggs were placed in each aquarium and they will hatch become the larvae after 18-24 hours in room temperature. The fertilization rate and hatching rate were calculated by using the following formulas[41]:

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

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

2.5. Survival and Mortality Rates

After hatching upon the two days old, the larvae just consumed the egg yolk in their bodies as a food reserve. Afterward the larvae were fed with *Daphnia*sp. three times a day for 13 days. At 15 days old, the pellet was given twice a day to increase the survival of the larvae. The larvae were kept in the aquarium for 30 days to observe their survival.The survival rateand mortality rate of larvae are calculated using the formulas[42]:

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

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

2.6. Length-Weight Relationship

The length-weight relationship of fish can be expressed in both allometric form and logarithmic equation[43]:

$W = aL^b$

Log W= Log a + b Log L

Where: W is the weight (g), L is the total length (mm), *a* is the constant showing the initial growth index and b is the slope showing growth coefficient. The b value has an important biological meaning; if fish retains the same shape, it grows increase isometrically (b = 3). When weight increases more than length (b> 3), it shows positively allometric. When the length increases more than weight (b< 3), it indicates negatively allometric[26].The coefficient of determination (R^2) and coefficient of correlation (r) of length-weight variables between male and female were also computed. Water quality parameters such as water temperature, pH,dissolved oxygen (DO), and ammonia (NH3) were also recorded.

3. Results And Discussion

The overallmeasurement resultsonthe fecundity, fertilization rate,hatching rate, survival rate and mortality rate of the Climbing perchtested in the experimental tanks were described in Table 2.Each treatment has own characteristic of performance and statistically comparable between parameters tested.

Table2.*The treatments and parameters observed for Climbing perch in the experimental tanks.*

Treatment	Mean and standard deviation of parameters observed				
	Fecundity (egg)	Fertilizationrate (%)	Hatching rate (%)	Survival rate (%)	Mortality rate (%)
A1B1	14,880 ± 8,532	69.73 ± 1.49	96.67 ± 2.64	18.22 ± 0.84	81.78± 0.83
A1B2	11,040 ± 4,320	72.07 ± 10.99	95.67 ± 5.27	21.14 ± 0.76	78.86± 0.75
A1B3	9,920± 8,881	74.63 ± 9.18	86.40 ± 11.60	12.76 ± 2.10	87.24± 2.10
A2B1	37,440± 19,970	81.92 ± 10.33	91.87 ± 7.13	22.41 ± 1.33	77.59± 1.33
A2B2	21,760 ± 6,337	80.69 ± 5.81	85.93 ± 8.60	22.50 ± 5.79	77.50± 5.78
A2B3	12,320 ± 4,355	81.46 ± 7.60	78.00 ± 17.42	13.70 ± 2.45	86.30± 2.45

A1: broodstock of 14 cm TL, A2: broodstock of 16 cm TL, B1: Eichhorniacrassipes,B2: Hydrillaverticillata, B3: without aquatic plants.

3.1. Fecundity

The highest fecundity was produced by A2B1treatment:broodstock of 16 cm TL with *E.crassipes*(37,440± 19,970 eggs), followed by A2B2:broodstock of 16 cm TL with *H.verticillata*(21,760 ± 6,337 eggs), A1B1:broodstock of 14 cm TL with *E.crassipes*(14,880 ± 8,532 eggs), A2B3:broodstock of 16 cm TL without aquatic plants (12,320 ± 4,355 eggs), A1B2: broodstock of 14 cm TL with *H.verticillata* (11,040 ± 4,320 eggs), and A1B3:broodstock of 14 cm TL without aquatic plants (9,920± 8,881 eggs).The variation in fish fecundity is not only due to fish length and weight, but also influenced by age, nutritional diet, broodstocks condition,environmental condition and food availability[10,22,30].

