Engineering culture using natural filter differences based on microsatellite to improve the quality of Snakehead (Channa striata)

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Abstract. The aim of the study was to examine various biological filters for hyacinth, hydrilla, apu apu and azzola media based microsatellite analysis of quality snakeheads (C. striata) production. The research was carried out at the integrated laboratory of Diponegoro University in January to April 2019. The snakehead used was size 4.83 ± 0.02 cm long of body and weighed 5.47 ± 0.12 g/fish, using artificial feed 35% protein content is given 3% perbiomas per day. The design used CRD 4 treatments and 3 replications were T1 (hyacinth), T2 (Hydrilla), T3 (apu apu), and T4 (Azzola) treatments, then for determining the quality the microsatellite genetic marker IS-GB1F 5'-CCC TGT ATT TCA TTT CTC CA-3' and IS-GB1 R 5'-ACC ACT AAC GCA ATC TCT CT-3', for measuring genetic variation, polymorphic, number allel. Data analysis used minitab version 11. The results showed that the best treatment was T3 with survival rate 96.67 ± 1.92%, absolute weight growth of 14.60 ± 2.41g, SGR 2.61 ± 0.22% / day. The quality of snakehead using microsatellite was T3(band10-12, 13-16) on the best quality of snakeheads fish on T3 (204 bps allel ladder with 215 bps).

1. Introduction

The advantage of snakeheads (Channa striata) is an important economical type of fish and the price is also expensive and tastes good, there are many in public waters and very potential to be developed (Herlina, [1]. Yulisman et al [2], reported that snakeheads can to be of economic value because it has many benefits. The use of snakeheads in the medical world, can accelerate the process of wound healing postoperatively, because snakeheads contain albumin serves to heal wounds and accelerate blood clotting.

Another advantage of this snakeheadsis that the current condition in Central Java contains a lot of albumin, namely protein in blood serum which is important to use post surgery and accelerate blood clotting, is needed in society and the world of medicine. Albumin is still imported from abroad, so the success of this basic research will produce genetic data (DNA) that are specific to snakeheads to be developed in snakeheads culture, so as to meet the needs of albumin in Indonesia. At the end of this research activity, new theories and methods were found to support the findings of snakeheads culture technology to support the fishing industry.

Based on the superiority of snakeheads that is very potential, it was necessary to develop it by conducting good quality snakeheads culture. To determine good quality genetic marker analysis test is needed with microsatellite method to obtain genetic variation data, polymorphisms, DNA weights. By evaluating DNA variations in snakeheads, genetic engineering using snakeheadsmicrosatellite can be found which has slow, normal and fast growth variability of the parent (Year I) and seed (Year II) which are geographically different and are expected to be used as indicators for selection in order to improve the genetic quality of the parent, Nevertheless in the maintenance of snakeheads seeds until they reach a certain size (consumption) there are still some obstacles. The problem with snakeheadsis the high mortality rate in snakeheads seeds from 80 to 90%. The high mortality of snakeheads was influenced by several things, one of which was the
decrease in water quality in the snakeheads seed maintenance media due to the high ammonia in the maintenance media, and the quality of snakeheads seeds that have not been radicalized by marker genetic testing through microsatellite methods using plants as bifilter can absorb N, P, and K. Ammonia levels in the maintenance media become high due to the amount of metabolic waste and food waste. If there is a buildup of high ammonia levels it will be toxic to snakeheads. The snakeheads maintenance is relatively high, ie above 70% [3]. Moreover, Hartini et al.[4] explained that the problem faced in maintenance was the high level of mortality in snakeheads seed stage. The high mortality rate of snakeheads seeds at the maintenance stage is due to several reasons. One of them is a decrease in the quality of living media water.

Water quality in aquaculture media is one aspect that can increase the growth of snakeheads. Water is the most important media or habitat for fish life. Adequate water supply will solve various problems in fish farming. In addition, good water quality is one of the keys to success in fish farming and can increase growth [5].

Lestari et al [6] explained that the addition of water quality plays an important role in the field of fisheries, especially for aquaculture activities. Water quality can decrease due to the accumulation of organic and inorganic materials derived from metabolic waste and leftovers that are not consumed, so it is necessary to improve water quality using biological biofiltration technology, whereas to find out the quality of seeds or the size of snakeheads consumption using genetic marker test using the micro satellite method. Technology to create optimal environmental conditions by using filtration with a recirculation system. Water recirculation in fish maintenance media functions to help biological balance in water, maintain temperature stability, help oxygen distribution and maintain the accumulation or accumulation of toxic metabolites so that toxic levels or power can be suppressed [7].

