Survival and growth performance the catfish *Clarias gariepinus* in high density nurseries using recirculating aquaculture system (RAS)

Cecilia Eny Indriastuti[1*], *Beata* Ratnawati[2], *and Ivone* Wulandari Budiharto[2]

[1]College of Vocational Studies of IPB University, Study Program of Production Technology and Management of Aquaculture, Cilibende Jalan Kumbang No 14, Bogor, Indonesia
[2]College of Vocational Studies of IPB University, Study Program of Environmental Engineering and Management, Cilibende Jalan Kumbang No 14, Bogor, Indonesia

Abstract. The recirculating aquaculture system (RAS) is a cultivation system that minimizes water changes and can optimize high stocking densities (SD). The purpose of this study was to determine the effect of nursery culture in high stocking density with RAS on survival rate (SR), specific growth rate (SGR), relative length (LR), weight gain (WG), length gain (LG)and feed utility efficiency (FUE) of juvenile catfish. The study used a completely randomized design with 4 treatments and 3 replications: R3000 (SD of 3,000 fish/m^2 with RAS); NR3000 (SD of 3,000 fish/m^2 without RAS); R4000 (SD of 4,000 fish/m^2 with RAS); NR4000 (SD of 4,000 fish/m^2 without RAS). Juvenile catfish measuring 3.3±0.2 cm were reared for 21 days and water changes were carried out every 2 weeks in the RAS and every 2 days in the non-RAS. The results showed that the SR was not significantly different for all treatments. The highest SGR, LR, WG, LG and FUE were shown in the R4000 treatment ($p<0.05$). Water quality parameters, especially total ammonia nitrogen in the RAS treatment had a lower value than without RAS. The abundance of total bacteria in the RAS treatment was more than without RAS. This study showed that the density of 4,000 fish/m^2 with the RAS was able to provide better catfish nursery production performance than without RAS.

1. INTRODUCTION

Catfish *Clarias gariepinus* is the leading freshwater fish commodity in Indonesia. This fish ranks second highest in production after tilapia. The demand for catfish from year to year always increases, as shown from the 2016 production data, which was 764,796 tons, increasing to 1,125,526 tons in 2017 [1]. The increase in catfish meat production will of course be accompanied by an increase in the need for catfish seeds as an input for enlargement. To meet catfish seeds ready to be raised, a series of maintenance is needed from larval rearing to rearing at the nursery level.

* Corresponding author: cecilia_eny@apps.ipb.ac.id

Nursery for catfish is still mostly done in the conventional way, namely raising catfish without changing water and using low stocking densities. So that the survival and productivity of catfish seeds is still low. In general, catfish nursery is done *outdoors* using soil ponds, fiber tanks or tarpaulin tanks, without changing water and using low stocking densities. This condition usually requires a large area of land and water conditions that are not ideal resulting in growth and survival that are not optimal, so technology with a more intensive maintenance system, saving land and water is needed, to get a better production value.

RAS is one of the applications of aquaculture which can control the discharge of waste into the environment [2]. The existence of filtering in the recirculation system will reduce metabolic results and reduce the accumulation of organic matter leftover feed, feces contained in the rearing container, thereby increasing fish appetite. The nitrification process is the main process in RAS to remove ammonia and nitrite which are toxic to fish. Ammonium is converted to nitrite and to nitrate which is low in toxicity so that water can be reused [3-4].

In order to offset production costs and make the recirculation system more efficient, the density of catfish fry per unit area should be as much as possible. According to SNI [5], the stocking density of juvenile catfish is 1,000-1,500 fish/m^2. The stocking density commonly used by conventional catfish nursery cultivators is in the range of 1,500-2,000 fish/m^2 .It is hoped that by using RAS, the stocking density can be increased.

With a recirculation system, besides being able to optimize narrow land, it can also save water use which affects production efficiency. Recirculation can also be an alternative for catfish farmers who live in cities, where the availability of water sources is limited. In a recirculation system, an optimal environment can be obtained for seed maintenance which has an impact on survival and optimum growth [6]. The purpose of this study was to determine the effect of RAS on catfish nursery using high stocking densities on the survival, growth performance, feed efficiency, abundance of bacteria and water quality of the media.

2. MATERIALS AND METHODS

2.1 Ethics statements

All experiments in this study associated with fish complied with animal welfare and were handled under Indonesia accreditation SNI 6484.2:2014.

