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*Research Article*

# Nutrient removal from rainbow trout juveniles in fish ponds using integrated biofilter duckweed (*Lemna minor*) and freshwater mussel (*Anodonta cygnea*)

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**Keywords**

*Lemna minor*,
*Anodonta cygnea*,
*Oncorhynchus mykiss*,
Living Biofilter,
Aquaculture wastewater

**Abstract**

A biological filter (biofilter) is a technique that aims to degrade nutrients that are wasted in water bodies by utilizing living organisms such as plants and shellfish. The study aims to determine the potential of duckweeds (*Lemna minor*)-freshwater mussel (*Anodonta cygnea*) integrated biofilters in utilizing nutrients from wastewater from rearing rainbow trout (*Oncorhynchus mykiss*) juveniles. This study used the initial weight of 25±2.0g of rainbow trout juveniles, freshwater mussels (*A. cygnea*) with an initial weight of 58±2.0 g, and duckweeds (*L. minor)* with two different biomass weights (treatment 1: 270 g and treatment 2: 360 g) were used in the flow-through system. The water temperature was around 13-14°C during the experimental periods. Using duckweeds as a biofilter with two different stocking densities significantly affected ($p$<0.05) the pH, DO, nitrate, and total phosphorus values in the treatment tanks. This study's results also revealed that using duckweed-freshwater mussels integrated biofilters could minimize the concentration of ammonium and total phosphorus in wastewater from rainbow trout juvenile rearing tanks.

**Article info**

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## Introduction

The intensive application of aquaculture with artificial feed has increased the disposal of aquaculture waste. Aquaculture waste from leftover feed and metabolic waste (urine and feces) can cause various environmental problems when disposed directly into water bodies (Jones *et al*., 2001). The rest of the fish feed and solid waste will decompose through the decomposition process to form nutrient compounds, some of which are nitrogen compounds ($NH_3^-$, $NO_2^-$, $NO_3^-$), phosphorus (P), Total Suspended Solids (TSS), and carbon sources (C) (Yusoff *et al.*, 2011; Hlaváč *et al.*, 2014; Salin and Ataguba, 2018; Dauda *et al.*, 2019). Therefore, it is necessary to implement a sustainable cultivation system that is friendly to the environment.

One alternative that can be used in aquaculture waste management is to apply a biofilter system. Biofilter is a profitable biological waste treatment technique because it effectively treats waste from aquaculture activities and saves production costs (Shpigel and Neori, 2007). Several types of biofiltration can be used to treat aquaculture wastewater, including bacteria (Van Rijn, 1996), microalgae (Milhazes-Cunha and Otero, 2017); macroalgae (Hall and Martin, 2021), and suspension feeders (Zhou *et al.*, 2014). *Lemna minor* is a macrophyte plant widely used in waste treatment because it can absorb nutrients in the water and rapidly grow (Landesman *et al.*, 2005; El-Kheir *et al.*, 2007). In addition, the *Lemna minor* plant is a type of floating macrophyte that can be used as a feed source because it has a protein content ranging from 28-43%, fiber (5%) in dry weight, high concentrations of minerals such as phosphorus, potassium, xanthophyll, and carotene (Leng *et al.*, 1995; Landesman *et al.*, 2005). *Lemna minor* is also widely used in remediation methods to improve water quality. Bivalves are a filter feeder that effectively reduces the concentration of suspended organic matter in water bodies (Zhou *et al.*, 2006). In addition, bivalves are an essential component of aquatic ecosystems and function as bioindicators of pollution in waters because of their ability to survive in various environmental conditions. They can accumulate multiple substances found in aquatic environments (Helmholz *et al.*, 2016).

Research on the use of duckweeds in aquaculture activities has been carried out, such as its use as a source of additional feed nutrition in fish farming (Yılmaz and Günal, 2005; Olaniyi and Oladunjoye, 2012; Asimi *et al.*, 2018); biofilters in aquaculture waste disposal (Sipaúba-Tavares *et al.,* 2002; Paolacci *et al.*, 2021); as a biofilter in fish tanks (Ferdoushi *et al.*, 2008); Biofilter on the RAS system (Velichkova and Sirakov, 2013; Sumoharjo, 2015). *Lemna minor* is a phytoremediator plant found in tropical to subtropical areas with temperatures between 6-30$^o$C and optimal growth temperatures between 20-30$^o$C (Nafea, 2016). In contrast, the optimum temperature for growth of rainbow trout is between 12-20°C (Pepe-Victoriano *et al.*, 2021). This study aims to determine the ability of the integrated biofilter between duckweed (*Lemna minor*) and freshwater mussel (*Anodonta cygnea*) to utilize nutrients from juvenile rainbow trout

(*Oncorhynchus mykiss*) rearing wastewater carried out at 13-14ºC.

## Materials and methods

The animal ethics guidelines were strictly followed in this experiment, and approval for our study procedures and methods was taken in advance from the Istanbul University Local Ethics Committee of Experimental Animals (IUHADYEK; 2022/19).