One of the solution of solve the problem by Fadhil et al [8],explained that the circulation system is a process of water circulation. Filtering is a mechanical filter to remove suspended solids in water. The suspended solids are food scraps and metabolic waste. Biological filters consist of several solid media that function as surfaces where bacteria can stick and grow. According to Dwiputra [9], biofilter is a biological filter that has a function in filtering water containing nitrogenous waste using a substrate on a filter containing nitrifying bacteria. The occurrence of nitrification is the process of aerobic oxidation through two stages, the conversion of ammonia with oxygen to nitrite (nitritification), with ammonia aerobic oxidation bacteria namely nitrosomonas bacteria and further conversion of nitrite with oxygen to nitrate by nitrite oxidizing bacteria namely nitrobacter bacteria.

The biofilter used in the study was water hyacinth, hydrilla, azzola, and apu-apu. The four biological filters are used because they have different characteristics. Water hyacinth grows on the surface of the water, grows by sucking water and evaporating it back through plants that are exposed to sunlight through the evaporation process, the roots of water hyacinth are fibers. Water hyacinth has the ability to treat wastewater Moreover increase of survival rate of sanakeheads by (Mulyadi et al [7]. Explained that hydrilla water plants have the ability as a bioremediator. This plant is a plant whose entire body sinks below the surface of the water [10]. Azolla microphylla is one of many aquatic plants that can do bioremediation, which is an alternative treatment of wastewater containing heavy metals. Azolla microphylla is a water-nail plant that lives above the water surface [9]. Apu-apu (Pistia sp.) Is a kind of water weed that grows very fast and has adaptability to the new environment. This pest can be used to absorb toxic elements in wastewater. These apu-apu plants live above the surface of the water and have fiber roots [12].

The aim of the study was to examine the use of various biological filters for hyacinth, hydrilla, apu apu and azzola media based microsatellite analysis to improve the quality of snakeheads (C striata) production.
2. Material and Methods

The containers used in this study were 12 aquarium units measuring 60x40x40 cm³. Before being used, washed with salt water is used to sterilize the aquarium from fungi or parasites. Then rinse thoroughly. After cleaning the aquarium is dried. The aquarium is filled with water according to the aquarium's capacity. The filter used is dacron sheets, water hyacinth, hydrila, azola, and apu-apu. Aquarium pump for pumping water. Pump hose to connect the pump. Thermometer to measure water temperature. Dissolved oxygen in water is measured using a DO meter. Acid-base water is measured using a pH meter.

The filter container is where to put the filter. Scales for weighing fish. Feed (pellets) as a source of fish food. Heater to stabilize the temperature. Spectrophotometers are used for testing ammonia, nitrites, nitrates and phosphates.

This research was conducted in February 1, 2019 to May 24, 2019, at the Siwarak Freshwater Fish Seed Center, Semarang Regency.

2.1. Research material

The material used in this research is snakeheadsseed with a total length of 4.9 ± 0.05 cm / head as much as 30 fish per aquarium originating from the UPR hatchery of the Imam in Kudus. Water hyacinth as a biological filter weighing 2.5 g / L (T1) from ambarawa reel swamps. Hydrilla as a biological filter weighing 2.5 g / L comes from the ambarawa reel swamp (T2). Apu-apu as a biological filter weighing 2.5 g / L originating(T3), Azzola as a biological filter weighing 2.5 g / L originating(T4) from salatiga keboan. Apu-apu as a biological filter weighing 2.5 g / L originating from kebowan salatiga. Commercial feed with a size of 0.5-0.7 mm with a protein content of 39-41%.

2.2. Research procedure

Filter making of the filter container used is a 75x12x11 cm plastic gutter. Plastic gutters are divided into 3 parts. The first part is used to place biological filters (T1,T2,T3,T4), the second part to place dacron sheets, and the third part to place filtrated water (Figure.1).

Figure.1 Filtration schemes are listed in Figs. 1

Information: a. Pipe
T1. Water hyacinth
T2. Hydrila
T3. Apu-apu
T4. Azzola  
c. Dakron sheet  
d. Aquarium  
e. Water pump  
f. Snakehead

Container Preparation The maintenance container is an aquarium with a length of 75 cm, width 50 cm and height 50 cm. The aquarium is cleaned before being filled with water. Aquariums that have been cleaned filled with water as much as 60 L. Aquariums that have been filled with water were deposited for 1 week. Test Animal Preparation Test animals used are snakeheads seeds with a total length of 4.9 ± 0.05 cm / head and an average weight of 5.51 ± 0.05 g. Fish stocked with a density of 2 fish / liter. Each aquarium is filled with 30 snakeheads seeds. The snakeheads seed is adapted first in a maintenance container for 1x24 hours as an acclimatization process (adjusting to the new environment). Research methods The research method used was a Completely Randomized Design (CRD) with 4 treatments and 3 repetitions based on a modified method of Mahardika et al [3] and [4][6][7][13] explained that namely: Treatment T1: Circulation using water hyacinth (water surface) Treatment T2: Circulation using hydrila (in water) Treatment T3: Circulation using azzola (on water) Treatment T4: Circulation using apu-apu (Surface water).