2.2 Experimental design

The study was conducted for three weeks in Tania Akuakultur farm, Loji, Bogor. Water quality measurements were carried out at the Environmental Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences (FPIK), IPB. Bacterial counts were carried out at the Microbiology Laboratory, Vocational School, IPB.

The study used a completely randomized design with four treatments with three repetition, were applied: R3000; R4000; NR3000; NR4000 , R=recirculation, NR= non recirculation, 3000= SD 3,000 fish/m^2, 4000= SD 4,000 fish/m^2.

2.3 Experimental procedure

The research procedure is divided into 4 stages. These stages are preparation of rearing containers and recirculation systems, stocking of juvenile catfish, maintenance of juvenile catfish and sampling for measurement of test parameters.

2.3.1 *Preparation of the rearing container and recirculation system*

Preparation of the container began by preparing 18 tanks made of tarpaulin 700 cm ×100 cm ×40 cm in size and volume of 320 L. For catfish rearing, 12 tanks were used which were divided into 6 tanks installed in a RAS and 6 tanks non-RAS. While the other 6 tanks are used for the filtration system. These 18 tanks are connected by plastic pipes for inlet or outlet water lines which are driven by electric water pumps. The water used is well water that is stored in a reservoir. System stabilization was carried out for one week after the container was filled with precipitated water. The system is ready for use when the water quality parameters are in ideal conditions and sizes for the life and growth of the test fish (temperature: 28-30 °C, DO: 5-7 mg/l, pH: 6.5-9.0, ammonia: < 1.0 mg/l, alkalinity: = 0.1mg CaCO3/l).

2.3.2 Preparation of the recirculation system and filter

The recirculation and filter system uses 6 tanks, which have different functions, are presented in Figure 1. Tank no.1 collects dirty water from the fish rearing tank and then the water is passed to tank no. 2 which contains a dacron to filter coarse particles, then flowed into tank no 3. Tank no 3 is divided into 3 chambers, each containing sand, charcoal and zeolite. Sand serves to add minerals, charcoal to absorb ammonia odors, and zeolite to neutralize the pH of the water and increase oxygen. Then, the water flows into tank no. 4 which contains bioballs which are a biological filter as a home for bacteria. The water is then flowed into tank no. 5 which contains a tassel of raffia rope that serves to house bacteria. Finally, the water is channeled into tank no. 6 and is ready to be distributed to catfish rearing tanks with the help of a water pump. Water flow rate was 0.7 $l.min^{-1}$ and the complete water cycle took 43 min.

