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SHORT COMMUNICATION
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# Effectiveness of Green Water System as Larval Gourami (*Osphronemus gouramy*) Cultivation Technology - Efforts to Increase Larval Productivity

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## ABSTRACT

The crucial phase occurs at the larval stage, which often experiences mass mortality. The green water system is an aquaculture technology that offers a solution to increase larval survival and productivity. This study aims to determine the effectiveness of green water system technology in increasing larval survival and productivity. Experiments were carried out with three treatments: control without treatment, Green water using *Spirulina* sp. (T1), and *Chlorella* sp. (T2) with treatment replication units of 3 times each. The results showed that green water system cultivation technology had significant survival and final length compared to the control without treatment ($p<0.05$). Green water system technology effectively supports larval life by providing natural food and bioremediation mechanisms to convert organic matter in the environment so that a multi-trophic level balance occurs. This technology has a beneficial impact and has the prospect of being developed for broader applications in the future.

**Keywords:** Aquaculture, Biotechnology, Microalgae

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## INTRODUCTION

Gourami, with the Latin name *Osphronemus gouramy*, is one of the leading freshwater fish commodities. It has a high economic value and relatively stable selling price (DJPB, 2017). Banyumas Regency is a central producers larvae of gourami in Central Java, which is well-known nationally. Gourami hatchery activities in Banyumas Regency start from the first grower (GI), second grower (GII), third grower (GIII), fourth grower (GIV), and fifth grower (GV). The size of larvae produced from gourami hatchery activities varies from 1 to 11cm. However, as with other fish problems, mass mortality is a significant obstacle in gourami hatcheries due to physiological conditions and resistance, which are still very vulnerable at the larval stage (Hamre et al., 2013; Rønnestad et al., 2013).

Various efforts have been made as an alternative to the mortality problem, from feeding types to engineering cultivation media. Several studies have tried types of feed to meet the nutritional needs of fish larvae from natural to artificial feed, such as artemia (Conceição et al., 2010), copepods, rotifers (Mona et al., 2019), and microcapsule feed (Sukardi et al., 2019). Other efforts made by fish farmers are generally hatcheries made to make it easier to control, especially the cultivation environment. However, this requires relatively expensive costs and feeding, which requires intensive attention. Increasing the productivity of larvae and fish fry requires an effective and efficient technology with good quality feed and cultivation.

Green water system is a cultivation system that uses microscopic plants in the form of microalgae as a remediation agent (Pacheco et al., 2015). Naturally, microalgae have a role as one of the supplies of energy and oxygen in water through photosynthesis. In green

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water system technology, microalgae are engineered to be a bioremediation agent that utilizes dissolved organic materials to improve water quality (Muller-Feuga, 2000). In addition, fish can utilize microalgae as natural food in the culture media (Basford et al., 2021). Furthermore, fish in the media contribute important organic material to technology. Microalgae utilize the metabolic products of fish as a carbon source used in the metabolic process to produce oxygen products (Muller-Feuga 2000; Cunha et al., 2019). The breakdown of these compounds generally benefits fish farming, so it becomes the basis for innovation in developing effective and efficient fish larval culture.

Green water system technology has a high opportunity to increase productivity in the aquaculture industry, especially in the hatchery stage. Some studies have shown that green water system technology can significantly increase survival at the larval stage of fish (Van Der Meeren et al., 2007; Basford et al. 2021). This study experimented with creating a green water system technology using *Chlorella* sp. and *Spirulina* sp. in different containers as cultivation media to see the effectiveness in increasing the survival of gourami larvae. The success of this study is expected to be a reference in applying the cultivation system for gourami larvae as an alternative to the problems that occur in the field.

## MATERIALS & METHODS

### Research Design

This study used an experimental design to see the differences in the types of microalgae used in green water system media. The control treatment applied in this study serves as a comparison in the experiment. The following treatment of the green water system using *Spirulina* sp. in this study is treatment 1, and *Chlorella* sp., is treatment 2. Each treatment was repeated three times. Each experimental unit included 100 carp larvae. The parameters of survival and final fish length were measured to see the effectiveness of the experimental media on the parameters measured. This experiment was conducted in the aquaculture laboratory of Muhammadiyah Purwokerto University, Banyumas Regency, Cental Java, Indonesia

### Preparation of Green Water System Media

The green water system media was prepared using the formulation listed in Table 1. Next, each media was aerated and left for 14 days. This is the microalgae enrichment process in the system. Monitoring of the enrichment process was carried out daily to ensure the success of media preparation.