2.3. Research data collection
2.3.1. Volumetric TAN removal rates (VTR)
Volumetric TAN removal rates are calculated using the formula from Peng [14] as follows:

\[ \text{VTR} = 1.44 \times (\text{TAN}_{\text{in}} - \text{TAN}_{\text{out}}) \times \frac{Q}{V} \]

Information:
- VTR = Volumetric TAN removal rates (g m\(^{-3}\) day\(^{-1}\))
- \(\text{TAN}_{\text{in}}\) = Total ammonia concentration in the inlet biofilter (mg / l)
- \(\text{TAN}_{\text{out}}\) = Total ammonia concentration in the biofilter outlet (mg / l)
- Q = Total water flow through the filter (m\(^3\) / day)
- V = filter place volume (m\(^3\))
- 1.44 = Concentration

2.3.2. Survival (SR)
Survival rate is the percentage of fish that live, calculating the survival rate of fish using the formula by Effendi [15], as follows:

\[ \text{SR} = \frac{N_t}{N_0} \times 100 \]

Information:
- SR = degree of survival (%)
- \(N_t\) = number of individuals at the end of the t-day treatment (tail)
- \(N_0\) = number of individuals at the beginning of the treatment day 0 (tail)

2.3.3. Specific Growth Rate
The specific biomass growth rate (SGR) is% of the difference between the final weight and initial weight, divided by the length of time. SGR can be calculated formula by Weatherley [16] as follows:

\[ \text{SGR} = \frac{\ln W_t - \ln W_0}{t} \times 100\% \]
2.3.4. Absolute Length
Absolute length is the increase in fish length each day during maintenance. The absolute length increase is shown in units of cm / day by Weatherley [16]:
\[ PM = Pt - Po \]
Information:
\( PM \) = absolute increase (cm / day)
\( Pt \) = average length of fish at the end (cm)
\( Po \) = average length of fish at the beginning (cm)

2.3.5. Absolute Weight
Absolute weight growth is weight gain (weight difference end and initial weight) during maintenance time. Absolute weight growth can be calculated by the formula by Weatherley [16]:
\[ W = Wt - Wo \]
Information:
\( W \) = Growth in absolute weight (grams)
\( Wt \) = Average fish weight at the end of treatment (grams)
\( Wo \) = average weight of fish at the beginning of the treatment (grams)

2.3.6. Water Quality Parameters
Measurement of water quality parameters include oxygen solubility (DO), acidity (pH), temperature, ammonia, nitrite, nitrate, and phosphate dam. Measurement of temperature water quality parameters is done every 3 times a day ie 08.00, 12.00, and 16.00 WIB in the aquarium. Water pH measurement is done once a day. DO measurement is done every 7 days. Ammonia, nitrite, and nitrate measurements were carried out at the beginning, middle and end of the study. Growth measurements are carried out once every 7 days with observational parameters in the form of the length and weight of snakeheadsseeds by measuring as many as 5 fish samples per aquarium. Feeding is carried out at 08.00, 12.00 and 16.00 WIB, using the at satiation method.

2.3.7. Data analysis
Data analysis was performed on data on absolute weight growth value, absolute length growth, specific growth rate, and survival rate. Before analyzing the variance, the normality test, homogeneity test and additivity test are carried out to ensure that the data are spread normally, homogeneous and additive in nature. Then the data continued with analysis of variance (ANOVA) with 95% confidence interval, 99% to see the effect of treatment. After analysis, if the treatment is found to have a significant effect (P <0.05) or a very significant effect (P <0.01), then Duncan’s test is performed to determine differences in the mean values between treatments. Data analysis was performed using SPSS version 23.0 and Ms excel 2010. Water quality such as ammonia, nitrite, nitrate, phosphate, temperature, Do, pH, volumetric removal rate of NH3, NO2 and NO3 were analyzed descriptively to see the feasibility of supporting fish survival and growth snakeheads.

Ingredients for preparations
The material used in the development of snakeheads culture for microsatellite analysis of snakeheads used modified by Suresh et al. [17] [18] explained that seed extract samples, using PCR Kit reagents such as: 10 x PCR Buffer, 2.5 mM dNTP mix, OPA primer 4, 0.5 uL.
Polymerase tags, aquadest and mt-DNA genome in 0.2 ml PCR Tube, Universal primary OPA 4, 1% agarose gel in 1 x TBE (tris boric acid EDTA) buffer, 100 bp DNA ladder, ethidium bromide, UV transilluminator, enzyme Hind III restriction (A'AGCTT); Bam HI (GGATCC); EcoR V (GAT'ATC) and HAE III (GGCC), 10 x buffer buffer, 100 x BSA, restriction enzymes and aquadest and mt-DNA templates, 1.5% agarose gel in 1 x TBE buffer.

