Survival of Bada (Rasbora sp.) and Daphnia sp. using biofloc from Lake Maninjau as natural feed

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Abstract. Biofloc is one of the water bioremediation agents in aquaculture. A consortium of microorganisms in biofloc, which include heterotrophic bacteria, phytoplankton and zooplankton, can also function as natural food sources for aquatic biota. This study aims to investigate the potential of biofloc microorganisms consortium as natural food for commercial fish feed-less aquaculture. The study was conducted using Lake Maninjau water taken from two locations at Lake Maninjau, namely Nagari Sungai Batang and Nagari Maninjau, as test samples. Maninjau Lake was chosen because it had a high content of organic compounds and various microorganisms which could be used as an indigenous biofloc starter. Daphnia sp. and Bada (Rasbora sp.) were used as an experimental biota without periodical additional feed during the trial. The results showed that biofloc could grow well using Maninjau Lake water media. Bada and Daphnia could live in the biofloc system for 20 days without supplementary feeding and afterwards entering the death phase. The decline in the population of Bada and Daphnia was thought to be caused by the decrease of biofloc role as natural feed and its ability in maintaining the water quality.

1. Introduction
The fish feed from floating net cages (KJA) fishery activity in Lake Maninjau is one of the organic matter sources in the lake water body. Loading of the organic matter could trigger the growth of heterotrophic bacterial populations and various aquatic microorganisms in the lake. Heterotrophic bacteria and microorganisms found in Lake Maninjau can be used as a starter for biofloc systems for bioremediation purposes in aquaculture. Biofloc systems utilize a heterotrophic bacterial activity to degrade nutrient and organic matter by adjusting the ratio of organic carbon to nitrogen (C:N ratio) in a ratio ranging from 15 - 20:1 [1][2]. Biofloc systems in aquaculture can maintain water quality longer than conventional aquaculture so that cultivation with this system is water-efficient. The application of biofloc technology in aquaculture has been widely applied to various commodities including catfish [3], tilapia [4], Vannamei shrimps [5] [6], and Eel [7] [8].

Another advantage of biofloc systems is that biofloc itself can function as natural feed sources for cultivated biota so that it can reduce the value of the feed conversion ratio (FCR) [9]. This study aims to study the potential of a consortium of microorganisms, including indigenous heterotrophic bacteria from Lake Maninjau as a biofloc starter for the maintenance of Bada, a local name for Rasbora sp., and Daphnia sp. without commercial fish feeding. The concept of aquaculture without commercial
fish feed aims to reduce the intake of organic material into the water bodies. Bada was chosen in this test because this fish can be reared in a fertile pool of plankton without the addition of the feed from outside [10].

2. Methods
The study was conducted at Unit for Technology Transfer of Lake Restoration, Maninjau District, Agam West Sumatra from April to August 2018. Testing was carried out in three phases, namely (1) biofloc growth, (2) Bada rearing, and (3) Daphnia rearing. The lake water used in this study was taken from two regions with different characteristics, namely Nagari Sungai Batang and Nagari Maninjau. Water from the Nagari River Batang region was chosen because in that area the number of floating net cages was relatively less and based on 2017 data (Suryono T, personal communication) the water quality in the area was better compared to other regions. Whereas in the Maninjau area the water quality was lower than the Nagari River Batang area and there were more floating cages compared to Nagari Sungai Batang. Biofloc for Bada rearing was made by adding molasses to the lake water of the Nagari Sungai Batang and Nagari Maninjau areas (Figure 1) with or without the addition of urea then acclimatized for six days with aeration (table 1). The treatment of adding urea was aimed to see its effect on the formation of biofloc.

The second phase of research, namely Bada rearing, began on the seventh day when biofloc had been formed. Bada rearing time was carried out for 73 days using the initial stocking density of fish was 30 individuals/60 L. During the rearing periods, molasses were added 0.5 g every seven days and no water was replaced. But to compensate the water lost through vaporization, water was added weekly to obtain the same volume with the initial condition. During the Bada rearing study, no additional (commercial) feeding was carried out.

The biofloc for Daphnia rearing experiment in the third phase of the study was made by adding 5 g of molasses to the test medium which contains a mixture of 5 L biofloc water from the stage 2 testing and 25 L of lake water from the Nagari Sungai Batang and Nagari Maninjau. In the Daphnia rearing experiment medium, the urea was not added because of the purpose of the third stage of the research was aimed to study the growth patterns of daphnia in the biofloc system. The acclimatization of biofloc growth for this test was carried out for six days. Daphnia rearing time was carried out for 35 days. The initial stocking density of Daphnia was 100 individuals/60 L. During the rearing periods, molasses was added 0.5 g every seven days and no water was replaced. During the Daphnia rearing, no additional (commercial) feeding was carried out. In addition to Daphnia, there was also no periodic harvesting until the end of the test.