**Table 1:** Formulation of green water system media

| Ingredient | Treatment | | |
|---|---|---|---|
| | Control | T1 | T2 |
| *Spirulina* sp L/Tank | - | 2.00 | - |
| *Chlorella* sp L/Tank | - | - | 2.00 |
| KCl g/L | - | 0.20 | 0.20 |
| Urea g/L | - | 0.10 | 0.10 |
| Fertilizer ZA g/L | - | 0.80 | 0.80 |
| Dolomite chalk g/L | - | 0.01 | 0.01 |
| Worm food g * | 100.00 | 100.00 | 100.00 |

*worm feed after 14 days

### Preparation and Cultivation of Gourami Larvae

The gourami larvae used were newly hatched larvae from the eggs. The larvae were counted carefully. As many as 100 larvae were reared in each tank. Gourami larvae were obtained from the UPTD-Balai Budidaya Ikan Tawar (BIAT) Kutasari Purbalingga. Gourami larvae were cultured for 24 days with worm feed every morning and evening. Then, the aerator system was run as an oxygen supply during maintenance.

### Water Quality Test

Water quality measured in this study included pH, temperature, dissolved oxygen, and total dissolved solids. Measurements were made every week during the experimental period. In addition, the color and aroma of water in each cultivation medium were observed to describe the cultivation medium in the experiment.

### Final Lenght of Gourami Larvae

The final length of gourami larvae was measured using ten gourami larvae from each treatment replicate unit placed in a millimeter block. Larvae were positioned upright and measured using a caliper. Then, the data were written down for further analysis.

### Survival Rate of Gourami Larvae

Observations of larval mortality were made daily to determine the number of deaths. The total survival of carp larvae was calculated at the end of rearing following the study (Manduca et al., 2020) as follows:

$$SR = \frac{total\ of\ fish\ larvae\ in\ the\ end}{\text{total of fish larvae at the beggining}} \quad \text{X } 100\%$$

### Data Analysis

The gourami larvae survival data obtained were analyzed using ANOVA analysis of variance with a 95% confidence level. A Duncan test was followed at the same confidence level if there were significant differences. The analyzed data are displayed in Tables and Graphs. Then a complex description of the cultivation technology used in the treatment is based on the studies conducted so that a comprehensive conclusion is obtained.

## RESULTS

### Overview and Condition of the Green Water System

Green water system cultivation media is prepared by calculating the formulation of organic materials dissolved in water as an initial carbon source for microalgae culture. Some materials are basic materials used by microalgae to develop. Some of these materials include urea, lime, and fertilizer. Microalgae are cultured in experimental tanks before being used for cultivation. The description and quality of the microalgae culture are shown in the Table below.

The picture of the green water system in this study shows a different color appearance (Fig. 1). The control treatment without microalgae culture shows a transparent color. The treatment of adding *Spirulina* sp. culture shows

a greenish color, while the treatment of adding *Chlorella* sp. culture shows a green color that tends to be dense. Overall, water quality, such as dissolved oxygen, and temperature tend to be no different. However, in contrast, pH in the Spirulina treatment tends to be lower than the control, and *Chlorella* sp. treatment tends to be higher than the control (Table2). However, overall, the making of the Green water system shows a good microalgae culture in both treatments. Then, providing live worms in each treatment aims to be the leading food for gourami larvae. Worm feeding is done once and given continuously when the worms in the treatment have run out.

At the end of rearing, gourami larvae in each experimental unit were measured. The results showed that the overall average final length of carp fry ranged from 2.07-2.49cm. The control treatment had the lowest final seed length value of 2.07-2.10cm. The treatment using *Chlorella* sp. water media ranges from 2.09-2.44cm. The highest value of the average range of the final length of carp fry is found in the treatment using Spirulina maintenance media, which is 2.35-2.49cm, the results of which are listed in Table 3.