2.3.8. Equipment

Equipment used in the development of snakehead (seedling) cultivation using microsatellite analysis includes surgical instruments (tweezers, knives, scissors, watch glasses, petri dishes, glass objects, dropper pipettes, hotplates, tissue paper, microscopes (1000x magnification), (1500 x) and mt-DNA extraction and purification equipment, PCR amplification of mt-DNA genome, Restriction Fragment Length Polymorphism, such as: eppendorf tube, waterbath, heating device, centrifuge more than 13,000 rpm, PCR application, PCR tube 0 2 ml, UV transilluminator, camera gel, electrophoresis.

2.3.9. Research Methods

The method used in the first year research, in 2019 (superior seeds of snakeheads) with descriptive research design and randomized complete basic design with 4 treatments and 3 replications.

Data collection methods and instruments

1. Strategies for selecting superior quality snakehead seeds on the basis of genetic markers

   In this study, samples of superior snakehead seeds were used as the final results of various treatments T1, T2, T3 and T4 on the total length weight measurement 5.5 cm and a weight of 6.5 gr, a total length of 20 cm and a weight of 250 gr. Each group of 30 samples.

2. Extraction and Purification of mt-DNA

   The method of extraction and purification of mt-DNA from snakeheadswas carried out by microsatellite genome obtained through extraction following the modification of the Balkhis et al [19] reported that Snakeheadsmeat (both seed and consumption size) made extracted crushed in 500 mL of a 10% Chelex-100 solution which was put in an eppendorf tube and added 5 mL proteinase kinase (10 mg / mL) and heated 55°C in a water bath for 3-4 hours. Then the solution was reheated at 89°C for 8 minutes and cooled at room temperature to cool before adding 55 mL TE (Tris-EDTA) buffer to pH 8.0. The mt-DNA genome can be obtained by centrifugation for 5 minutes at a speed of 13,000 rpm. The solution in the upper layer and clear colored DNA genome was transferred into a new eppendorf tube and stored at -20°C for further analysis.

3. Amplification of DNA Genome PCR

   PCR amplification of DNA genomes by PCR amplification of snakeheadsgenome DNA samples from each treatment was initiated by mixing several PCR kit reagents (Qiagen) consisting of 10 x PCR buffers, 2.5 mM dNTP mix, primers, 0.5 µL Taq polymerase, aquadest and DNA genome in a 0.2 mL PCR tube and incubated in a 38 cycle PCR machine. In this amplification the initial denaturation temperature is 94°C for 2 minutes and the final denaturation temperature is 94°C for 40 seconds. For annealing temperature 60°C was used for 1 minute and continued with an initial extension temperature of 72°C for 5 minutes and a final extension temperature of 72°C for 5 minutes. The universal primers used in snakeheads DNA amplification were determined by species, to determine the banding pattern produced from DNA amplification, poly acrylamide and 1% agarose gel were used in 1 x TBE (tris boric acid EDTA) buffer with 25-30 minutes electrophoresis time. As a molecular marker 100 bp DNA ladder is used, for coloring it is used ethidium bromide with immersion for 10 minutes and washing with water for 10 minutes. The results obtained were observed under a UV transilluminator and documented with a camera gel.

4. Microsatellite Polymorphisms

   Microsatellite polymorphisms are known by using restriction enzymes on DNA templates of PCR amplification products cut with Hind III restriction enzymes (A'AGCTT); Bam HI
(G'GATCC); EcoR V (GAT'ATC) and HAE III (GG’CC). DNA template cutting begins by preparing a solution of 10 x buffer, 100 x BSA, restriction enzymes and aquadest as well as a DNA template for PCR amplification products with a certain concentration. Subsequently incubated in a water bath at 37 oC for 2.5 - 3 hours. The use of 1.5% agarose gel in 1 x TBE buffer and the electrophoresis process for 30-35 minutes and staining with ethidium bromide for 10 minutes, then obtained fragment pieces from each DNA template. As a molecular marker, 100 bp DNA ladder is used, while for control, a DNA template that is not cut is used. The results obtained were observed under a UV-transilluminator at 320 nm and documented with camera gel. Confirmation of frequency genotyping is done by GEN-POP Program analysis. The parameters measured include the total length and weight of the abalone, the composition and weight of DNA molecules, heterogeneity, genotype and allele frequency.

5. Data analysis

Data including growth in length and total weight of snakeheads (seed and consumption size) were analyzed by F test (ANOVA), and knowing length length relationship using Minitab 11 software, while data for DNA molecular weight composition, genotypic heterogeneity, egg diameter and color calculation, the development of the gonad was carried out by ecostatic and descriptive analysis.