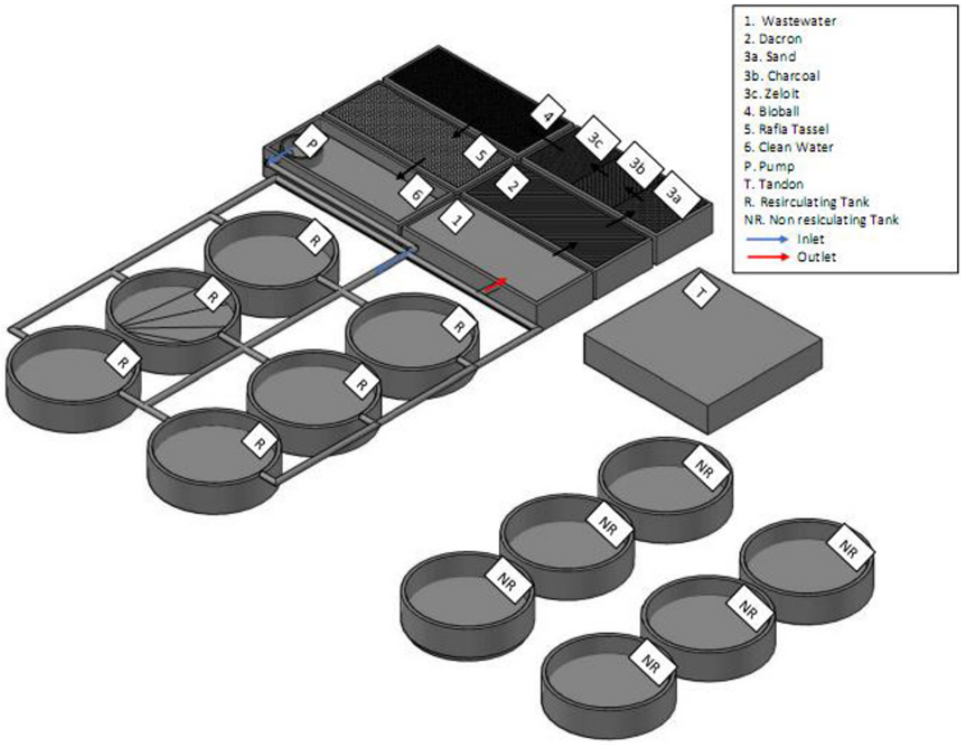

Fig. 1. Recirculating aquaculture system and rearing tanks of juvenile catfish

2.3.3 Juvenile catfish stock

The juvenile catfish used came from fish farmers around the IPB campus. The total juvenile catfish used amounted to 30,600 fish, three weeks old with a length of 3.2 ± 0.2 cm. Prior to stocking, the juveniles were acclimatized in an aquarium for 3 days, to adapt to new environmental conditions. Catfish stocked in each tank with different density levels, i.e., 3,000 fish / m^2 and 4,000 fish / m^2 were placed randomly. The R3000 and NR3000 treatments were filled with 2,250 fish/tank, while the R4000 and NR4000 treatments filled with 2,850 fish.

2.3.4 Catfish seed maintenance

Juveniles Catfish were reared for 21 days, given artificial feed with a protein content of 35-38%, as much as 5% by weight of biomass. Feeding was done twice in the morning and evening. Water changes were carried out every 2 weeks for the recirculation treatment and every 2 days for the non-recirculating treatment. During maintenance, several parameters were observed, namely weight, length and number of dead fish, bacterial density and water quality.

2.4 Variables evaluated

The variables measured were the survival rate (SR), specific growth rate (SGR), relative length (RL), weight gain (WG), length gain (LG), feed utilization efficiency (FUE), bacterial density and water quality parameters.

2.4.1 Survival rate (SR)

The survival rate (SR) was calculated at the end of the study (day 21), using the equation in Zonneveld [7].

$$\text{SR (\%)} = \frac{\text{final number of fish}}{\text{initial number of fish}}\ x\ 100$$

2.4.2 Weight gain (WG) and lLength gain (LG)

Weight gain (WG) and length gain (LG) are the differences between the final average fish weight or average fish length and the initial average fish weight or average fish length. The weight was measured using a digital scale with an accuracy of 0.0001 g, while the length was measured using a digital caliper with a precision of 0.01 mm. The weight and length of catfish were measured at the beginning of the study, the 7th day, 14th day and 21st day (the end of the study). The weight gain and length gain was calculated using this formula:

$$\text{Weight gain (g)} = final\ average\ fish\ weight\ \text{-}\ initial\ average\ fish\ weight$$

$$\text{Length gain (mm)} = final\ average\ fish\ length\ \text{-}\ initial\ average\ fish\ length$$

2.4.3 Specific growth rate (SGR)

Specific growth rate parameters of fish were measured once every seven days by weighing and measuring the fish length of 30 fish for each repetition. The specific growth rate (SGR) was calculated using the formula in [7]

$$\text{SGR (\% g day}^{-1}) = \frac{\text{ln final weight} - \text{ln initial weight}}{\text{days number of experiment}}\ \text{x 100}$$

2.4.4 Relative length (RL)

The relative length growth (RL) is the difference between the final average length and the initial average length divided by the initial average length, calculated using the following formula:

$$\text{LR (\%)} = \frac{\text{the average length of fish at the time t (mm)} - \text{the average length of fish at the beginning (mm)}}{\text{the average length of fish at the beginning of the study (mm)}}\ \text{x 100}$$

2.4.5 Feed utilization efficiency (FUE)

According to Tacon [8], the calculation of feed utilization efficiency (FUE) is as follows:

$$\text{FUE (\%)} = \frac{\text{fish biomass at the end of the study (gram)} - \text{fish biomass at the beginning (gram)}}{\text{amount of feed consumed during the study (gram)}}\ \text{x 100}$$

2.4.6 Total bacterial abundance and presence E. coli bacteria

Bacterial count was carried out at the end of the study. The bacterial parameters observed in this study included the parameters of *E. coli* bacteria and common carried out once a week. The method used is the *total plate count*, where a sample of treatment water is taken, then a gradual dilution is carried out from 10^{-1},$10^{-2,}10^{-3}$,10^{-4} and 10^{-5} from each sample to be tested as much as 1 ml. . 4, then 0.1 ml was taken from each sample using a micropipette at dilutions of 10^{-3},10^{-4} and 10^{-5} which were isolated on spread plates on PCA media (media for bacterial growth in general) and EMBA (special media for growth of bacteria). gram negative bacteria, namely *E. coli*). Next, incubation was carried out for 1x24 hours to determine whether or not there was bacterial growth as an indicator.