The ANOVA test showed that the treatment has a significant effect on the fecundity of fish. Further analysis with Duncan Multiple Range Test (DMRT) revealed that the treatment of A1B2 or A1B3 was significantly different from A2B1, butit was not significantly different from A2B2, A1B1, A2B3, and A1B2.Dealing with the breeding process, it was clearly observed that the broodstock of Climbing perch preferred with the presence of *E.crassipes*as compared to *H.verticillata*. This because *E.crassipes* have rootsthat stretch down, lush, flexible, smooth, and float on the waterserved as a good shelter and allow for ovulation process become faster. While *H. verticillata*were a type of sinkable aquatic plants that have a rough and lush texture resulted in the broodstockssenseinconveniently around them so that spawning process may takeslightly longer. On the other words, the presence of these aquatic plants in the breeding process showing better results than without them. The broodstock sizes used in the present study are within the range of sizes suggested by other research workers i.e. 12.4-19.2 cm TL [10] and 16.3-19.2 cm TL [44].The fecundity produced by the broodstocks of 14-16 cm TL in this research ranged from 4,320 to 59,520eggs, which is in agreement with the finding of Marimuthu[10] that is between 3,120 and 84,690 eggs generated by female of 12.4-19.2 cm TLsampled from the river Layar Tengah near Sungai Petani, Kedah, Malaysia. However, it was considerably lower than the fecundity of climbing perchtaken from Rupali Fish Hatchery, Bangladesh [2], which is ranged from 50,610 to 227,378in individuals of total length from 19-24 cm, indicating that the bigger size of fish the higher fecundity (Figure 1).In addition, Slamatand Pahmi[44] strongly recommended selecting the broodstocks with the body size of≥ 300 gr per individual in order to produce offspring greater than their broodstocks. The idea is supported by Wahyudewantoro and Haryono[45]. Fecundity can be used to estimate the number of offspring to be born in a spawning season, broodstockproductivity and the age class of the fish[44].No interaction between the broodstock sizes and the aquatic plants to the fecundity of fish was observed (F_{count} = 1,470<F_{table} = 3.885).

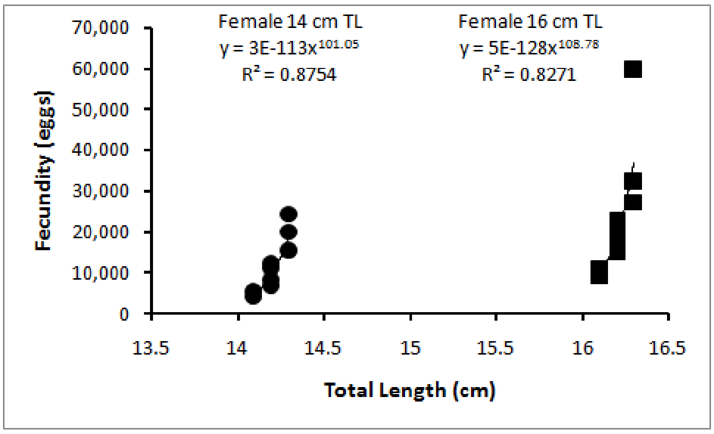

Figure1. *The relationship between total length and fecundity of Climbing perch female broodstocks with different sizes.*

3.2. Fertilization Rate

The treatment of A2B1 generated the highest fertilization rate (81.92 ± 10.33%), followed by A2B3 (81.46 ± 7.60%), A2B2 (80.69 ± 5.81%), A1B3 (74.63 ± 9.18%), A1B2 (72.07 ± 10.99%), and the lowest one was A1B1 (69.73 ± 1.49%).The fertilization rate obtained in the present study was similar to induced breeding of climbing perch in laboratory, Siliguri, India [20]. 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 tobroodstockshealth, Gonadosomatic index (GSI), environmental condition, broodstocksorigin, age and size of broodstocks and nutrients supply during pregnancy[3,21].Analysis of variance showed that the fish length significantly affects the fertilization rates of fish (F_{count} = 5.651>F_{table} = 4.747), but aquatic plants did not.No interaction between the broodstock sizes and the aquatic plants to fertilization rates was observed (F_{count} = 0.165 <F_{table} = 3.885).The fertilization ratein the egg cell is strongly influenced by the quality of sperm, the egg cell produced,movement of spermatozoa to the micropyle hole of the egg cell,and also water quality particularly water temperature [46,47]. The fertilization and the egg hatchabilitycan be used as indicator to find out the optimal spermatozoa in fertilizing ovum generated by broodstocks.

3.3. Hatching rate

The treatment of A1B1 was observed to have the highest hatchingrate (96.67 ± 2.64%), followed by A1B2 (95.67 ± 5.27%), A2B1 (91.87 ± 7.13%), A1B3 (86.40 ± 11.60%), A2B2 (85.93 ± 8.60%), and A2B3 (78.00 ± 17.42%).There was no significant difference between treatments and hatchingratesof fish (F_{count} = 2.638<F_{table}=4.747), as well as interaction between the sizes of broodstock and aquatic plants towards the hatching rates (F_{count} = 0.098 <F_{table} = 3.885). The hatching rates of 85-96% with the sex ratio of male to female 2:1 in the present study was higher than those of 40-85% with the sex ratio 1:1 reported by Slamatet al.[48]. WhileBurmansyahetal.[31]found no significant differences in the sex ratios (1:1, 1:2, 1:3, 1:4) toward the hatching rates of Climbing perch by mean of semi-natural breeding.The hatching rates of fish are greatly influenced by some factors such as the type of fish, age, nutrient availability, level of gonad maturity, the quality of sperm, fish health, the egg condition, and environmental conditionparticularly water temperature, water salinity[20,21,49,50].