3. Results and Discussion

3.1. Results
3.1.1. Filter effectiveness

The results of the observation of the effectiveness of the filter include data on ammonia, nitrite, nitrate and water quality data, namely temperature, pH, and dissolved oxygen in the snakeheads (C.striata) rearing media for each treatment during the study (Table.1).

Table 1. Water Quality Variable Values (temperature, DO, pH, ammonia, nitrite, nitrate, and phosphate) Snakeheads that are kept with different biological filters during maintenance

| Treatment     | Average Value of Water Quality Variables |
|---------------|------------------------------------------|
|               | Temperature (°C) | pH | DO (mg/L) | Ammonia (mg/L) | Nitrite (mg/L) | Nitrate (mg/L) | Phosphate (mg/L) |
| T1(hyacinth)  | 29,51           | 7  | 7,11      | 0,00218       | 0,06           | 0,709         | 0,05          |
| T2(hydrilla)  | 29,43           | 7  | 7,12      | 0,00205       | 0,058          | 0,561         | 0,05          |
| T3(apu-apu)   | 29,79           | 7  | 7,1       | 0,0016        | 0,021          | 0,429         | 0,04          |
| T4(azzola)    | 29,57           | 7  | 7,13      | 0,00202       | 0,032          | 0,541         | 0,07          |
| Reference     | ≤32,7a          | ≤9a| ≥3,7a     | ≤0,54b        | ≤0,1b          | 0,1-5b        | ≤0,124b       |

Information : a)Hartiniet al., (2013)[4]
               b)Hidayattullah et al.,(2015)[13]

Based on the above table it can be concluded that the average value of water quality variables at the inlet temperature, DO, pH, ammonia, nitrite, nitrate, and phosphate are still in the optimal range for the maintenance of snakeheads.

Feasibility of water quality

The results of the measurement of water quality parameters show that the value of water quality parameters of temperature, dissolved oxygen, pH, ammonia, nitrite, nitrate and phosphate during the study are still in a decent condition to be used as a media of snakeheads (C.striata) based on literature about the optimum water quality conditions for snakeheads(C.striata) (Table.2).

The results of research on the differences in biological filters on survival (SR), growth in absolute weight, growth in absolute length, and specific growth rate (SGR). Presented in Table 2.
Table 2. Result of Research Survival rate, absolute weight, absolute length and specific growth rate (SGR) of snakeheads used different of biology filters

| Treatment | T1(hyacinth) | T2(hydrilla) | T3(apu-apu) | T4(Azzola) |
|-----------|--------------|--------------|-------------|------------|
| 1.Survival Rate (%) | 81,11±11,7^c | 93,33±3,85^ab | 96,67±1,92^a | 77,78±5,10^bc |
| 2.Absolute weight (g±SD) | 8,76±1,34^bc | 12,99±3,00^ab | 14,60±2,41^a | 8,24±1,98^c |
| 3.Absolute length (cm±SD) | 2,63±0,25^bc | 3,15±0,25^a | 3,35±0,31^a | 2,47±0,39^c |
| 4.Specifc growth Rate (hari) (±SD) | 1,93±0,22^b | 2,48±0,32^a | 2,6±0,22^a | 1,83±0,24^b |

3.2. Results of Microsatellite Analysis of Snakeheads.

Results of microsatellite analysis on various snakeheads seeds which were the result of mating inhibition from snakehead brood from different waters in Central Java. Microsatellite analysis results (Figure 2).

Figure 2. The results of polymorphism analysis using microsatellites on snakeheads in various treatments T1 (water hyacinth filter), T2 (hydrilla filter), T3 (apu-apu) and T4 (azzola filter) quality of snakeheads products differed in genetic variation from the results of genetic analysis marker method microsatellite ie (T1, bands 1-3), (T2 bands 4-6), (T3, bands 10-12, 13-16) (T4, bands 7-9).

Then each treatment in snakeheads based on the results micro satellite analysis using IS-GB2 F OLGIO BASE TYPE 5-AGA AGA AGA AGA AGC CGA GT-3 primers and IS-GB2 R 5-GAA AAA CAG AGC AGG AAC AC-3 (INTEGRATED DNA TECHNOLOGIES SINGAPORCE),
bright bands found in the treatment T3 (band10-12, 13-16) snakeheads maintained using maintenance media filtered with azzola filter with a 202 bp ladder band with 214 bps alleles and to the right of 224 bps, as well as having high polymorphism. Snakeheads as a whole are caught in the waters of Central Java have a high heterozygot, so it has a good quality snakehead sparent product that grows well.

