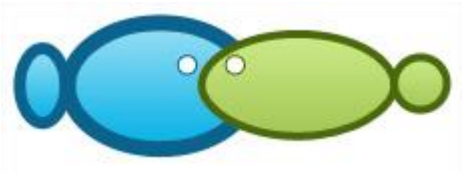

# Effluent from whiteleg shrimp (*Litopenaeus vannamei*) intensive culture increases *Artemia* biomass

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**Abstract**. High shrimp production is followed by a high effluent water discharge containing nutrients such as nitrogen and phosphorus. *Artemia* shrimps are non-selective filter feeders that can utilize the effluents from aquaculture as food. This study aimed to determine the appropriate stocking density, turbidity, and salinity using the effluent water of whiteleg shrimp (*Litopenaeus vannamei*) intensive culture as food for *Artemia*. The experiment included two setups [i.e., small-scale (100 L tank) and large-scale (1 $m^3$ concrete tank)]. Small scale was designed with two subtrials: (1) a two-factorial design, in which turbidity was 20, 25, and 30 cm and stocking densities were 200, 300, 400, and 500 individuals $L^{-1}$; (2) *Artemia* was cultured with 15, 20, 25, and 30 g $L^{-1}$ salinities. The best treatment in the first subtrial was expressed in terms of maximal *Artemia* biomass productivity, which was used to set up the second subtrial. Water quality parameters, such as temperature, pH, dissolved oxygen, and salinity, were recorded daily. Additionally, total ammonia nitrogen (TAN), $NO_2$-N, total nitrogen, total protein, and total suspended solids (TSS) were measured on days 7 and 14. The survival rate and growth (length) were measured on days 7 and 14, while biomass was collected at the end of the culture. Three microalga phyla were found throughout the experiment, including twelve species. A significant difference was observed ($p < 0.05$) in the main effects of the parameters on *Artemis* survival, growth, and biomass. In the first sub-trial, the best parameters were a stocking density of 300 individuals $L^{-1}$ and a turbidity of 25 cm. Meanwhile, the best parameters of the second subtrial were 30 g $L^{-1}$ salinity, with the highest biomass of 1.5 $kg.m^{-3}$.

**Key Words**: *Artemia*, algal, growth, shrimp effluent, salinity, survival.

**Introduction**. In Vietnam, whiteleg shrimp (*Litopenaeus vannamei*) is the most common species cultured and exported, followed by black tiger shrimp (*Penaeus monodon*). It is characterized by intensive culture and, thus, became an issue for soil sustainability and water pollution (Hai et al 2015; Van Nguyen et al 2021).

*Artemia* is a small crustacean usually found in salty lakes (Atashbar et al 2010). It is an important live food for larviculture of fish and shellfish because of its essential fatty acid 20:5ω3, which is crucial for marine fish and crustacean larvae (Leger et al 1987; Sorgeloos et al 1998). Therefore, *Artemia* production increases continuously worldwide to satisfy market demands.

*Artemia* is a non-selective filter feeder of microalgae, organic detritus, and bacteria (Lavens & Sorgeloos 1996). Multiple studies have demonstrated that agricultural wastes, by-products, and aquaculture waste could be used as alternative foods to culture *Artemia* with different setups (McShan et al 1974; Santhanakrishnan & Emelda 2013; Lopes-dos-Santos et al 2019; Ogburn et al 2023). Tunvilai (1991) conducted an experiment using aquaculture wastewater to culture *Artemia*, resulting in better growth and *Artemia* biomass production. Our study aims to investigate the effects of stocking density, turbidity, and different salinities using effluent water from whiteleg shrimp intensive culture as food for *Artemia* to optimize the effluent water and increase *Artemia* biomass production.

## Material and Method

***Effluent preparation***. Effluent was collected from a whiteleg shrimp intensive culture pond in an *Artemia* field station (Vinh Chau, Soc Trang, Vietnam). Shrimps were reared for 60 days in 500 $m^2$ ponds with a stocking density of 200 individuals $m^{-3}$. They were fed with commercial pellets containing 40% of proteins. The feeding rate was 3-5% of total shrimp biomass, five times daily. For this study, the effluent was taken from the supernatant fraction of the effluent collection pond before treatment.

The effluent was pumped into a 4-ton tank through a 50 µm mesh and left for five days with aeration for indigenous microalga growth. No nutrient was added to the effluent tank. The effluent was distributed into experiment tanks through 50 µm mesh to separate large particles that were not suitable for *Artemia* (Dobbeleir et al 1985).