2.4.7 Water quality parameters

Water quality parameters observed during this study included temperature, pH and DO parameters which were measured twice a day using thermometer, DO-meter and pH-meter instruments. Ammonia, alkalinity, BOD, COD parameters were measured every seven days using titration, spectrophotometer and phenate methods.

2.5 Data analysis

Data were tabulated using Microsoft Excel 2010. Parameters of survival rate, specific growth rate, relative length, weight gain and length gain were analyzed for variance using one-way ANOVA at level P=0.05 with the help of SPSS 21 software. If the difference is significant, the data is further tested using Duncan's test. Inhomogeneous data, tested by nonparametric test using Kruskal Walis. Data on water quality and bacterial composition were analyzed descriptive in tabular form.

3. RESULT

3.1 Survival rate, specific growth rate, relative length, weight gain, length gain and feed utilization efficiency

Results demonstrated that there were no significant differences related to the survival rate of fish. Treatment R4000 resulted in the highest survival value (91.03±0.90%) and treatment NR3000 with the lowest value (89.93±0.40%), but all treatments were not significantly different (Table 1).

The highest SGR was demonstrated by the treatment R4000 at 7.71±0.19%, and the lowest was demonstrated by the treatment NR3000 at 5.92±0.31% ($P< 0.05$). The highest LR was demonstrated by the treatment R4000 at 0.61±0.02%, and the lowest was demonstrated by the treatment NR3000 at 0.44±0.03% ($P< 0.05$; Table 1).

The highest WG were demonstrated by the treatment R4000 at 1.67 ± 0.08g, and the lowest were demonstrated by the treatment NR3000 at 1.04 ± 0.09g ($P< 0.05$). The highest LG were demonstrated by the treatment R4000 at 20.86±0.57mm , and the lowest were demonstrated by the treatment NR3000 at 14.96±1.10mm ($P< 0.05$; Table 1).

The highest FUE were demonstrated by the treatment R4000 at 88.45±2.03%, and the lowest were demonstrated by the treatment NR3000 at 69.49 ± 4.39% ($P<0.05$; Table 1).

Table 1. Survival rate, specific growth rate, relative length, weight gain, length gain and feed utilization efficiency of juvenile catfish reared under condition of different stocking density and system recirculation for 21 days

Treatments	Survival Rate (%)	Specific growth rate (%)	Relative Length (%)	Weight Gain (g)	Length Gain (mm)	Feed Utilization Efficiency (%)
R3000	90.30 ± 0.20^{a}	6.68 ± 0.79^{a}	0.50 ± 0.06^{a}	1.30 ± 0.28^{a}	17.26 ± 1.88^{a}	78.43 ± 5.78^{a}
R4000	91.03 ± 0.90^{a}	7.71 ± 0.19^{b}	0.61 ± 0.02^{b}	1.67 ± 0.08^{b}	20.86 ± 0.57^{b}	88.45 ± 2.03^{b}
NR3000	89.93 ± 0.40^{a}	5.92 ± 0.31^{a}	0.44 ± 0.03^{a}	1.04 ± 0.09^{a}	14.96 ± 1.10^{a}	69.49 ± 4.39^{a}
NR4000	90.10 ± 0.53^{a}	6.10 ± 0.31^{a}	0.46 ± 0.06^{a}	1.10 ± 0.10^{a}	15.81 ± 2.21^{a}	71.08 ± 6.52^{a}

Different superscript letters within a single treatment column showed significant differences among treatments (Duncan's Test: $P < 0.05$).

3.2 Total bacterial abundance

The total bacterial density increased significantly with treatment R3000 and R4000 at each water sample. NR4000 treatment only few bacteria grow, whereas in the NR3000 treatment no bacteria grow. Meanwhile, for observations of bacteria *E. coli,* none grew in all treatment samples (Table 2).

Table 2. Abundance of total bacterial and presence of *E. coli* during 21 days rearing of juvenile catfish reared under condition of different stocking density and system recirculation

Treatments	Total bacteria			*E. coli* bacteria		
	H7	H14	H21	H7	H14	H21
R3000	+ (TMTC)	+ (TMTC)	+ (TMTC)	-	-	-
R4000	+ (TMTC)	+ (TMTC)	+ (TMTC)	-	-	-
NR3000	-	-	-	-	-	-
NR4000	+ (TFTC)	+ (TFTC)	+ (TFTC)	-	-	-

Noted :
(+) = bacteria detected
(-) = no bacteria detected
TFTC = Too few to count
TMTC = Too many to count

3.4 Water Quality

The results of water quality measurements showed that the quality of the media was still within the feasible range to support the survival and growth of catfish seeds (Table 3).