3.2. Discussion

3.2.1. Filter effectiveness

The results of the measurement of filter effectiveness include ammonia, nitrite, nitrate and phosphate concentration data and water quality of each maintenance container. Ammonia, nitrite, nitrate, and phosphate concentrations in each treatment is still in optimal condition. Ammonia, nitrite, nitrate, and phosphate concentrations were lowest in treatment T3 (apu-apu) and highest in treatment T4 (azzola). This is reinforced by the results of Balkhis et al [19] reported, that the results obtained during the study showed the concentration of ammonia, nitrite, phosphate and turbidity of 45 grams treatment showed the most effective results[4][6][20-24]. The value of survival rate, daily growth rate, absolute length, and feed efficiency showed significantly different results (p <0.05) between treatments using phytoremediation of apu and control plants. This is because the presence of apu wood plants as phytoremediators in the maintenance media can absorb ammonia directly from fish metabolism and can adsorb particles that cause turbidity.

Difference results from inlet-outlet ammonia concentration in treatment T1 (0.00218 mg / l), treatment T2 (0.00205 mg / l), treatment T3 (0.0016 mg / l) and treatment T4 (0.00202 / l) the highest ammonia inlet and outlet differences in treatment T3 (filter used apu apu) can be concluded that treatment T3 has higher ammonia absorption power compared to other treatments. The highest volumetric removal rate of NH3 in azzolla (T4) treatment and the lowest volumetric removal rate of NH3 in azzola treatment. This was confirmed by Putri et al [25] states that the ideal ammonia concentration for fish is no more than 1 mg / L. Fish cannot tolerate too much free ammonia because it can interfere with the binding process of oxygen by the blood. Azzola plants have advantages compared to other plants such as high germination, fast growth, absorption rate or absorption of nutrients and water that are large, easy to find and high adaptability to climate [24-26]

Difference results from inlet-outlet nitrite concentrations in treatment T1 (0.06 mg / l), treatment T2 (0.058 mg / l), treatment T3 (0.021 mg / l) and treatment T4 (0.032 mg / l). The results of nitrite concentration during treatment are still in optimal conditions. This was confirmed by Lestari et al [6][26] explained that nitrite concentrations shows were good for cultivation must be less than 0.1 mg / L. According to Setijaningsih and Suryaningrum [26], showed that the level of nitrite in water is generally not more than 1 mg / L. Even though nitrite waters are present in small amounts, nitrite levels in excess of 0.5 mg / L will be toxic to some sensitive aquatic organisms. The source of nitrite in the waters is ammonia, but in this case the ammonia binds to water reacts to ammonium used directly by plants as fertilizer, so the nitrification process of ammonia to nitrite takes place is not optimal.

Difference results from inlet-outlet nitrate concentrations in treatment T1 (0.709 mg / l), treatment T2 (0.561 mg / l), treatment T3 (0.429 mg / l) and treatment T4 (0.541 mg / l). Nitrate concentrations during the study were still in the optimal range. This was confirmed by Setijaningsih and Suryaningrum [26],[27] stated that the residual nitrate concentration measured in maintenance media was 0.665-1.558 mg / L. Nitrate value tends to decrease at the end of maintenance, this is due to the process of new nitrification and nitrate which is a nutrient for new growth utilized by plants. Nitrate concentrations in waters range from 0.1 to 5 mg / L. According to Ni’ma et al.(2014)[24] reported that, the use of plants as biological filters can change the form of inorganic nitrogen which is harmful to fish (ammonia and nitrite) into forms that are not harmful to fish (nitrates). Plants become substrates as a place to attach bacteria so that the nitrification process
goes well. Nitrifying bacteria utilize ammonia and nitrite as food ingredients that produce the final product in the form of nitrate. Furthermore, nitrate is used by aquatic plants as a source of nutrients for its growth.

Difference results from inlet-outlet phosphate concentrations in treatment T1 (0.05 mg / l), treatment T2 (0.05 mg / l), treatment T3 (0.04 mg / l), and treatment T4 (0.07 mg / l). The results of phosphate concentrations during the study are still in the optimal range. According to Nirmala et al. 2016 [12][27] showed that the measure phosphate concentration in water was 0.032–0.08 mg / L. Phosphorus is generally absorbed by plants as orthophosphate (H2PO4) and secondary phosphate (HPO42-). As with ammonium and nitrate, phosphate concentrations decrease at the end of maintenance because phosphorus is used by plants to grow. Phosphorus levels in natural waters range from 0.005 to 0.02 mg / L.

Based on the results obtained that the use of different biological filter media on snakehead significantly affected the survival rate. The highest value was treatment T3 (apu - apu) (96.67 ± 1.92%) and the lowest value in treatment T4 (Azzola) (77.78% ± 5.10). This is reinforced by the study of Nirmala et al 12 that the highest value of the survival rate of gouramy fish during 30 days of maintenance was achieved in the 45 g apu apu wood treatment of 96.33% and the lowest in the control treatment of 24.5%. Based on the variance statistical test (ANOVA) at 95% confidence interval, the control value was significantly different from the survival rate of 45 g, 90 g and 135 g apu apu wood treatments (P <0.05), while the 45 g apu apu wood treatment was significantly different from 90 g and 135 g.