3.4. Survival Rate

The ANOVA test showed that the treatment significantly affects the survival rates of the fish (F_{count} = 84,129>F_{table} = 3.885).The best performance for the survival rate was given by the treatment of A2B2 (22.50 ± 5.79%), followed by A2B1 (22.41 ± 1.33%), A1B2 (21.14 ± 0.76%), A1B1 (18.22 ±0.84%), A2B3 (13.70 ± 2.45%), and A1B3 (12.76 ± 2.10%). The highest survival rate for A2B2 treatment positively corresponds to fecundity, fertilization and hatching rates obtained. The presence of aquatic plants significantly affects the survival rates of the fish. The treatment of A1B2, A2B1 or A2B2 was considerably higher than that of A2B3, indicating thatthe presence of aquatic plants can help increasing the survival rates of larvae up to 22.5% or almost two times higher than treatment with no aquatic plants, other than *Daphnia*sp. supply. The survival rates of Climbing perch in the present study are greatly lower as compared to other studies in terms of dietary approaches [16,29,51].

3.5. Mortality Rate

Mortality is the number of dead fish during culture process. The highest mortalityrate was observed in the treatment of A1B3 (87.24± 2.10%), followed by A2B3 (86.30± 2.45%), A1B1 (81.78± 0.83%), A1B2 (78.86± 0.75%), A2B1 (77.59± 1.33%), and lastly was A2B2 (77.50± 5.78%).The ANOVA test showed that the treatment significantly affects the mortality rates of the fish (F_{count} = 16,140>F_{table} = 3.885).Further analysis was found that the treatments of A1B2, A2B1, and A2B2 were significantly different from A1B3, A2B2, and A2B3.While the treatments of A2B3 and A1B1 were not significantly different from A1B2 and A1B3.No interaction between the broodstock sizes and aquatic plants towards the mortality rates was detected (F_{count} = 0,599<F_{table} = 3.885).The presence of aquatic plants can help reducing mortality rate of larvae up to 18% as compared to the absence of them; this because aquatic plantsprovided a good supply of water quality, served as shelter from cannibalism attacks, and can be used as an agent of bioremediation.

It is recognized that making into the fingerling size is crucial to success due tomortality question at larval stage.The critical period of larvae occurs at day 7 to day 14 following the development of larval mouth opening, when the larval yolk has run outand larvae need feed from the outside. If the mouth opening is not well-developed, the larvae will take the trouble with its dietary and potentially causes cannibalism leading to the death.Moreover the average size of *Daphnia*sp. given to the larvae was about 125µm, which is bigger than the mouthopening of larvae observed at day 3 to day 7. Thus the high mortality rate in the present study was more attributable to this circumstance rather than water quality condition.Rukmini[52] reported that the average mouth opening of larvae at day 3 and day 7 was 103.11 µm and106.02 µmrespectively, and suggested that the natural feed given should be less than these values.

Figure 2 shows the relationship between the treatments and growth rate of the larvae during observation periods. The results clearly demonstrated that the treatment of A2B1 produced the best performance in term of larval growth rate (21.98%), followed by A1B1 (20.88%), A2B2 (16.48%), A1B2 (15.38%), A2B3 (13.19%) and A1B3 (12.09%), indicating that the presence of aquatic plantswas beneficial for larvae to grow faster than without aquatic plants. Overall, the larval growth obtained ranging from 0.22 to 0.40 g weight per individual. The use of aquatic plants also capable of increasing the larval survival of catfish in the tank by 95.49% and specific growth rates of 1.53% in length for 28 days [53].

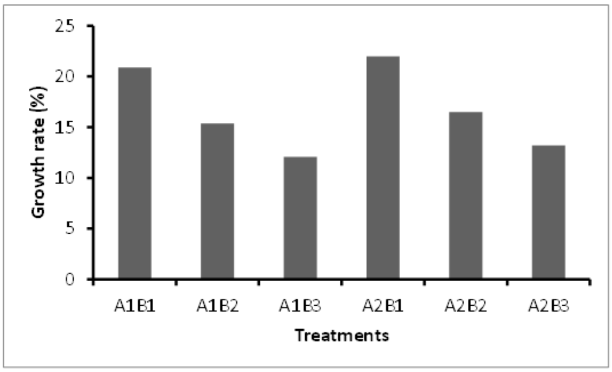

Figure2.*The relationship between growth rate and treatments given to Climbing perch associated with the aquatic plants during research periods.A1: broodstock of 14 cm TL, A2: broodstock of 16 cm TL, B1: Eichorniacrassipes, B2: Hydrillaverticillata, B3: without aquatic plants.*