Table 3. Water quality range during 21 days of rearing juvenile catfish reared under condition of different stocking density and system recirculation

Treatments	Parameter					
	Temperature (°C)	pH	DO ($mg.L^{-1}$)	TAN ($mg.L^{-1}$)	Amonia ($mg.L^{-1}$)	Alkalinity (mg CaCO3/L)
R3000	25,58-27,12	7,26-7,36	3,60-4,10	0,30	0.033	104,59
R4000	25,58-27,12	7,25-7,26	4,10-4,70	0,25	0.027	122,96
NR3000	25,58-27,12	7,11-7,21	1,80-4,40	0,66	0.072	121,55
NR4000	25,58-27,12	7,09-7,22	1,60-4,20	0,73	0.080	135,68
Reference	28 – 31 [9]	6,5 – 8,5 [5]	Min 3,0 [5]	Maks 4 [10]	< 0,1 mg/L [11]	30-100 mg CaCO3/L [12]

4. DISCUSSION

Recirculating aquaculture system (RAS) is proven to increase the density of catfish seeds. In addition, RAS can also improve production performance and feed efficiency. Recirculating and non-recirculating treatments at all densities did not give a significant value to the survival rate. Increased density of 3,000 fish/m^2 to 4,000 fish/m^2 did not show any significant difference in the death of the fish, so the value of SR did not differ significantly. This is in accordance with a study conducted by Haylor [13] which found that catfish larvae reared with densities of 50, 100 and 150 L/larvae did not increase mortality. The same thing was shown by Siregar [14] and Rahmadiah [15] that the increase in stocking density did not cause mortality and cannibalism in juvenile catfish.

Specific growth rate of juvenile catfish reared in recirculation tanks with a high density of 4,000 fish/m^2 is better than the other interventions. The same results were also obtained for other growth parameters; weight gain, relative length and length gain. In general, SGR is influenced by stocking density (SD), this is related to competition for space, feed and maintenance water conditions [16].

Catfish have territorial aggressiveness, the tendency to attack their relatives to defend their territory which causes high levels of cannibalism. In this study, at a lower stocking density (3,000 fish/m^2), the rearing media still had space that allowed the catfish to attack their friends. At SD 4,000 fish/m^2 the room is getting narrower, catfish are more alert to other fish attacks, so that aggressive behavior decreases. Catfish with high stocking density reduces the territorial aggressiveness [17-18]. This also affects the diet so that at a stocking density of 4,000 fish/m^2 with recirculation, catfish are more efficient in utilizing their feed.

At SD 4,000 fish/m^2 metabolism results, feces will be more than SD 3,000 fish/m^2, but by using RAS it is able to have a significant effect on improving water quality than non-recirculating systems so that with SD 4,000 fish/m^2 can produce significantly better growth performance and feed efficiency.

This is possible because in the RAS, dirty water resulting from metabolism, fish feces and feed residues originating from the rearing media undergo physical, chemical and biological filtering so that they meet the quality standards of fish maintenance and can be used and channeled back to the rearing media. Poor water quality can cause stress for fish so that fish are susceptible to disease or reduce their growth rate [19].

The effect of RAS also has an effect on feed efficiency parameters. The recirculation treatment with a density of 4,000 fish/m^2 had the highest percentage of 88.45% which was significantly different from the recirculation treatment of 3,000 fish/m^2 and the non-recirculating treatment. This is in accordance with other studies which state that the use of a recirculation system in catfish has a significant effect on feed efficiency compared to water exchange systems and circulation systems [6]. This influence is thought to be due to the water quality of a good rearing medium that can affect fish in maximizing the utilization of well-digested feed. In addition, the rearing water in the recirculation system is possible for the presence of good bacteria and natural food in the water which comes from organic waste that is broken down by bacteria in the waters and flowed back into the rearing media. The water quality of the rearing media is an important factor related to feed efficiency, as stated by Effendi [16] that one of the factors that determine feed efficiency is water quality (especially oxygen, temperature, pH and ammonia) besides that it also depends on species (feeding habits, size/stadia), and type of feed (quality and quantity).