**Table 2:** Water quality of microalgae culture

| Parameter | Average of Water Quality Value | | |
|---|---|---|---|
| | Control | T1 | T2 |
| Water pH | 9.1 | 8.8 | 9.5 |
| Temperature (°C) | 30.8 | 30.5 | 30 |
| dissolved oxygen (ppm) | 5.7 | 4.2 | 4.8 |
| Smell | No odor | No odor | No odor |
| Water Color | clear | green | Deep green |

**Table 3:** Length of gourami larvae at end of rearing

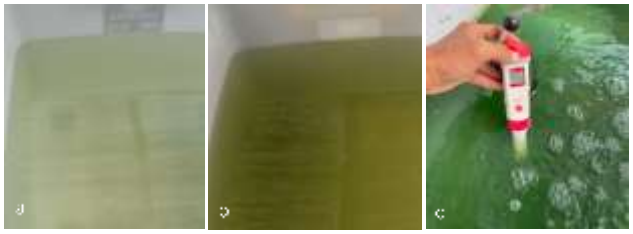
| Treatment | R1 | R2 | R3 | Average |
|---|---|---|---|---|
| Control | 2.07 ± 0.12 | 2.09 ± 0.09 | 2.10 ± 0.11 | 2.08 |
| T1 | 2.49 ± 0.27 | 2.35 ± 0.15 | 2.39 ± 0.13 | 2.41 |
| T2 | 2.29 ± 0.20 | 2.09 ± 0.17 | 2.44 ± 0.16 | 2.27 |

**Table 4:** Survival Rate of Gourami Larvae

| Treatment | Repeat Treatment | Survival Rate (%) |
|---|---|---|
| Control | R1 | 0 |
| | R2 | 0 |
| | R3 | 0 |
| T1 | R1 | 63.5 |
| | R2 | 56.5 |
| | R3 | 69.5 |
| T2 | R1 | 66.5 |
| | R2 | 73.5 |
| | R3 | 43.0 |

Data on the final length of larvae were subjected to statistical testing to see in more detail the effect of the green water system (Fig. 2). The results of the analysis showed that the carp larvae rearing media using the green water system with Spirulina and Chlorella showed significantly different length values compared to the control. This is because Spirulina and Chlorella can provide additional nutrients for larvae to meet nutritional needs as an energy source for growth. In addition, the green water system media improves water quality so that it can support the larval growth process.

The average survival rate of carp fry reared without treatment (control) is 0%. Death in the control treatment occurs as a whole on the 24th maintenance day. This is thought to be due to saturated water conditions so that it can no longer sustain the life of fish seeds. Different things were shown in the *Spirulina* sp. and *Chlorella* sp. treatments, which had a high percentage of survival, namely 56.6-69.5% and 43.0-73.5% (Table 4). This shows that the Green Water System maintenance media using *Spirulina* sp. and *Chlorella* sp., gourami larvae, can be utilized as a natural food that can be used to maintain survival and growth. The nutritional content contained in *Spirulina* sp. and *Chlorella* sp., as well as the size that is right for the mouth opening of carp fry, can be a source of nutrition after the fry is released from the yolk and before eating silkworms. Nutritional needs that are always met can increase the growth and durability of seeds in the future.



a = Control, b = *Spirulina* sp., c = *Chlorella* sp.

**Fig. 1:** Green water system cultivation media.

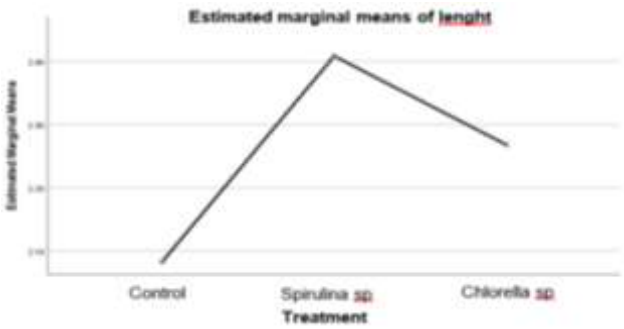
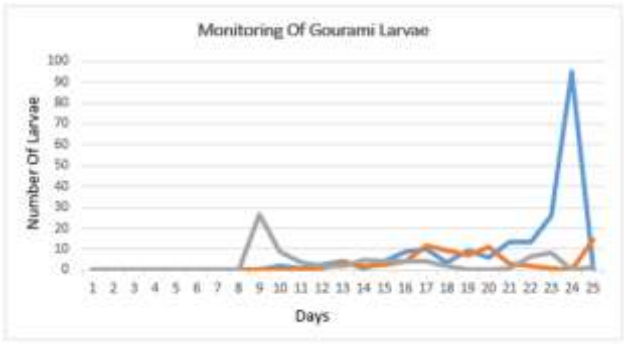