***Experimental design***. Two outdoor experiments were conducted: small-scale (100 L tank) and large-scale (1 $m^3$ concrete tank) cultures of *Artemia franciscana* strain Vinh Chau (Hoa 2003). The small scale comprised two subtrials. The first trial studied the effect of stocking density (200, 300, 400, or 500 individuals $L^{-1}$) and turbidity (20, 25, or 30 cm by Secchi disc) or *Artemia* growth, and the second subtrial analyzed the effect of different salinity levels (15, 20, 25, and 30 g $L^{-1}$). The best treatment, based on the highest production of *Artemia* biomass (kg $m^{-3}$) collected at the end of the culture period, was then applied for the second subtrial. The best treatment from the second subtrial was scaled up in 1 $m^3$. All experimental treatments were randomly distributed with three replicates each. Each experiment was conducted for 14 days. The experiments were carried out during January-February, 2024.

During the experiment, the tank was covered with a nylon net to reduce the ambient heat. Thirty percent of the water volume was exchanged daily to maintain the experiment's turbidity and compensate for evaporation. If the desired turbidity was not reached, microalgae from the effluent tank were filtered and supplied into culture tanks based on the designed treatment.

The stocking density and turbidity interaction effect can be classified as additive, synergistic, or antagonistic. Synergistic interaction occurs when the response to the treatment by the two factors A and B ($R_{AB}$) is greater than the sum of the response to each ($R_A$ and $R_B$). The opposite is called an antagonistic interaction (Slinker 1998). Additionally, if the response and the sum of the responses are equal ($R_{AB} = R_A+R_B$) or two lines in the interaction plots are parallel, the interaction is called additive.

***Water quality analysis***. Water temperature, pH, and dissolved oxygen (DO) were monitored in situ twice a day at 7 am and 2 pm, and salinity was monitored after water exchange. The parameters were checked using a portable DO meter (Digital DO meter DO9100), a pH meter (pH and temperature Hanna Model- HI98127), and a refractometer (Atago model 2491-master's Japan) respectively. Samples were taken on days 7 and 14 from the experimental tanks before water exchange to monitor alkalinity, total ammonia nitrogen (TAN), and $NO_2$-N. The latter were analyzed by multi-spectrophotometers (Hanna Model-HI83303). Additionally, total nitrogen (TN), total phosphorus (TP), and total suspended solids (TSS) were measured following the 59 Standard Method procedures (APHA Method 4500-N, 4500-P, and 2540, respectively) for the examination of Water and Wastewater (APHA 1999). Samples were sent and processed in a water quality laboratory at Can Tho University.

***Algal identification***. Alga samples were collected daily before the water exchange at 10 am. The frequency was counted with a Sedgewick rafter cell (SRC). SRC is a rapid method to quantify samples with high cell numbers (LeGresley & McDermott 2010). A 1 mL aliquot was placed in an SRC using a pipette and observed under a light microscope (Nikon Eclipse E200 with magnification 10x). The algae were counted based on the following formula (Hossain et al 2007):

$$N = (A \times 1000 \times C) / (V \times F \times L)$$

where N is the number of plankton cells or units per mL, A is the total number of plankton counted, C is the final volume of the sample (mL), V is the volume of a field ($mm^3$), F is the number of fields counted, and L is the volume of original water (L).

Algal identification was performed according to Shaari et al (2011). Algae were preserved in Lugol, examined under a light microscope (Nikon Eclipse E200, magnification 40x), and identified based on morphological characteristics from Shirota (1966). Appearance frequency was based on Scheffer & Robinson (1939). When the frequency was > 60%, algae were marked as "high density" (+++). They were marked as "medium density" (++) at 30-60% and as "low density" (+) at < 30%.

***Survival and growth (length)***. *Artemia* survival rate was calculated with the following formula (Toi et al 2013):

$$\text{Survival rate (\%)} = \frac{\text{Final number of } \textit{Artemia}}{\text{Initial number of } \textit{Artemia}} \times 100$$

*Artemia* growth was measured by the length from the anterior tip to the base of the furca (Vanhaecke & Sorgeloos 1980) with a digital caliper millimeter ruler (resolution 0.1 mm).