5. CONCLUSION

Juvenile catfish rearing using recirculating aquaculture system can increase stocking density so that it can optimize production with limited land. By using recirculating aquaculture system, there is a possibility that stocking density can be increased as evidenced by treatment with SD 4,000 with recirculating aquaculture system, giving significantly better results on growth and feed efficiency than other treatments.

REFERENCES

1. KKP, Kelautan dan perikanan dalam angka tahun 2018, Pusat data dan statistik, (2018)
2. N.M. Ramli, M.C.J. Verdegem, F.M. Yusoff, M.K. Zulkifely, J.A.J. Verreth. Removal of ammonium and nitrite in recirculating aquaculture systems by the epiphyte *Stigeoclonium nanum* immobilized in alginate beads. Aquacult Environ Interact. 9 :213-222 (2017)
3. J.V. Rijn. Waste Treatment in Recirculating Aquaculture Systems. Aquacultural Engineering. 53:49-56 (2013)
4. W. Pungrasmi, P. Phinitthanaphak, S. Powtongsook. Nitrogen Removal From a Recirculating Aquaculture System Using Apumice Bottom Substrate Nitrification Denitrification Tank. Ecological Engineering. 95:357-363 (2016)
5. SNI. Standar Nasional Indonesia. Ikan lele dumbo (*Clarias* sp.) Bagian 4: Produksi benih. SNI: 6484.4: 2014 (2014)
6. A.W. Primaningtyas, H.S. Subandiyono. Performa produksi ikan lele (*Clarias gariepinus*) yang dipelihara dalam sistem budidaya berbeda. Journal of Aquaculture Management and Technology. 4(4):51-60 (2015)
7. N. Zonneveld, EA. Huisman, JH. Boon, Prinsip-prinsip Budidaya Ikan. Terjemahan. Jakarta (ID): PT Gramedia Pustaka Utama. (1991)
8. A. Tacon, The Nutrition and Feeding of Farmed Fish and Shrimp, A Training Manual (2) Nutrient Source and Composition, FAO, Brasilia, (1987)
9. E. Baras, T. Raynaud, J. Slembrouck, D. Caruso, C. Cochet, M. Legendre, Interactions between temperature and size on the growth, size heterogeneity, mortality and cannibalism in cultured larvae and juveniles of the Asian catfish, *Pangasianodon hypophthalmus* (Sauvage), Aquaculture Research, 42, 260–276, (2010)
10. N.M. Stone, H.K. Thomforde, Understanding your fish pond water analysis report, Chicago (US): University of Arkansas Cooperative Extension, (2004)
11. G.A. Wedemeyer, Fish Hatchery Management, 2nd Edition, Bethesda, Maryland, American Fisheries Society, (2001)
12. C.E. Boyd, Water Quality in Warm Water Fish Ponds, Fourth Printing, Alabama (US): Auburn University Agriculture Experiment Station, hlm 359, (1988)
13. G.S. Haylor, Controlled hatchery production of *Clarias gariepinus* (Burchell 1822): growth and survival of fry at high stocking density, *Aquaculture Research*, 22(4), 405-422, (1991)
14. K.N. Siregar, Pengendalian kanibalisme benih ikan lele Afrika *Clarias* 24 *gariepinus* menggunakan hormon estradiol-17β dan pengaturan padat tebar, tesis, Bogor (ID): Institut Pertanian Bogor, (2017)
15. T. Rahmadiah MJr. Zairin, Alimuddin, I. Diatin, Aggressive and cannibalistic behaviour of african catfish larvae: effect of different doses of methyltestosteron injecting to female broodstock and larval stocking densities, *JAI,* 18(2), 182-192, (2019)
16. H. Effendi, Telaah Kualitas Air Bagi Pengelolaan Sumberdaya dan Lingkungan Perairan, Penerbit Kanisius, Yogyakarta. (2003)
17. T. Hecht, S. Appelbaum, Observations on intraspesific aggression and coeval sibling cannibalism by larva and juvenile *Clarias gariepinus* (Clariidae: Pisces), *Journal of Zoologi* 214, 21-44, (1988)
18. H. Kaiser, O. Weyl, T. Hecht, Observations on agonistic behavior of *Clarias gariepinus* larvae and juveniles under different densities and feeding frequencies in a controlled environment, *J of Appl Ichth*, 11(1-2), 25-36, (1995)
19. S. Diansyah, T. Budiardi, AO. Sudrajat, Kinerja pertumbuhan *Anguilla bicolor bicolor* bobot awal 3 g dengan kepadatan berbeda, Jurnal Akuakultur Indonesia, 13(1), 46-53, (2014)