Monitoring of water quality was carried out for pH, temperature, dissolved oxygen, dissolved and suspended solids, total ammonium nitrogen (TAN), nitrite-nitrogen, and chlorophyll-a [11]. Sampling in stage 1 test (biofloc growth) and the other tests were carried out every two and seven days, respectively.
Figure 1. Water sampling locations at Nagari Sungai Batang and Nagari Maninjau. The water was used as biofloc growth medium.

Table 1. Dose of molasses and urea added to Bada rearing container for the biofloc growth.

| Water Source  | Treatment          | Molasse (gr) | Urea (gr) | Water Volume (L) |
|---------------|--------------------|--------------|-----------|------------------|
| S. Batang (SB)| Without urea (TU)  | 0.248        | 0         | 60               |
|               | With urea (U)      | 6.664        | 0.499     | 60               |
| Maninjau (Man)| Without urea (TU)  | 0.364        | 0         | 60               |
|               | With urea (U)      | 7.772        | 0.577     | 60               |
Figure 2. Preparation of containers, water medium, and fish at Unit for Technology Transfer of Lake Restoration, Maninjau District, Agam West Sumatra.

3. Results and Discussion

3.1 The growth of biofloc

The urea addition treatment at the beginning of the experiment was subjected to provide an overview of Lake Maninjau water quality conditions in 2017 when the maximum TAN concentration was obtained (Suryono T, personal communication). Therefore, molasses added were different between with and without urea treatment experiment in order to obtain C:N ratio of 15-20:1.

Figure 3 presents the changes in water quality during the biofloc formation stage, i.e. from the beginning to the sixth day of testing. Suspended solids (SS) gave an overview of biofloc formation that consists of a consortium of the aquatic heterotrophic bacteria and other microorganisms. From the suspended solids profile, it could be seen that in both urea added-media from Sungai Batang and Maninjau had higher SS concentration than treatment without urea (figure 3D). While from the chlorophyll-a profile, it can be seen that the addition of urea has an effect on the increase of phytoplankton abundance (figure 3E). Based on these conditions it can be seen that biofloc could grow well in Lake Maninjau water with low (0,165 mg/L) or high (0,705 mg/L) TAN content (figure 3A). Increased concentrations of urea and molasses added to Lake Maninjau water medium influenced the increase in the amount of biofloc formed. The composition of heterotrophic bacteria against phytoplankton in biofloc was influenced by the initial concentration of TAN and organic carbon as molasses [1]. This could reflect that the increase of the environmental ammonium could trigger the growth of microorganism’s population. Nitrite was decreased at the 10th days in all of the treatments (figure 3B) and this phenomenon was an indication that biofloc was ready to be used for aquaculture and bioremediation purposes.

The appearance of water on phase 1 (the first and fourth day) and phase 2 (the 79th day) research are showed in Figure 4A. On the fourth day, biofloc growth was seen as if the water began to look cloudy in all test containers both given urea and those not given urea. However, the addition of urea treatment gives a faster formation of biofloc compared to the treatment without the addition of urea (figure 4B).
Figure 3. Profiles of (A) Total ammonium nitrogen (TAN), (B) nitrite-nitrogen, (C) heterotrophic bacteria, (D) suspended solids, and (E) chlorophyll-a.
Figure 4. (A) Biofloc formation from the first day until 79th day and (B) the difference of water content between with and no urea addition at the fourth day (100x magnification).

3.2 Survival of Bada Fish

During the Bada fish experiment period, the parameters of temperature, dissolved oxygen, and pH in all maintenance media were in the appropriate range for fish maintenance [12] (figure 5). However, in the middle of the experiment, there were conditions where dissolved oxygen was very low in all treatments, which was around 3 mg/L.

![Figure 5. Profiles of (A) temperature, (B) dissolved oxygen, (C) pH, and (D) total dissolved solids during the study of Bada survival in biofloc system (phase two) .](image)

The results showed that Bada fish were able to survive for 20 days without the addition of commercial fish feed and subsequently the survival was decreased by day 78th (figure 6). Bada fish reared in biofloc made from Nagari Maninjau water were able to survive better than fish reared in biofloc made from Nagari Sungai Batang water. This was presumably because the water is taken from Nagari Maninjau had a richer consortium of microorganisms including zooplankton than Nagari Sungai Batang River so natural food was more available. The number of zooplankton species in Maninjau biofloc ranged from 9-10 species, while in Sungai Batang ranged from 5-8 species. The zooplankton diversity index (H) in Maninjau biofloc also tend to be higher (2.14 - 2.79) compared to Sungai Batang biofloc (1.16 - 2.45).