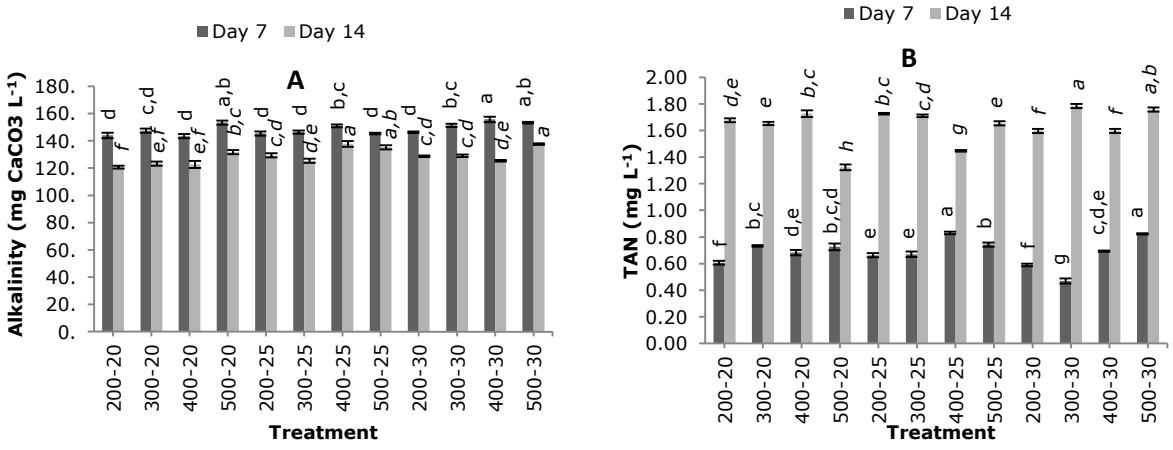
***Microbial load***. Total heterotrophic bacteria (THB) were determined using soy agar. Thiosulphate citrate bile salt (TCBS) is a selective media, to a certain degree, for the genus *Vibrio*. A serial dilution of the cultured water was spread on a plate in duplicate. THB counts were determined during the large-scale *Artemia* culture on days 7 and 14.

***Statistical analyses***. The dataset was analyzed with a two-way analysis of variance (ANOVA) for the experiment's first subtrial and a one-way ANOVA for the second subtrial. A significance level of 0.05 was used, followed by a confirmation of normality and homogeneity of variance. The *Artemia* survival data used arcsine transformation to satisfy the normal distribution and homoscedasticity. Additionally, Tukey's post hoc procedures were performed to detect differences among the groups for multiple comparisons. All statistical tests were performed using SPSS statistics version 28.0 and Minitab statistic software 21.4.1.

## Results

### *Effect of stocking density and turbidity*

*Water quality.* The water quality, including temperature, pH, and DO, were stable during the experiment. No significant difference ($p > 0.05$) was observed in these parameters. In contrast, alkalinity, TAN, $NO_2$-N, TN, and TSS were significantly different ($p < 0.05$) among different treatments. Except for TP on the first week, no interaction was observed between stocking density and turbidity ($p > 0.05$) (Figures 1 and 2). Among eighteen water quality indicators, eleven combinations interact (Table 1).



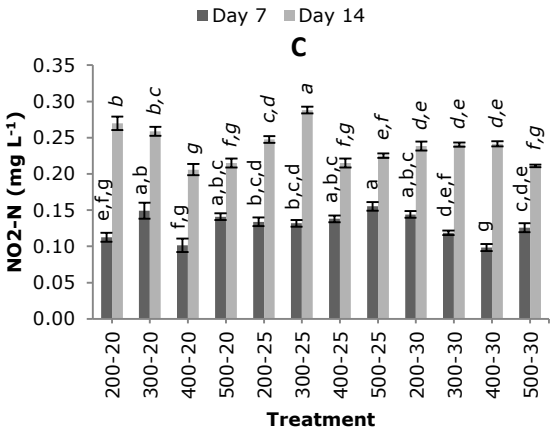



Figure 1. (A) Alkalinity, (B) TAN, (C) Nitrate-nitrogen ($NO_2$-N) measured in the first subtrial. Different superscripts indicate significant differences ($p < 0.05$) among treatments.