### *Experimental design*

Fish rearing will be carried out using the flow-through system. Fish rearing was carried out in four round tanks with a water volume of ±250L. One round tank with a volume of 200L (sump tank); One round tank with a volume of 200L (sump tank); Then, six rectangular filtration tanks with a volume of 100L (200×50×10cm), which function as biofiltration media. Water was circulated using a water pump (5L min$^{-1}$). The water flow speed in the biofilter tanks was 45 seconds L$^{-1}$. The water well was channeled into Tank 1 (fish tank). To Tank 2 (sedimentation tank), then into Tank 3 (sump tank), then into Tank 4 (filtration tanks containing biofilter species: freshwater mussel and duckweed), and then out into Tank 5 (waste disposal tank) (Fig.1). The aeration system (26 m$^{-3}$ hours$^{-1}$) is also added to the fish and biofiltration tanks. The average water flow speed in the living biofilter tank was approximately 35 seconds L$^{-1}$. This study used two treatments (T1 and T2) and three replications.

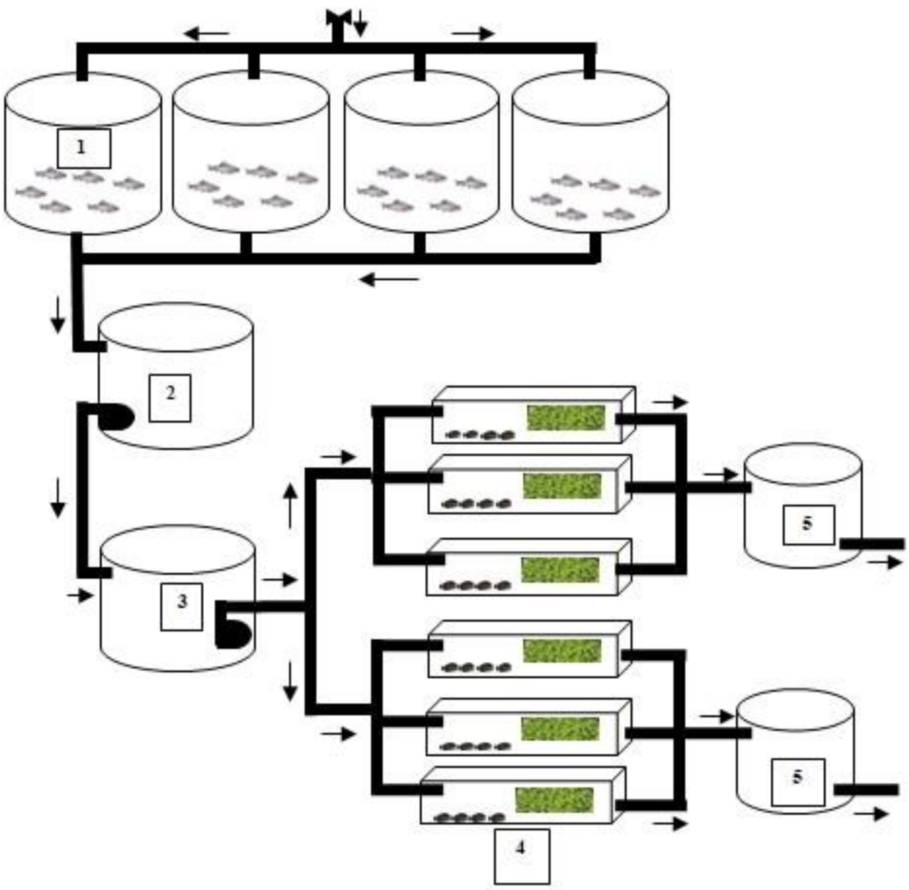


**Figure 1: The layout of rearing rainbow trout fish with the flow-through system.**

The treatments given to the study were *Lemna minor* biomass weights (T1: 270g and T2: 360g) with a large cover area used approximately 75% of the area of the biofiltration tank in all treatments. The freshwater mussels to be used had a stocking density of 30 individuals/ $m^2$ with an average weight of 58±2.0g in T1 and T2 (Retnosari *et al.,* 2019). The freshwater mussels were collected from the Maşukiye Stream in Sapanca. The duckweed used was obtained from the Sapanca Lake. Then, the duckweed and freshwater mussels were adapted for six days in rearing tanks before the experiment. During the study, irradiation was also carried out on macrophytes with a ratio of light and dark conditions of 10:14 hours. The process will be provided using an LED lamp (HYG05) with a light intensity of around 6600lux.

*Fish stocking and management*

The research was conducted at the Aquaculture Laboratory in the Istanbul University Aquatic Vertebrate Live Experiment Unit (İÜSUCAN) Sapanca. The rainbow trout (*Oncorhynchus mykiss*) juveniles to be used have an average size of 25±2.0g with a stocking density of 15 kg $m^{-3}$ (Çelikkale *et al.,* 1999). Fish maintenance was carried out for 30 days. During the experiment, fish were fed using commercial extruder pellets of 3% fish body weights daily (Kamalam *et al.,* 2020). Feeding is given twice daily, in the morning (08.00) and afternoon (16.00). The proximate composition in the commercial rainbow trout extruded diets can be seen in Table 1.