**Fig. 2:** The Final Length of Body Larvae Gourami




Based on the graph of daily mortality in gourami larvae shows that death begins to occur on the ninth day of maintenance. The death occurred in treatment 2 with the provision of Chlorella. The next few days, death occurred in other treatments, which occurred alternately. However, peak mortality occurred massively and thoroughly on the 24th maintenance day in the control treatment. The results of previous observations show that in the control treatment, there are symptoms caused in the larvae, such as inactive movement. This indicates that the cultivation media experienced poor water quality in the control treatment, so the fish could not survive in these

conditions. This contrasts the green water system treatment with *Spirulina* sp. and *Chlorella* sp., where the gourami larvae survive better than the control on the 24th maintenance day. This shows that the Green water system media can improve water quality so that the larvae live more stable.

## DISCUSSION

Green water systems can be done with various types of microalgae (Basford et al., 2021). This study uses *Spirulina* sp. and *Chlorella* sp. plankton species as remediation and natural food in gourami larvae media. The results of the green water system experiment using spirulina and *Chlorella* sp. showed a significant final length in gourami larvae compared to the control or no treatment. This cultivation system has been proven to increase growth significantly and effectively. Although this study only looked at the final length of the fish, it can illustrate that the green water system positively impacts growth. Previous experiments conducted on tilapia showed that this system has a great opportunity in the future because it is highly effective in multi-trophic integrated aquaculture, which can increase aquaculture productivity (Gilles et al., 2013). This technology is the right one to use in applying larval stadia fish. The natural feed in this technology is very supportive of larval growth and can complement nutritional needs (Basford et al., 2021) and last but not least, the natural feed in this technology has a size that matches the mouth opening of the larvae (Nandi and Saikia, 2015).

The survival percentage in the green water system treatment showed a higher value than the control. The high survival rate is likely due to the green water system media being able to improve water quality (Pacheco et al., 2015). Microalgae in the system act as bioremediation to break down organic compounds in the environment, which are utilized as carbon in photosynthesis to produce oxygen, which is beneficial for fish (Muller-Feuga, 2000). This role has a good impact on larvae, especially fish health. One of the health improvements is triggered by good environmental quality so that the respiration and metabolism processes can run well (Gilles et al., 2013; Mohd Nasir et al., 2019).

The green water system is an integrated cultivation technology that can be applied to mass mortality in larvae due to failure to consume feed that does not match the mouth opening. Then, based on daily monitoring, mortality began to occur on day 9 of the rearing period in the green water treatment. However, mortality occurred alternately in each treatment, and complete mortality occurred on day 24 of the control treatment. The mortality in the control treatment shows that the maintenance media without treatment cannot support larval life due to decreased water quality. In contrast to the green water system treatment, fish larvae are more stable and able to live because of adequate oxygen supply and organic content that has been processed in the system (Muller-Feuga 2000; Basford et al. 2021).

Green water systems offer many advantages in aquaculture applications. A series of multi-trophic complexes and integrations produce products that support increased aquaculture survival and productivity (Muller-Feuga 2000; Gilles et al. 2013). In addition, these systems are known to enhance fish growth and immunity (Alagawany et al., 2021). Furthermore, chlorella microalgae have been widely used as a decomposer of aquaculture waste, a significant problem, especially in shrimp farming. Broadly, microalgae have been widely used in aquaculture. This research includes one application of microalgae services in supporting the survival of gourami larvae. The use of green water systems can be developed not only in the application of gourami larvae cultivation but further study regarding the microorganisms that play a role in the system so that the utilization of technology is maximized.

### Conclusion

Green water system technology shows good effectiveness in applying gourami larval culture. The survival rate and final length of larvae treated with the green water system were significantly higher than the control treatment. Green water systems provide advantages in aquaculture and have the potential to be developed in the future.

### Authors Contribution

IP contributed to coordinating, analyzing, and producing the initial version of the article. S, RN, and MN prepared a draft article, including template frameworks, and modified it to the final submitted version. All authors also contributed to reading and reviewing the final manuscript.

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### Competing Interests

The authors declare that they have no competing interests.

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