The use of different biological filters obtained different results in life. This biological filter is a means of breeding microorganisms to carry out their biological functions. Biofilter is used to condition and maintain water quality in closed and open circulation systems. According to Dwiputra [9] explained that biofilter is a biological filter that has a function in filtering water containing nitrogenous waste using a substrate on a filter containing nitrification bacteria. This was confirmed by Wen et al [21] reported nitrification is a two-step aerobic oxidation process, the conversion of ammonia to nitrite (nitritation) with ammonia toxic oxidation bacteria and the further conversion of nitrite to nitrate by nitrite oxidizing bacteria. This is also reinforced by Sesuk et al [22] [28] Nitrification is a biological process that is studied aerobically changing ammonium and nitrite into nitrates which are non-toxic to aquatic animals.

The best survival value in treatment T4 is using Azzola biology filter. The high survival of apu - apu treatment is supported by the results of the most optimal water quality among other treatments. Optimal water quality is one factor in the survival of fish. This was confirmed by Setijaningsih and Suryaningrum [26] which states that the availability of high quality water is one of the most important factors in aquaculture. Various steps can be taken to regulate good environmental conditions. Water quality remains in the optimal range proven to improve the growth and survival of aquatic organism

3.2.2. Growth

Data taken during research on snakeheads growth are absolute weight growth, absolute length growth and specific growth rate. Based on the results of the study showed that absolute weight growth, absolute length growth, and growth rate had a significant effect (P <0.05) on different biological filters. The use of biological filters is used to improve water quality in aquaculture environments. This is confirmed by Nirmala et al. [12][15][29][30][31-33] that many factors that affect growth include the number of fish, available food sources and water quality factors. Poor water quality factors cause stress on the body of the fish so that it reduces appetite, therefore with good water quality it will increase the appetite of the fish[12][34-35].

The results of the growth showed that the absolute weight value, absolute length, and the highest growth rate in treatment T3 (apu - apu) were equal to (14.60 gram ± 2.41), (3.35 cm ± 0.31), and (2 , 6 grams ± 0.22), while the lowest treatment in treatment T4 (azzola azzola) with values (8.24 grams ± 1.98), (2.47 cm ± 0.39) and (1.83 ± 0.24 ). This is reinforced by the study of Setijaningsih and Suryaningrum [26] [36-37], which states that the daily growth rate and absolute
length growth in the treatment of 45 gram *Pistia stratiotes* give results that are significantly different (p <0.05) and higher growth compared to controls and other treatments. These values were 3.46 ± 0.19% and 0.93 ± 0.036 cm, respectively. This is presumably because judging from the results of measurements of water quality treatment of 45 grams also showed the best results among the controls and others. Yusoff et al [38][39-42] reported many factors affect growth including the amount of fish, available food sources and water quality factors. The condition of water quality in treatment T3 (Apu-apu) in better condition can be seen from the water quality results of ammonia, nitrite, nitrate, and phosphate with lower yields compared to treatments T1 (water hyacinth), T2 (Hydrila), and T4 (Azzola), besides supporting factors such as temperature, pH and Do also affect fish growth. According to Hartini et al [4][43-45], that the parameters of water quality which are quite influential on growth are temperature, nitrite, nitrate, ammonia, dissolved oxygen, and phosphate. Temperature variability is very important because generally aquatic organisms have a degree of tolerance within a certain range. The cultivation environment by Herlina [1] showed must have an optimal pH content. Aquatic conditions that are too acidic or too alkaline will endanger the survival of the organism because it results in impaired metabolism and respiration. In addition Weatherly [16][46-52] High pH values will cause the balance between ammonium and ammonia in water to be disturbed, while too high ammonia concentrations are toxic to organisms[51-52].

### 3.2.3. Microsatellites

This is in accordance with the opinion of Prasetiyono and Tasliah (2004) stating that differences in the number of microsatellite are the basis for the analysis of polymorphisms in the genome of an organism.

This is reinforced by the opinion According to Prasetiyono and Tasliah [53] [54-56] microsatellite is a simple sequence that is repeatedly abundant in the genome of a species. Microsatellites have repeated sequences of two to 4 nucleotide sequence motifs as conservative sequences. Suresh et al [17][28-30][53-59] reported that this marker is very useful as a genetic marker because it has a codominant nature, so that it can detect alleles diversity at a high level, easily and economically in its application because it uses the PCR process. Simple repetitive forms of DNA sequences that make microsatellite markers often called simple sequence repeat (SSR), short tandem repeats (STRs) or simple sequence length polymorphisms (SSLPs), which are now one of the most widely used markers for genetic mapping, genetic diversity analysis, and evolutionary studies Suresh et al [17]. These markers appear as markers that are very varied and easily repeated, making it ideal for genome mapping. This microsatellite is a type of repetitive polymorphism, which is usually grouped into simple tandem repeat polymorphism (STRP), because of genetic differences between DNA molecules that contain copies of short DNA sequences that are repeated several times. STRPs that have 2-9 base pairs are often called microsatellite, whereas STRPs with 10-60 base pairs are often called minisatites or variable numbers of tandem repeats (VNTR) (Prasetiyono and Tasliah,2004)[49], showed that mention microsatellites are scattered throughout the genome, whereas most minisatites are centered near telomeres.