**Table 1: Proximate composition of rainbow trout juveniles' diet (3 mm pellet size).**

| Proximate composition | Contents (%) |
|---|---|
| Crude protein | 46.0 |
| Crude fibre | 1.9 |
| Lipid | 18.0 |
| Ash | 10.2 |

*Water quality measurements*

Water was brought to the experimental unit by passing through a mechanical filter and a UV filter to use in this system. Water quality sampling was carried out in the morning before the feeding process. Measurements of temperature, pH, and Dissolved Oxygen (DO) were carried out *in situ* using a multiparameter instrument (WTW Multiparameter 3620 IDS) and TDS (Total Dissolved Solids) using a multiparameter tool (ISO lab multiparameter). Measurements of Ammonium ($NH_4^+$), Nitrite ($NO_2^-$), Nitrate ($NO_3^-$), Total Phosphorus (T. Phosphorus), and Silica dioxide ($SiO_2$) were measured once every ten days using the APHA (2017).

*Growth performance parameters of fish*

A sampling of fish and mussel growth parameters was carried out once every ten days. At the same time, the sampling of *L. minor* growth was carried out at the beginning (day 0), mid (day 15), and late (day 30). Parameters measured in fish during the study included Survival Rate (SR), Weight Gain (WG), Total Biomass (TB), and Feed Conversion Ratio (FCR). In comparison, the test parameters measured on mussels were Average Body Weight (ABW), Survival Rate (SR), Specific Growth Rate (SGR), and Total Biomass (TB). Parameters measured in duckweed were the average fresh biomass of initial and final biomass, Relative Growth Rate (RGR), and the percentage of increase and decrease in biomass during the rearing periods.

*Data analysis*

Data were presented as mean value and standard deviation (±SD). The data obtained were processed using the Excel workshop program and tested for normality and homogeneity. Then, the data were analyzed statistically using an independent sample t-test. Statistical comparisons were evaluated with the IBM SPSS software 20th version for Windows (SPSS Inc., Chicago, IL, USA).

## Results

*Water quality data between the treatments*

Based on the results of 30 days of maintenance, water quality data were obtained in the filtration media with different treatments, which can be seen in Table 2. Table 2 shows that using duckweed plants with different wet biomass influences water quality. During the 30 days of rearing, DO, nitrate, and T. Phosphorus concentration values in the T1 treatment showed a significant difference ($p<0.05$) compared to the T2 treatment on day 10 and day 20. At the same time, nitrate and T. Phosphorus values show significantly different values at T1 compared to T2 on day 20 and day 30. The results of ammonium, nitrite, nitrate, T. Phosphorus, and silica dioxide measurements carried out every ten days on the rearing medium can be seen in Figure 2.

**Table 2: The mean water quality (Temperature, pH, DO, TDS, ammonium, nitrite, nitrate, T. phosphorus, and Silica dioxide values in biofiltration tanks with different duckweed treatments during the 30-day maintenance period.**

| Water parameters | T1 (270 g) | | | | T2 (360 g) | | | |
|---|---|---|---|---|---|---|---|---|
| | Day-0 | Day-10 | Day-20 | Day-30 | Day-0 | Day-10 | Day-20 | Day-30 |
| Temperature (°C) | 13.64±0.05 | 13.72±0.03 | 13.53±0.05 | 13.41±0.08 | 13.66±0.57 | 13.70±0.05 | 13.47±0.11 | 13.50±0.10 |
| pH | 7.73±0.06 | 7.83±0.02 | 7.84±0.03* | 7.69±0.02 | 7.77±0.04 | 7.85±0.02 | 7.92±0.02 | 7.66±0.01 |
| DO (mg $L^{-1}$) | 9.70±0.03 | 9.82±0.02 | 9.34±0.03 | 9.72±0.03 | 9.67±0.05 | 9.82±0.03 | 9.47±0.03* | 9.73±0.15 |
| TDS (mg $L^{-1}$) | 166.0±5.0 | 218.8±1.4 | 217.0±5.0 | 213.2±1.1 | 166.0±7.0 | 218.7±1.0 | 217.5±1.1 | 213.6±2.2 |
| Ammonium (µg $L^{-1}$) | 279.3±0.0 | 198.2±16.2 | 25.5±8.9 | 22.4±9.8 | 274.3±0.0 | 195.6±10.4 | 24.7±5.6 | 24.7±7.0 |
| Nitrite (µg $L^{-1}$) | 1.7±0.0 | 15.2±7.7 | 9.7±1.0 | 27.4±7.0 | 1.9±0.0 | 13.6±4.6 | 10.4±0.6 | 16.7±4.9 |
| Nitrate (µg $L^{-1}$) | 692.6±0.0 | 759.1±115.0 | 800.5±83.4 | 895.8±7.4 | 784.6±0.0 | 766.1±82.5 | 788.5±51.0 | 849.4±4.0* |
| T. Phosphorus (µg $L^{-1}$) | 27.2±0.0 | 24.1±4.3* | 33.8±37.1 | 31.9±4.4 | 37.7±0.0 | 47.6±29.0 | 14.4±1.0* | 20.9±1.7* |
| $SiO_2$ (mg $L^{-1}$) | 5.4±0.0 | 5.6±0.1 | 5.8±0.1 | 5.2±0.1 | 5.4±0.0 | 5.5±0.0 | 5.8±0.1 | 5.1±0.1 |

DO: Dissolved oxygen; TDS: Total dissolved solids; $SiO_2$: Silica dioxide. Mean values (± Standard deviation)

Based on the maintenance period, the ammonium value decreased at the end of the maintenance period in all treatments. The nitrite value fluctuated during the 30 days of the rearing period in all treatments but tended to increase at the end of the rearing period.