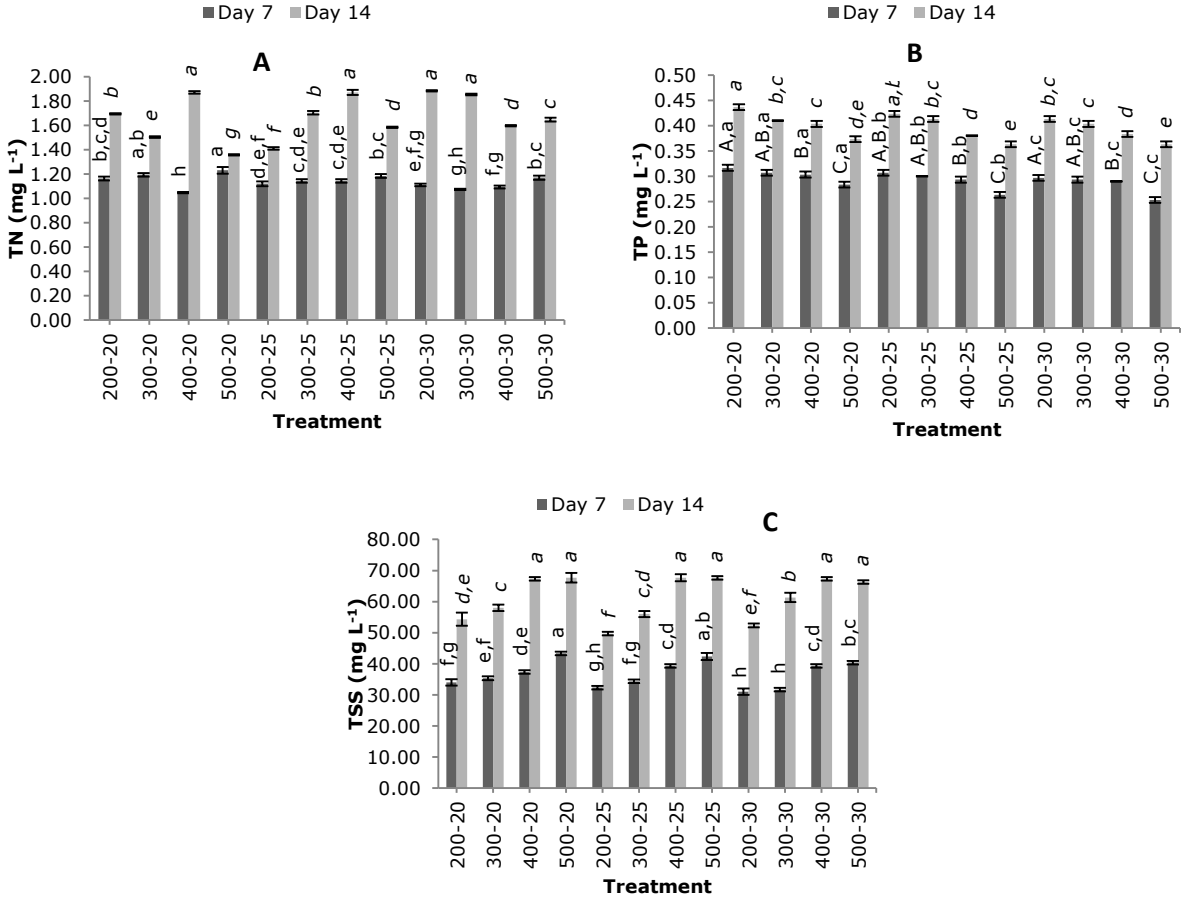



Figure 2. (A) TN, (B) TP, and (C) TSS measured during the first subtrial. Different superscripts indicate significant differences ($p < 0.05$) among treatments.