Zooplankton in the Maninjau biofloc generally had higher mean abundance compared to the Sungai Batang biofloc, except in the Sungai Batang SBU-1 (table 2). The higher abundance of zooplankton in the SBU-1 biofloc experiment was due to the domination of *Frontonia angusta*. *F. angusta* is a species of free-living unicellular ciliate protists belong to the genus of *Frontonia* (Kingdom Chromista; Phylum Ciliophora; Class Oligohymenophorea; Order Hymenostomatida; Family Frontoniidae) [13]. According to type of food consumed, *F. angusta* is classified as algophage ciliate protist which preys phytoplankton as their food [14]. The dominance of *F. angusta* in SBU-1 might relate to lower chlorophyll-a content in that location compared to Man-U which had the same bioflocs treatment with SBU. The similar phenomenon was found by Filip et al [15] in their research on effects of ciliate...
consumer diversity and nutritional mode on algal-prey biovolume and evenness. The research was conducted using *F. angusta* as purely heterotrophic consumers and it was found that prey biovolume was significantly lower in the treatments since *F. angusta* was known as a very strong grazer [15].

**Table 2.** Zooplankton diversity index in the biofloc grown on water from S. Batang and Maninjau District on the 7th day.

| Species                  | Water Source |
|--------------------------|--------------|
|                          | SB TU-1      | SB TU-2      | SB U-1      | SB U-2      | Man U-1      | Man U-2      | Man TU-1     | Man TU-2     |
| **Anuraeopsis fissa**    | 12           | 32           | 28           |             |             |             |             |             |
| **Anuraeopsis navicula** | 4            | 8            | 4            |             |             |             |             |             |
| **Anuraeopsis prodonta** | 40           | 4            | 4            |             |             |             |             |             |
| **Arcella discoides**    | 56           | 32           | 4            |             |             |             |             |             |
| **Ascomorpha saltans**   | 20           | 16           | 8            |             |             |             |             |             |
| **Asplachna brightwelli**| 4            | 8            | 4            |             |             |             |             |             |
| **Asplachna prodonta**   | 4            | 8            | 4            |             |             |             |             |             |
| **Branchionus calyciflorus** | 72         | 16           | 52           | 64          | 88          |             |             |             |
| **Bursaridium pseudobursaria** | 4       |             |             |             |             |             |             |             |
| **Centropyx aculeata**   | 4            | 4            | 204          | 4           | 24          | 8            |             |             |
| **Euchlanis aropha Gosse** | 4          |             |             |             |             |             |             |             |
| **Frontonia altra**      | 8            |             |             |             |             |             |             |             |
| **Frontonia angusta**    | 336          |             |             |             |             |             |             |             |
| **Gastropus hyptorus**   | 4            |             |             |             |             |             |             |             |
| **Haltera sp.**          | 8            |             |             |             |             |             |             |             |
| **Keratella procurva**   | 4            |             |             |             |             |             |             |             |
| **Lacinularia elongata** | 4            |             |             |             |             |             |             |             |
| **Lembadion bullinum**   | 40           |             |             |             |             |             |             |             |
| **Monostyla bulla**      | 40           | 12           | 16           | 88          | 16          |             |             |             |
| **Monostyla lunaris**    | 4            |             |             |             |             |             |             |             |
| **Notholca laurentiae**  | 4            |             |             |             |             |             |             |             |
| **Notholca squamula**    | 4            |             |             |             |             |             |             |             |
| **Pompholyx sulcata**    | 16           | 16           |             |             |             |             |             |             |
| **Stentor araucanus**    | 4            |             |             |             |             |             |             |             |
| **Trachelomonas sp.**    | 4            |             |             |             |             |             |             |             |
| **Trichocerca Iata**     | 16           |             |             |             |             |             |             |             |
| **Trichocerca mucosa**   | 4            |             |             |             |             |             |             |             |
| **Trichodina pediculus** | 4            |             |             |             |             |             |             |             |
| **Chydorus sp.**         | 4            |             |             |             |             |             |             |             |
| **Calanoid nauplius**    | 4            | 4            | 4            |             |             |             |             |             |
| **Copepoda molt**        | 4            | 4            | 4            |             |             |             |             |             |
| **Heterocope septentrionalis** | 8      |             |             |             |             |             |             |             |
| **Number of species**    | 6            | 8            | 5            | 8           | 9           | 10           | 10           | 9            |
| **Abundance (individu/liter)** | 76          | 168          | 556          | 128         | 72          | 172          | 216          | 172          |
| **H**                    | 1.44         | 2.33         | 1.16         | 2.45        | 2.84        | 2.80         | 2.24         | 2.14         |