*Water quality in all tanks*

The average value of water quality obtained from the 30-day maintenance period for all experimental tanks can be seen in Table 3. Based on Table 3. The average DO in the fish tank is lower than in other tanks. The average value of ammonium in the disposal pond is higher than in different tanks.

Nitrite values fluctuate, with the lowest values found in fish-rearing tanks. The same thing is also found in nitrate values, which fluctuate but tend to decrease in the disposal pond. The T. Phosphorus value also showed a decrease in the waste pond. The average $SiO_2$ values were stable in all study tanks during the 30 days of the rearing period.

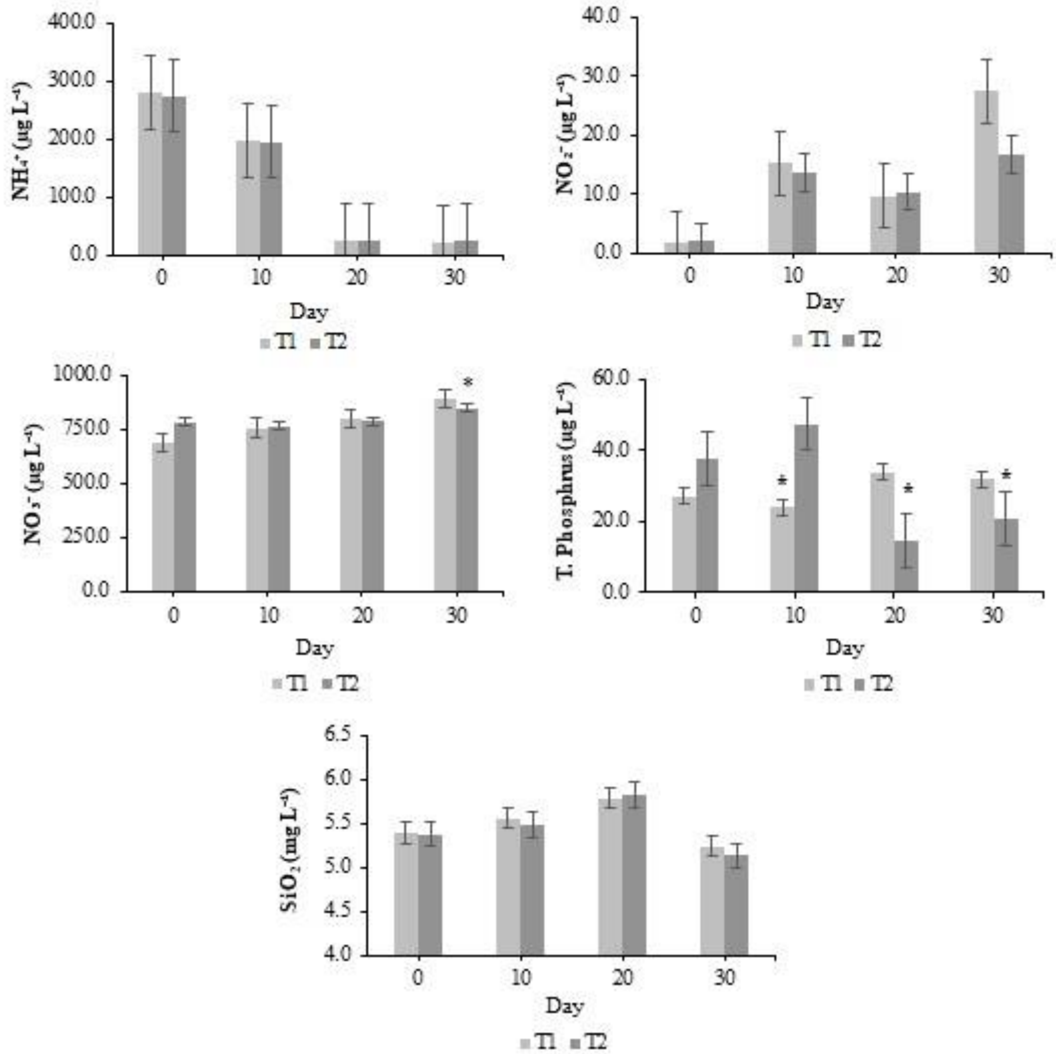


**Figure 2: The values of ammonium, nitrite, nitrate, T. Phosphorus, and silica dioxide during 30 days of rearing. Vertical bars indicate the standard error (n=3). "*" indicates a significant difference (*p*<0.05). Error bars are the standard error of the mean (*n* = 3) for all panels.**

*Growth parameters of trout juveniles, duckweed, and freshwater mussel*

From the 30-day rearing period, data on the growth of rainbow trout juveniles were obtained, as seen in Table 4. The growth parameters of freshwater mussels and duckweed carried out during the 30-day rearing period can be seen in Tables 5 and 6. Based on the rearing period, which was carried out for 30 days, it was shown that the kept freshwater mussel had an SGR of between 0.05-0.08% with a final average weight of 60.07±0.58g (T1) and 58.90±0.88g (T2).