Table 1

Water quality parameters during the first sub-trial culture of *Artemia*

| *Parameters* | | *Treatments (NT)* | | | | | | | | *P value* | *Interaction* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *200-20* | *300-20* | *400-20* | *500-20* | *200-25* | *300-25* | *400-25* | *500-25* | | |
| Temperature (°C) | 7:00 | 26.5-28.2 | 26.8-28.1 | 26.5-28.2 | 26.5-27.9 | 26.6-28.3 | 26.5-28.0 | 26.8-27.9 | 26.9-28.0 | | |
| | | 27.6±0.5$^{a}$ | 27.6±0.5$^{a}$ | 27.5±0.5$^{a}$ | 27.5±0.5$^{a}$ | 27.6±0.6$^{a}$ | 27.6±0.5$^{a}$ | 27.6±0.4$^{a}$ | 27.6±0.5$^{a}$ | 0.995 | ns |
| | 14:00 | 29.3-31.5 | 29.5-31.6 | 29.4-31.8 | 29.7-31.7 | 29.4-32.1 | 29.2-32.0 | 29.5-31.4 | 29.4-31.5 | | |
| | | 30.6±0.7$^{a}$ | 30.6±0.7$^{a}$ | 30.7±0.7$^{a}$ | 30.8±0.7$^{a}$ | 30.8±0.8$^{a}$ | 30.8±0.8$^{a}$ | 30.6±0.6$^{a}$ | 30.6±0.7$^{a}$ | 0.707 | ns |
| pH | 7:00 | 6.9-8.2 | 7.0-8.2 | 6.9-8.2 | 6.8-8.2 | 7.0-8.2 | 6.9-8.2 | 6.9-8.2 | 7.0-8.2 | | |
| | | 7.6±0.3$^{a}$ | 7.6±0.3$^{a}$ | 7.6±0.3$^{a}$ | 7.5±0.4$^{a}$ | 7.5±0.3$^{a}$ | 7.6±0.3$^{a}$ | 7.6±0.3$^{a}$ | 7.5±0.3$^{a}$ | 0.991 | ns |
| | 14:00 | 7.6-8.1 | 7.5-8.1 | 7.5-8.1 | 7.3-8.2 | 7.6-8.2 | 7.3-8.1 | 7.2-8.1 | 7.40-8.00 | | |
| | | 7.8±0.1$^{a}$ | 7.2±0.2$^{a}$ | 7.8±0.2$^{a}$ | 7.7±0.2$^{a}$ | 7.8±0.2$^{a}$ | 7.3±0.2$^{a}$ | 7.7±0.2$^{a}$ | 7.7±0.2$^{a}$ | 0.818 | ns |
| DO (mg $L^{-1}$) | 7:00 | 5.9-6.8 | 6.0-7.0 | 6.0-6.9 | 5.7-6.6 | 5.5-6.8 | 5.9-8.0 | 5.9-8.0 | 5.7-8.2 | | |
| | | 6.4±0.3$^{a}$ | 6.4±0.3$^{a}$ | 6.34±0.24$^{a}$ | 6.19±0.25$^{a}$ | 6.16±0.32$^{a}$ | 6.50±0.52$^{a}$ | 6.46±0.51$^{a}$ | 6.40±0.63$^{a}$ | 0.267 | ns |
| | 14:00 | 6.2-7.6 | 6.2-7.5 | 6.1-7.4 | 5.6-7.4 | 6.1-7.9 | 6.2-7.5 | 5.8-7.2 | 5.5-7.4 | | |
| | | 6.9±0.4$^{a}$ | 6.9±0.3$^{a}$ | 6.9±0.4$^{a}$ | 6.8±0.5$^{a}$ | 7.1±0.4$^{a}$ | 7.0±0.4$^{a}$ | 6.8±0.5$^{a}$ | 6.6±0.6$^{a}$ | 0.512 | ns |
| Alkalinity (mg $CaCO_3$ $L^{-1}$) | Day 7 | 144.00± 2.00$^{d}$ | 147.33± 1.53$^{c,d}$ | 143.33± 1.53$^{d}$ | 153.33± 1.53$^{a,b}$ | 145.33± 1.53$^{d}$ | 146.33± 1.15$^{d}$ | 151.00± 1.00$^{b,c}$ | 145.33±0.58$^{d}$ | 0.000 | Antagonistic |