3.6. Length-Weight Relationship

The estimated *b* values obtained from the length-weight relationship equations were 2.68 for male and 2.63for female (Figure 3), indicating that Climbing perch broodstocks used in the present study grew negatively allometric (b< 3), which means that the length increases more than weight.The R^2 values ranged from 0.7898 and 0.9947representing that more than 78% of variability of the weight is explained by the length. The coefficient of correlation(r) of male and female were 0.8887 and 0.9973,found to be higher than 0.5, showing the length-weight relationship is positively correlated.Negative allometric growth pattern in this study was also reported by other research workers [17,26,54].

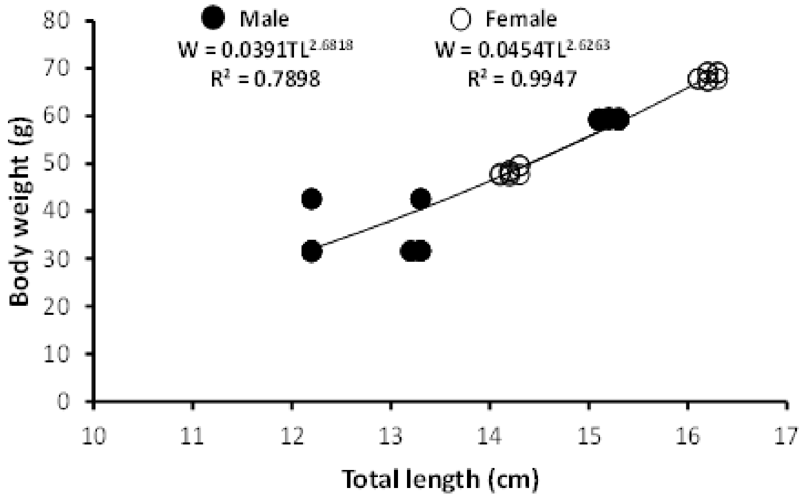

Figure3. *The relationship between total length and body weight of Climbing perch broodstocks that showing negative allometric growth pattern.*

3.7. Water Quality

The measurement results of water quality in the experimental tanks for Climbing perch were presented in Table 3.A suitable water quality parameter is an important prerequisite for healthy aquatic environment, better production and breeding success[2].During the research, water qualities were in tolerance range for Climbing perch larvae rearing.According to Widodoet al.[55], the optimum water temperature for the growth of Climbing perch ranges of 25-30°C, andwater temperature measured in this research (26-30°C) was fixed accordingly.The pH value obtained during the study rangesof 6-7. While Widodoet al.[55] reported that Climbing perch can grow normally in waters with a pH ranged of 4-8. The dissolved oxygen (DO) was recorded between 3.4-4.0 mg L^{-1}. Since Climbing perch are typically labyrinth species, they can still survive even DO less than 3mg L^{-1} [4]. Level of Ammonia (NH3) was measured in the ranged of 0.001 - 0.043 mg $^{L-1}$. Boyd [56] suggested that the Ammonia concentration should be less than 0.1 mg L^{-1}.There were no significant differences in the water temperature, pH,DOand NH3 in different timeof the measurement during the study periods.

Table3.*Measurement results ofwater quality parameters during observation periods*

Parameter	Initial	Middle	End
Temperature (°C)	26 - 29	28 - 30	26 - 29
pH	6 - 7	6 - 7	6 - 7
DO (mg L^{-1})	3.4 - 3.8	3.6 - 4.0	3.6 - 3.9
NH3 (mgL^{-1})	0.001 - 0.041	0.001 - 0043	0.002 - 0.043

4. Conclusion

The largerbroodstockand *Eichhorniacrassipes*(A2B1) provided the best performance especially in fecundity and fertilization rates of Climbing perchtested in the experimental tanks.Inability of larvae to consume natural food at early days resulted in low survival, while water quality did not affect and was in tolerance range for larvae rearing.

Acknowledgment

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

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Citation: *Syamsuddin, et.al., "Variation of Broodstock Sizes and Types of Aquatic Plantstoward Breeding and Survival of Newly Hatched Larvae of Climbing Perch (Anabas testudineus Bloch 1792)". International Journal of Innovative Studies in Aquatic Biology and Fisheries, 5(2), pp.11-20. DOI: http://dx.doi.org/10.20431/2454-7670.0502003*