**Table 3: The average water quality (temperature, pH values, DO, TDS, ammonium, nitrite, nitrate, silica) values in rainbow trout rearing tanks using the flow-through system.**

| Water parameters | Experimental tanks average (Min-Max) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Well water | Fish tank | Sump tank | T1 | T2 | T1 Waste disposal | T2 Waste disposal |
| Temperature (ºC) | 13.3 (13.2-13.6) | 13.5 (13.2-13.8) | 13.5 (13.3-13.7) | 13.6 (13.4-13.8) | 13.6 (13.5-13.8) | 13.6 (13.3-13.8) | 13.6 (13.4-13.9) |
| pH | 7.7 (7.2-7.9) | 7.5 (7.3-8.0) | 7.7 (7.4-8.0) | 7.8 (7.4-8.0) | 7.8 (7.4-8.0) | 7.8 (7.7-7.9) | 7.8 (7.7-7.9) |
| DO (mg $L^{-1}$) | 9.6 (9.6-9.9) | 8.4 (7.3-9.0) | 8.5 (7.7-9.6) | 9.7 (9.0-10.1) | 9.7 (9.1-10.1) | 9.7 (9.2-9.9) | 9.6 (9.3-10.0) |
| TDS (mg $L^{-1}$) | 201 (163-216) | 201 (166-216) | 195 (165-217) | 203 (166-219) | 203 (166-218) | 202 (166-218) | 202 (166-218) |
| Ammonium (µg $L^{-1}$) | 77.2 (12.9-184) | 132.2 (12.9-226) | 91.7 (30.8-202) | 145.4 (51.1-279) | 137.1 (25.4-274) | 151.6 (30.8-268.7) | 142.2 (30.8-275.9) |
| Nitrite (µg $L^{-1}$) | 1.56 (0.4-3.0) | 0.86 (0.4-1.2) | 2.11 (0.0-4.6) | 6.9 (1.0-15.2) | 6.3 (0.0-13.6) | 5.0 (1.9-8.1) | 3.5 (1.3-6.4) |
| Nitrate (µg $L^{-1}$) | 598 (118-822) | 786 (725-822) | 739 (646-803) | 768 (693-820) | 790 (766-819) | 742 (674-803) | 743 (649-813) |
| T. Phosphorus (µg $L^{-1}$) | 9.2 (3.5-20.4) | 13.5 (3.5-20.4) | 16.5 (8.3-21.1) | 25.0 (15.0-33.8) | 29.2 (14.4-47.6) | 17.5 (8.3-25.2) | 18.7 (8.3-23.9) |
| $SiO_2$ (mg $L^{-1}$) | 5.5 (5.4-5.8) | 5.5 (5.3-5.8) | 5.5 (5.4-5.7) | 5.5 (5.3-5.8) | 5.5 (5.4-5.8) | 5.5 (5.4-5.7) | 5.5 (5.4-5.7) |

DO: Dissolved oxygen; TDS: Total dissolved solids; $SiO_2$: Silica dioxide.

**Table 4: Data on the average growth of rainbow trout during the 30 rearing days.**

| Growth parameters | Rainbow trout |
|---|---|
| Initial Weight (g) | 25.74±0.46 |
| Final Weight (g) | 59.99±1.74 |
| SR (%) | 100.00±0.00 |
| Weight gain (g) | 34.24±2.10 |
| SGR (% $day^{-1}$) | 2.82±0.15 |
| Final Biomass (g $tank^{-1}$) | 8313±24 |
| Total Feed (g $tank^{-1}$) | 3631±16 |
| FCR | 0.82±0.07 |

SR: Survival rate; SGR: Specific growth rate; FCR: Feed conversion ratio. Mean values (±Standard deviation).

The 30 days of maintenance showed that the final percentage of fresh biomass of duckweeds in both treatments showed a decreasing trend. Relative growth occurred in treatment T1 but not in treatment T2. The final fresh biomass of the duckweed in all treatments experienced a decrease in fresh biomass of 23.24% (T1) and 19.86% (T2).

The reduction in the percentage of new biomass from duckweed during the maintenance period can be seen in Figure 3. Figure 3 shows that all fresh biomass from duckweed decreased until the end of the 30-day rearing period. Although, in general, it declined, the percentage of fresh biomass in the T1 increased by 2.25% from 74.51% (day 15) to 76.76% (day 30). Meanwhile, T2 decreased until the 30th day, with a biomass percentage value of 80.14% (30th day). Overall, the T1 experienced a more significant decrease of 23.24% compared to the T2 (19.86%).

**Table 5: Average growth of mussels during 30 days of the rearing period.**

| Growth parameters | T1 (270 g) | T2 (360 g) |
|---|---|---|
| Initial weight (g) | 58.72±1.21 | 57.97±1.49 |
| Final weight (g) | 60.07±0.03 | 58.90±0.88 |
| Survival rate (%) | 100±0.0 | 100±0.0 |
| SGR (% day$^{-1}$) | 0.08±0.07 | 0.05±0.04 |

SGR: Specific Growth Rate. Mean values (± Standard Deviation).