| | Day 14 | 120.67± 1.15$^{f}$ | 123.33± 1.53$^{e,f}$ | 122.67± 2.52$^{e,f}$ | 131.67± 1.53$^{b,c}$ | 129.33± 1.53$^{c,d}$ | 125.33± 1.73$^{d,e}$ | 137.67± 2.08$^{a}$ | 135.33±1.53$^{a,b}$ | 0.000 | Antagonistic |
| TAN (mg $L^{-1}$) | Day 7 | 0.61± 0.02$^{f}$ | 0.73±0.01$^{b,c}$ | 0.68±0.02$^{d,e}$ | 0.73± 0.03$^{b,c,d}$ | 0.66±0.02$^{e}$ | 0.67±0.02$^{e}$ | 0.83±0.01$^{a}$ | 0.74±0.02$^{b}$ | 0.000 | Antagonistic |
| | Day 14 | 1.68± 0.02$^{d,e}$ | 1.65±0.01$^{e}$ | 1.73±0.03$^{b,c}$ | 1.32±0.02$^{h}$ | 1.73±0.01$^{b,c}$ | 1.71±0.01$^{c,d}$ | 1.45±0.01$^{g}$ | 1.65±0.02$^{e}$ | 0.000 | Antagonistic |
| $NO_2$–N (mg $L^{-1}$) | Day 7 | 0.11± 0.01$^{e,f,g}$ | 0.15±0.01$^{a,b}$ | 0.10±0.01$^{f,g}$ | 0.14± 0.00$^{a,b,c}$ | 0.13± 0.01$^{b,c,d}$ | 0.13± 0.00$^{b,c,d}$ | 0.14± 0.00$^{a,b,c}$ | 0.16±0.01$^{a}$ | 0.000 | Antagonistic |
| | Day 14 | 0.27± 0.01$^{b}$ | 0.26±0.01$^{b,c}$ | 0.21±0.01$^{g}$ | 0.22±0.01$^{f,g}$ | 0.25±0.00$^{c,d}$ | 0.29±0.00$^{a}$ | 0.22±0.01$^{f,g}$ | 0.23±0.00$^{e,f}$ | 0.000 | Antagonistic |
| Total nitrogen (mg $L^{-1}$) | Day 7 | 1.16± 0.02$^{b,c,d}$ | 1.19±0.02$^{a,b}$ | 1.05±0.01$^{h}$ | 1.23±0.03$^{a}$ | 1.12± 0.02$^{d,e,f}$ | 1.14± 0.02$^{c,d,e}$ | 1.14± 0.02$^{c,d,e}$ | 1.18±0.02$^{b,c}$ | 0.000 | Antagonistic |
| | Day 14 | 1.69± 0.01$^{b}$ | 1.50±0.01$^{e}$ | 1.87±0.01$^{a}$ | 1.36±0.01$^{g}$ | 1.41±0.01$^{f}$ | 1.70±0.02$^{b}$ | 1.87±0.02$^{a}$ | 1.58±0.01$^{d}$ | 0.000 | Antagonistic |
| Total phosphorus (mg $L^{-1}$) | Day 7 | 0.32± 0.01$^{A,a}$ | 0.31± 0.01$^{A,B,a}$ | 0.30± 0.01$^{B,\ a}$ | 0.28±0.01$^{C,a}$ | 0.31± 0.01$^{A,B,a}$ | 0.30± 0.00$^{A,B,b}$ | 0.29± 0.01$^{B,b}$ | 0.26±0.01$^{C,b}$ | 0.122 | ns |
| | Day 14 | 0.44± 0.01$^{a}$ | 0.41±0.00$^{b,c}$ | 0.40±0.01$^{c}$ | 0.37±0.01$^{d,e}$ | 0.42±0.01$^{a,b}$ | 0.41±0.01$^{b,c}$ | 0.38±0.00$^{d}$ | 0.36±0.01$^{e}$ | 0.005 | Antagonistic |
| TSS (mg $L^{-1}$) | Day 7 | 34.00± 1.00$^{f,g}$ | 35.33± 0.58$^{e,f}$ | 37.33± 0.58$^{d,e}$ | 43.33±0.58$^{a}$ | 32.33± 0.58$^{g,h}$ | 34.33± 0.58$^{f,g}$ | 39.33± 0.58$^{a,b}$ | 42.33±1.15$^{c,d}$ | 0.000 | Antagonistic |
| | Day 14 | 54.33± 2.08$^{d,e}$ | 58.00±1.00$^{c}$ | 67.33±0.58$^{a}$ | 67.67±1.53$^{a}$ | 49.67±0.58$^{f}$ | 56.00± 1.00$^{c,d}$ | 67.67± 1.15$^{a}$ | 67.67±0.58$^{a}$ | 0.000 | Antagonistic |