Also added by Poiko et al [55] that in snakeheads (*Channa striata*) or *Ophiocephalus striata* that the variation in the capacity of fish species to biosynthesize n-3 polyunsaturated fatty acids (HUFA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids have a very important role important for growth and survival as well as influencing genetic variation in snakeheads [58-59]. Likewise the role of fat desaturation and elongation of enzyme processes - mediated has cloned, characterized and identified different genes for fatty acyl desaturase and elongase (FAD and FAE, respectively) enzymes from snakeheadsspecies. Also added that DNA extraction or mRNA genes can use snakeheadstissue such as: muscle, ovaries, testes, gills and skin, and different genes of fatty acyl desaturase and elongase (FAD and FAE) can be identified, respectively. Enzymes in snakeheads.

Also added by Balkhis et al [19] that the analysis of the use of genetic markers with microsatellite in snakeheads in Malasia obtained results that did not significantly affect the
presence of perlokus alleles between 2-7 with heterozygotes ranging from 0.120 to 0.880 with the Hardy-Weinberg deviation in single locus (CSI-C07) has a significant effect on CS1-C07, and is useful in assessing the level of diversity and population modulation structure for cork breeding and conservation strategies. 

According to Balkhis et al [19][55][59] that Channa striata or, known as "Haruan", is economically important in the fisheries and aquaculture industries in countries around Asia. DNA sequencing techniques, in snakeheads, are very important in determining genetic variation based on a partial segment of the cytochrome oxidase c 1 (CO1) gene, used to determine genetic variation in C. Striata. Samples, at different geographies, especially on the west coast Peninsular Malaysia has a nucleotide, and the haplotype is the highest diversity of population with a value ( <0.05) in all population pairwise comparisons. So that the genetic mapping of snakeheads(C.striata) provides information on genetic variation about the origin of snakeheads geographically different ..

Also added by Balkhis et al [19] that genetic analysis using microsatellite in snakeheadscan distinguish genetic variations in different geographical origins as well as the value of heterozygotes and genetic diversity.

Compared to core DNA Qiufen et al [44], that mitochondrial DNA is as a genetic marker, in genetic-differentiation studies. Mitochondrial cytochrome c oxidase 1 (CO1) becomes prime-bar DNA coding region to identify fish taxonomies viz snakeheads [56][58][60], also added by Yu et al [57] that the use of DNA sequencing from CO1 mt DNA can investigate the pylogenetic group of snakeheads (C. striata) along the west coast of Peninsular Malaysia.

4. Conclusion
1. The use of different biological filters in the maintenance of snakeheads with a recirculation system has a significant effect (p <0.05) on survival, absolute weight growth, absolute length growth, and specific growth rate.
2. Treatment T3 with apu apu media gives the best results with the highest Volumetric removal rate of NH3 at treatment T3 (0.0016 mg/l ). The highest volumetric removal rate of NO2 results in treatment T3 (0,021 mg/l). The highest volumetric removal rate of NO3 results in treatment T3 (0.429 mg/l), phosphate (0.04 mg/l), survival rate (96.67 ± 1.92%), growth in absolute weight 14.60 ± 2.41g, growth in absolute length 3.35 ± 0.31 cm, SGR 2.61 ± 0.22.
3. The results of polymorphism analysis using micro satellitese on snakeheads in various treatments T1 (water hyacinth filter), T2 (hydrilla filter), T3 (apu apu filter) and T4 (azzola filter) quality of snakeheads products differed in genetic variation from the results of genetic analysis marker method microsatellite ie (T1, bands 1-3), (T2 bands 4-6), (T4, bands 7-9) (T3, bands 10-12, 13-16) and then each treatment in snakeheads based on the results micro satellite analysis using IS-GB2 F OILG0 BASE TYPE 5-AGA AGA AGA AGA AGC CGA GT-3 primers and IS-GB2 R 5-GAA AAA CAG AGC AGG AAC AC-3 (INTEGRATED DNA TECHNOLOGIES SINGAPORE), bright bands found in the treatment T4 (band 4,5,6) snakeheads maintained using maintenance media filtered with azzola filter with a 202 bp ladder band with 214 bps alleles and to the right of 224 bps, as well as having high polymorphism. Snakeheads as a whole are caught in the waters of Central Java have a high heterozygot, so it has a good quality snakeheads product that grows well.

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