**Table 6: Duckweed fresh biomass during the rearing period.**

| Variable | T1 (270 g) | T2 (360 g) |
|---|---|---|
| Initial fresh biomass (g) | 270±0.0 | 360±0.0 |
| Final fresh biomass (g) | 226±9.4 | 320±19.4 |
| RGR (day$^{-1}$) | 0.002±0.00 | -0.005±0.01 |
| Percentage of fresh biomass (%) | 83.8±10.1 | 88.9±8.5 |
| Percentage of decreased fresh biomass (%) | 23.2±6.3 | 19.9±4.1 |

RGR: Relative Growth Rate. Mean values (± Standard Deviation).

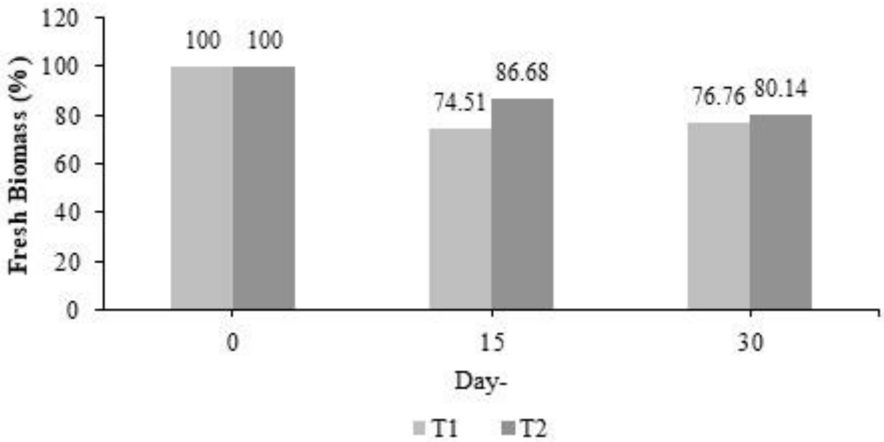


**Figure 3: The percentage of fresh biomass of duckweed on days 0, 15, and 30 of the rearing periods based on different treatments (T1: 270 g and T2: 360 g).**

## Discussion

The water temperature during the study ranged between 13.4-13.8°C in all biofilter tanks, which is the optimal temperature for freshwater mussels but not for duckweed. The optimal temperature for the growth of early adult freshwater mussels grows optimally at 14°C in their natural habitat (Lima *et al.*, 2004). Meanwhile, the optimal temperature for maximum growth of *Lemna* plants. Ranges between temperatures of 19-30°C (Nafea, 2016). Temperature conditions that are not optimal will cause plants to experience a decrease in average growth and can even stop growing and reduce duckweed biomass (Culley *et al.*, 1981). Some types of duckweed will experience a reduction of average growth and biomass when it is at temperatures below 17°C and stops growing at temperatures below 8°C and above 35°C. The decrease in temperature also affects the clones produced (Culley *et al.*, 1981; Zimmo *et al.*, 2005; Lasfar *et al.*, 2007; Paterson *et al.*, 2020).

The pH values during the study in all biofilter tanks ranged from 7.69-7.84 in T1 and 7.66-7.92 in T2 (Table 2). The average pH value decreased at the end of the maintenance period in both treatments. The chlorosis process began on the 10th day of the rearing period and continued until the end. This reduction in biomass can be caused by less than the optimal water temperature and a fluctuating pH value during the maintenance period. Duckweed has a wide tolerance for pH, which varies with values between 3.5 and 9.0. However, the ability to tolerate it depends on the type of species. The optimal pH value for growth is 6.5-7.5 (Leng *et al.*, 1995; Hasan and Chakrabarti, 2009; Nafea, 2016). The pH value of water affects the ratio of ammonia and ammonium in wastewater (Paterson *et al.,* 2020). Increasing the pH value of water also impacts shifting the ammonium concentration towards ammonia (Tchobanoglous *et al.*, 2003). High ammonium content in water can also disrupt the process of transporting anions into the duckweed membrane, thereby inhibiting duckweed growth (Caicedo *et al.,* 2000).

DO values during the study period showed significantly different values ($p<0.05$) between T1 and T2. DO levels on T1 showed a lower value than on T2 on the 10th day. However, overall, it indicates that the DO value between the two treatments was similar. The decrease in DO occurs on the 10th day between the two treatments. This can be caused by the high content of organic matter originating from the duckweed plant, which dies and settles at the bottom of the tank, thereby affecting the consumption of microorganisms in the decomposition of organic matter. Although, in general, the DO value is still in optimal conditions for fish maintenance. In mineralization, microbial respiration can increase water oxygen absorption (Bianchini *et al.,* 2011; Boyd, 2015).