SB: Sungai Batang District  
TU: without urea addition  
Man: Maninjau District  
U: with urea addition
Zooplankton found in the biofloc consortiums that have the potential to be used as fish feed was Brachionus (1 SB; 4 Man), Cladocera, and Copepoda (2 SB and 3 Man). Zooplankton is very important to be present in open waters as a natural feed for the fish juvenile and other aquatic biota at least in the first ten days after the egg yolk reserves run out. This happens because zooplankton is a provider of important nutrients needed in early fish life [15, 16]. Said & Hidayat [10] states that the natural feed of Bada fish is plankton, worms, water fleas, tiny shrimp, and mosquito larvae.

Mortality of Bada in the first week was caused by the adaptation to the water and as the impact of the transportation process from the rearing tank to the experiment container. In the next week until the 20th days of the experiment there was no significant death of Bada. The significant decrease of Bada fish was found after 20 days of experiment (figure 6). The decreased was thought to be caused by the decrease in the number of microorganisms as a source of natural food due to consumption by Bada fish continuously, the decrease of water quality, or because of Bada life cycle itself.

**Figure 6.** Population profile of Bada fish grown in biofloc systems without commercial feed.

### 3.3 Survival of Daphnia sp.

Biofloc for Daphnia experiment was grown using biofloc from the container used for Bada survival experiments (stage 2). The treatment was aimed to accelerate the acclimatization period of biofloc growth as found by Sadi et al [17] that the use of ex-cultivation biofloc can shorten the biofloc acclimatization time in new ponds. The acceleration of biofloc acclimatization was caused by the presence of the living microorganism consortium of mature biofloc in large amount in the ex-cultivation water so they can readily assimilate TAN and other nutrients. The pH profile in phase one experiment (biofloc growth) showed that at the beginning of biofloc growth there was a surge in pH reduction from the range of 9.6 - 9.9 to 8.4 - 8.6 within 24 hours in all test media (figure 5C). This indicated that the condition of water quality was not yet stable and could not be used for cultivation. Different condition was found in the Daphnia experiment test medium (phase three). The pH value during the acclimatization period of biofloc growth was stable in the range of 8.4 - 8.5 in all test media (figure 7C). Furthermore, during the Daphnia experiment period, temperature, dissolved oxygen content and pH were in the appropriate range for fish growth [12].
Figure 7. Profile of (A) Water temperature, (B) dissolved oxygen, (C) pH profiles, and (D) dissolved solids during the Daphnia rearing period in biofloc systems.

Daphnia could reproduce well until the 20th day and subsequently, the population was decreased for the next 15 days (figure 8). The density of Daphnia which was originally 2 individuals / L increased rapidly to 6,500 individuals / L in the Maninjau biofloc medium and 10,000 individuals / L in the Sungai Batang medium on the 21st day. In contrast to Bada fish, Daphnia populations reared in Sungai Batang biofloc were higher than those reared in Maninjau biofloc. It was suspected that bacterial floc grown in the Sungai Batang water were optimally consumed by Daphnia because there are no competitors or fewer competitors than the water from the Maninjau.

Figure 8. Growth patterns of Daphnia sp. grown in biofloc systems.

The finding in this research opens the opportunity that Bada aquaculture could be done using the biofloc system cultivated in Maninjau Lake water. This Bada aquaculture is possible to be done in the commercial feed-less method is accompanied by the addition and restocking of Daphnia periodically. This type of aquaculture could be considered as integrated multitrophic aquaculture and have bioremediation purposes since the biofloc could maintain the water quality during the aquaculture process.
4. Conclusions

Biofloc could be grown in Lake Maninjau water both in low or high TAN concentration season. Bada fish and Daphnia could live and reproduce (Daphnia only) in the biofloc system for 20 days without supplemental feeding and afterward undergo a phase of death. The decline in Bada and Daphnia populations was thought to be caused by the decrease of biofloc ability in maintaining water quality and its role as natural food. In order to maintain the ability of biofloc for water quality management and its role as natural feed, biofloc regeneration is needed throughout the treatment of settled bacterial floc.

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