| *Parameters* | | *Treatments (NT)* | | | | *P value* | *Interaction* |
|---|---|---|---|---|---|---|---|
| | | *200-30* | *300-30* | *400-30* | *500-30* | | |
| Temperature (°C) | 7:00 | 26.6-28.3 | 26.7-28.2 | 26.7-28.1 | 26.8-28.1 | | |
| | | 27.6±0.5$^{a}$ | 27.6±0.5$^{a}$ | 27.6±0.5$^{a}$ | 27.6±0.5$^{a}$ | 0.995 | ns |
| | 14:00 | 29.3-31.4 | 29.2-31.6 | 29.3-31.8 | 29.4-31.4 | | |
| | | 30.6±0.7$^{a}$ | 30.6±0.7$^{a}$ | 30.9±0.7$^{a}$ | 30.6±0.7$^{a}$ | 0.707 | ns |
| pH | 7:00 | 7.0-8.2 | 7.0-8.2 | 6.9-8.2 | 7.0-8.2 | | |
| | | 7.6±0.3$^{a}$ | 7.6±0.3$^{a}$ | 7.6±0.3$^{a}$ | 7.6±0.3$^{a}$ | 0.991 | ns |
| | 14:00 | 7.1-8.1 | 7.1-8.1 | 7.1-8.1 | 6.6-8.1 | | |
| | | 7.8±0.2$^{a}$ | 7.8±0.3$^{a}$ | 7.8±0.2$^{a}$ | 7.7±0.4$^{a}$ | 0.818 | ns |
| DO (mg $L^{-1}$) | 7:00 | 6.0-6.7 | 6.0-6.8 | 6.2-6.9 | 6.0-6.8 | | |
| | | 6.4±0.2$^{a}$ | 6.4±0.2$^{a}$ | 6.5±0. 2$^{a}$ | 6.4±0.2$^{a}$ | 0.267 | ns |
| | 14:00 | 6.3-7.5 | 6.1-7.4 | 6.2-7.5 | 5.7-7.5 | | |
| | | 7.0±0.3$^{a}$ | 7.0±0.3$^{g}$ | 7.0±0.4$^{c,d,e}$ | 6.6±0.5$^{a}$ | 0.512 | ns |
| Alkalinity (mg $CaCO_3$ $L^{-1}$) | Day 7 | 146.33±0.58$^{d}$ | 151.33±1.15$^{b,c}$ | 155.67±2.08$^{a}$ | 153.33±0.58$^{a,b}$ | 0.000 | Antagonistic |
| | Day 14 | 128.67±0.58$^{c,d}$ | 129.00±1.00$^{c,d}$ | 125.33±0.58$^{d,e}$ | 137.67±0.58$^{a}$ | 0.000 | Antagonistic |
| TAN (mg $L^{-1}$) | Day 7 | 0.59±0.01$^{f}$ | 0.47±0.02$^{a}$ | 0.69±0.01$^{f}$ | 0.82±0.01$^{a,b}$ | 0.000 | Antagonistic |
| | Day 14 | 1.60±0.02$^{f}$ | 1.78±0.02$^{b}$ | 1.60±0.02$^{a}$ | 1.76±0.02$^{a}$ | 0.000 | Antagonistic |
| $NO_2$-N (mg $L^{-1}$) | Day 7 | 0.14±0.00$^{a,b,c}$ | 0.12±0.00$^{d,e,f}$ | 0.10±0.00$^{g}$ | 0.13±0.01$^{c,d,e}$ | 0.000 | Antagonistic |
| | Day 14 | 0.24±0.00$^{d,e}$ | 0.24±0.00$^{d,e}$ | 0.24±0.00$^{d,e}$ | 0.21±0.00$^{f,g}$ | 0.000 | Antagonistic |
| Total nitrogen (mg $L^{-1}$) | Day 7 | 1.11±0.01$^{e,f,g}$ | 1.07±0.01$^{g,h}$ | 1.09±0.01$^{f,g}$ | 1.17±0.02$^{b,c}$ | 0.000 | Antagonistic |
| | Day 14 | 1.88±0.01$^{a}$ | 1.85±0.01$^{a}$ | 1.60±0.01$^{d}$ | 1.65±0.02$^{c}$ | 0.000 | Antagonistic |
| Total phosphorus (mg $L^{-1}$) | Day 7 | 0.30±0.01$^{a,c}$ | 0.29±0.01$^{a,b,c}$ | 0.29±0.00$^{b,c}$ | 0.25±0.01$^{c,c}$ | 0.122 | ns |
| | Day 14 | 0.41±0.01$^{b,c}$ | 0.40±0.01$^{c}$ | 0.38±0.01$^{d}$ | 0.36±0.01$^{e}$ | 0.005 | Antagonistic |
| TSS (mg $L^{-1}$) | Day 7 | 31.00±1.00$^{h}$ | 31.67±0.58$^{h}$ | 39.33±0.58$^{c,d}$ | 40.33±0.58$^{b,c}$ | 0.000 | Antagonistic |
| | Day 14 | 52.33±0.58$^{e,f}$ | 61.33±1.53$^{b}$ | 67.33±0.58$^{a}$ | 66.33±0.58$^{a}$ | 0.000 | Antagonistic |