The use of duckweed and freshwater mussels as integrated biofilter species on rainbow trout juvenile rearing for 30 days has been shown to absorb nitrogen sources, especially ammonium. At the same time, the nitrate concentration in the duckweed tanks in the two treatments fluctuated but tended to rise at the end of the rearing period. The following research results from Velichkova and Sirakov (2013) and Paolacci *et al.* (2021) show that *L. minor* plants are faster at utilizing nitrogen in the form of ammonium than in the form of nitrate, which is easier for *L. minor* plants to convert into protein. Hasan and Chakrabarti (2009) state that *L. minor* will utilize all available ammonium before assimilating nitrate. Several studies have shown that *Lemneacea* utilizes ammonium as a nitrogen source more than nitrate (Culley *et al.*, 1981; Cedergreen and Madsen, 2002). However, research by Paterson *et al.* (2020) showed that *L. minor* absorbs nitrate better than ammonium for growth. *Lemnaceae* is a type of plant that can utilize nitrogen in the form of nitrite, nitrate, ammonium, urea, and various amino acids. However, ammonium and nitrate are generally the primary nitrogen sources (Ardiansyah, 2018).

The nitrite and nitrate concentrations in the duckweed plant tanks in both treatments fluctuated but tended to increase at the end of the maintenance period. The results of this study were also obtained by Velichkova and Sirakov (2013) and Muvea *et al.* (2019), who showed an increase in nitrite and nitrate concentrations at the end of the maintenance period. The increase in nitrite and nitrate in waters can be caused by environmental factors such as temperature and biological factors such as the aging process of plants and chlorosis that occurred during the research period. In the opinion of Kuschk *et al.* (2003) and Akratos and Tsihrintzis (2007), stated that the activities of bacteria and vegetation, which are responsible for nitrogen removal, cannot function properly below a temperature of 15°C. Meanwhile, according to Lee *et al.* (2009), the concentration of nitrite and nitrate will decrease over time because macrophyte plants can use it to form biomass, but the concentration can increase due to the mineralization of ammonia and nitrogen as well as the reaction of nitrogen with dissolved oxygen in the wastewater.

During the 30-day maintenance period, there were significantly different ($p$<0.05) results in T. Phosphorus absorption between the two treatments. Treatment T2 was more effective in utilizing total phosphorus than treatment T1. Duckweed is one type of plant that has an excellent ability to absorb the nutrients contained in water bodies. The removal rate of duckweed in absorbing nutrients is 2.1 g $m^{-2}$ $d^{-1}$ for total nitrogen (TN) and 0.6 g $m^{-2}$ $d^{-1}$ for T. Phosphorus (Cheng *et al.*, 2002). Although the *L. minor* can remove phosphorus in water, it depends on the water temperature. During the winter season, the ability of macrophyte plants decreases seasonally, with the lowest phosphorus removal efficiency in winter and the highest in spring (Kalengo *et al.*, 2021). *L. minor* removed the phosphorus in the rearing medium within four days at 25°C. Whereas at 8°C, the *L. minor* could absorb half of the phosphorus found in the rearing medium. Removing phosphorus will continue even though conditions cannot support biomass formation in *L. minor*. In addition, the removal of phosphorus is also influenced by the presence of nitrate (Paterson *et al.,* 2020).

Silica is an essential element in macrophytes, especially in some species. The value of $SiO_2$ in the duckweed tanks in both treatments continued to increase until the end of the rearing period. The availability of silica in the rearing media helps plants absorb nutrients and increases their tolerance to abiotic and biotic stress (Olle, 2017). Khondker *et al.* (1994), found a positive correlation between *L. gibba* biomass and phosphate and silicate concentrations.

*Water quality in all experimental tanks*

Based on Table 3. Shows that the water temperature, pH value, and TDS values were stable in all experimental tanks until the end of the maintenance period. The water temperature during the study period ranged between 13.3-13.6°C. It was the optimal temperature for the growth of juvenile rainbow trout and freshwater mussels but not optimal for the development of duckweed plants. The optimal temperature for rainbow trout ranges from 10-14°C (Gall and Crandell, 1992). The average pH value in all ponds is in standard and slightly alkaline conditions, between 7.5 and 7.8. According to Hinshaw (2000), the optimal pH range for rearing rainbow trout is 6.5-8.5. Meanwhile, for freshwater mussels, based on the research results of Lopes-Lima *et al.* (2014), the range of pH values during the research period in the natural habitat of *Anodonta cygnea* mussels ranged from 6.6 to 7.4. TDS values on all maintenance tanks are still in average condition. According to Summerfelt (2000), freshwater fish can tolerate the TDS value of less than 400 mg $L^{-1}$. The DO content in fish tanks is lower than in other tanks. However, it remains in optimal conditions for rearing rainbow trout. Barton (1996) states that the optimal DO concentration for cultivating rainbow trout is more than 5 mg $L^{-1}$. This study also follows the results of Ferdoushi *et al.* (2008) and Velichkova and Sirakov (2013), which show that adding duckweed plants to ponds can increase oxygen concentrations. The ammonium value in the disposal tank is higher than in other tanks. The influence of pH and water temperature can cause this. Ammonium concentration in water depends on the pH value and temperature, where ammonium will be more dominant at low pH and water temperature (Hargreaves and Tucker, 2004).

Nitrite values in all rearing tanks tended to fluctuate, with the lowest value found in fish-rearing tanks. However, the nitrite value in all tanks is still within optimal limits for rainbow trout juvenile-rearing activities. Nitrite is an essential intermediary product in nitrification and denitrification processes (Kroupova *et al.*, 2005). The concentration of nitrite in the waters tends to increase during winter and spring due to fluctuating temperatures, which causes the activity of plankton or bacteria to decrease (Durborow *et al.*, 1997). The nitrate and T. Phosphorus parameters in each pond have different values but show a decrease in the disposal tank compared to the treatment tank. The low nitrate and T. Phosphorus values in the disposal tank can be caused using nitrate and T. Phosphorus by microorganisms and *L. minor* plants. Nitrate and ammonium are sources of N and phosphorus used by phytoplankton and aquatic plants for growth (Walstad, 1999; Boyd, 2015).

The average $SiO_2$ concentration value tends to increase until the end of the rearing period in all experimental ponds with a range of 5.3-5.8 mg $L^{-1}$. Silica is essential in fish farming activities, especially shrimp because it is needed by phytoplankton, especially diatoms, for growth (Boyd, 2003) and can strengthen stems and leaves in higher plants (Boyd, 2014). The source of silica during the research was obtained naturally from water entering the pond. In conventional shrimp cultivation, the source of silica can come from pond bottom sediments (Cremen, 2007); however, in super-intensive cultivation using a plastic-lined pond system, the primary source of silica only comes from the intake water (Emerenciano *et al.*, 2022). The average silicon concentration in freshwater in $SiO_2$ ranges from 5-25 mg $L^{-1}$ (Boyd, 2014). $SiO_2$ affects plant growth and increases tolerance to unfavorable environmental conditions (Kara *et al.,* 2022).

During the 30-day rearing period, juvenile trout experienced growth with an average SGR value of 2.82±0.15%/day (Table 4). Rainbow trout grow optimally at temperatures between 7-18$^oC$ from seed to harvest size. The amount of food in rainbow trout increases with increasing temperature. However, trout's appetite decreases when the temperature is lower or higher than its optimal temperature range (Woynarovich *et* *al.*, 2011). The SGR value during the maintenance period is still in optimal condition. The results of research by Sonay and Başçınar (2017) showed an SGR value of 2.61±0.04%/day when rearing juvenile rainbow trout in monoculture.

The measurements showed that the fresh biomass in both treatments decreased until the end of the maintenance period. The final fresh biomass between the two treatments decreased until the end of the rearing period. The decrease in duckweed biomass occurred in all treatments until the end of the rearing period. However, at T1, there was an increase in biomass on the 30th day (Fig. 3). This decrease in duckweed biomass may have been influenced by water temperature, which was less than optimal for duckweed growth and density that was too high in all treatments, especially in the T2 treatment (360gr), and the absence of regular harvesting of *L. minor* plants during the maintenance period. Several factors can inhibit duckweed growth, including light intensity, water temperature, nutrients, pH, and mat density (Driever *et al.*, 2005; Jaiprasert, 2018). The chlorosis process started on the 10th day in all treatment tanks. This chlorosis process can be caused by the influence of abiotic factors in the form of water temperature that is not optimal and biotic factors in the form of high biomass density, which reduces the level of nutrient absorption. This follows the results of Chakrabarti *et al.* (2018), where production from duckweed decreased when the water temperature was less than 18.5$^o$C. *Lemna* growth slows down during winter in temperate climates. *Lemna* and *Landoltia* species experienced a decrease in average growth during winter. Meanwhile, *Spirodella* and *Wolffia* species died and formed turions at the bottom of the waters (Zhao *et al.*, 2014). In addition to temperature conditions, slow growth can also be caused by plant density (Driever *et al.*, 2005; Mkandawire and Dudel, 2007). The research results from Paolacci *et al.* (2018) showed that water temperature affects the RGR of *L. minor* and *L. minuta*, where the RGR of both will decrease gradually at low temperatures compared to high temperatures with a maximum RGR at 20$^o$C. Regular harvesting is also important because it can increase duckweed growth and nutrient removal (Xu and Shen, 2011) and reduce the release of ammonium and phosphorus originating from the decomposition of dead plant tissue (Sudiarto *et al.*, 2019).

During the 30-day rearing period, freshwater mussel growth experienced growth with an SGR value of 0.08% day-1 at T1 and 0.05% day-1 at T2. Generally, freshwater mussels gave similar results in the two treatments. The TDS value in both treatments during the maintenance period was lower than in the sump tank (Table 3). *Anodonta* is a genus of freshwater mussels widely used as a biofilter in aquaculture activities. Freshwater mussels are filter feeders that can absorb waste from aquaculture. The absorption rate of mussels and oysters in absorbing aquaculture waste in the form of (particulate-N) is more significant than 0.3 mg day$^{-1}$ gr$^{-1}$ tissue wet weight with assimilation efficiency between 18-26%. The absorption rate of shellfish is influenced by several factors, including flow rate, food concentration and water temperature (Shpigel and Blaylock, 1991), pH value, water velocity, and turbidity (Tuttle-Raycraft and Ackerman, 2018).

## Conclusions

The present study showed that an integrated living organism biofilter between duckweed (*L. minor*) and freshwater mussel *(A. cygnea)* could reduce the concentration of ammonium and total phosphorus in wastewater from rainbow trout juvenile rearing at a water temperature of around 13-14°C. However, using different fresh biomass weights affected the percentage of fresh biomass produced, with the best percentage increase in biomass found in treatment T1 (270g).

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