The results are the mean value and standard deviation (mean±SD). Each treatment was performed in triplicate. Mean values in the same row with different superscripts indicate significant differences ($p < 0.05$) among treatments. The interaction was according to the output from two-way ANOVA; ns: not significant ($p > 0.05$).

Three phytoplankton phyla - Chlorophyta, Cyanophyta, and Bacillariophyta - were identified, with two species and ten genera in total (i.e., *Nannochloropsis* sp., *Chlorella* sp., *Oocystis* sp., *Closterium* sp., *Oscillatoria* sp., *Pseudanabaena* sp., *Chroococcus* sp., *Trichodesmium lacustre*, *Cylotella meneghiniana*, *Navicula* sp., *Nitzschia* sp., and *Thalassiosira* sp. (Figure 3 and Table 2).

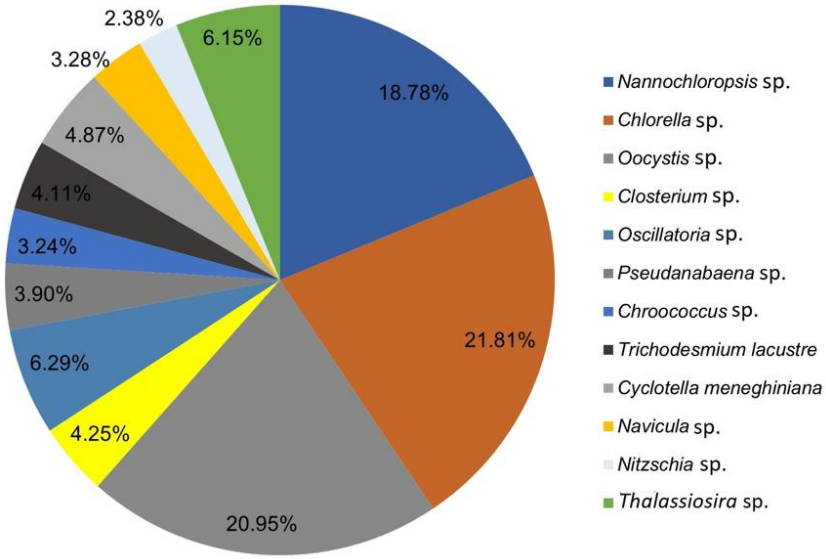



Figure 3. Composition in algal species during the first subtrial of *Artemia* culture.

Table 2

Alga appearance frequencies during the first subtrial of *Artemia* culture

| *Species* | *Frequency* |
|---|---|
| Chlorophyta | |
| *Nannochloropsis* sp. | + |
| *Chlorella* sp. | + |
| *Oocystis* sp. | + |
| *Closterium* sp. | + |
| Cyanophyta | |
| *Oscillatoria* sp. | + |
| *Pseudanabaena* sp. | + |
| *Chroococcus* sp. | + |
| *Trichodesmium lacustre* | + |
| Bacillariophyta | |
| *Cyclotella meneghiniana* | + |
| *Navicula* sp. | + |
| *Nitzschia* sp. | + |
| *Thalassiosira* sp. | + |

Note: +++: high density (> 60%); ++: medium density (30%–60%); and +: low density (< 30%).

*Artemia survival rate and growth.* The highest survival on the last culture day was with a density of 200 individuals $L^{-1}$ and a salinity of 25 g $L^{-1}$, with a survival rate of 68.9%. However, with a turbidity of 20 cm, the survival rate increased with the stocking density; with other turbidity levels (25 and 30 cm), the survival rate decreased when the stocking density increased. *Artemia* length in the first week ranged between 3.4 and 4.2 mm. In the second week, *Artemia* reached 5.1-6.5 mm. Survival and growth were significantly different ($p < 0.05$) in the first subtrial (Figure 4).

*Artemia biomass.* The highest biomass was collected from a treatment with a density of 300 individuals $L^{-1}$ and a salinity of 25 g $L^{-1}$ (1.3 kg $m^{-3}$). In contrast, the lowest biomass was when treated with 500 individuals $L^{-1}$ and a salinity of 30 g $L^{-1}$ (0.5 kg $m^{-3}$). Overall, a significant effect ($p < 0.05$) was observed among treatments (Figure 4).

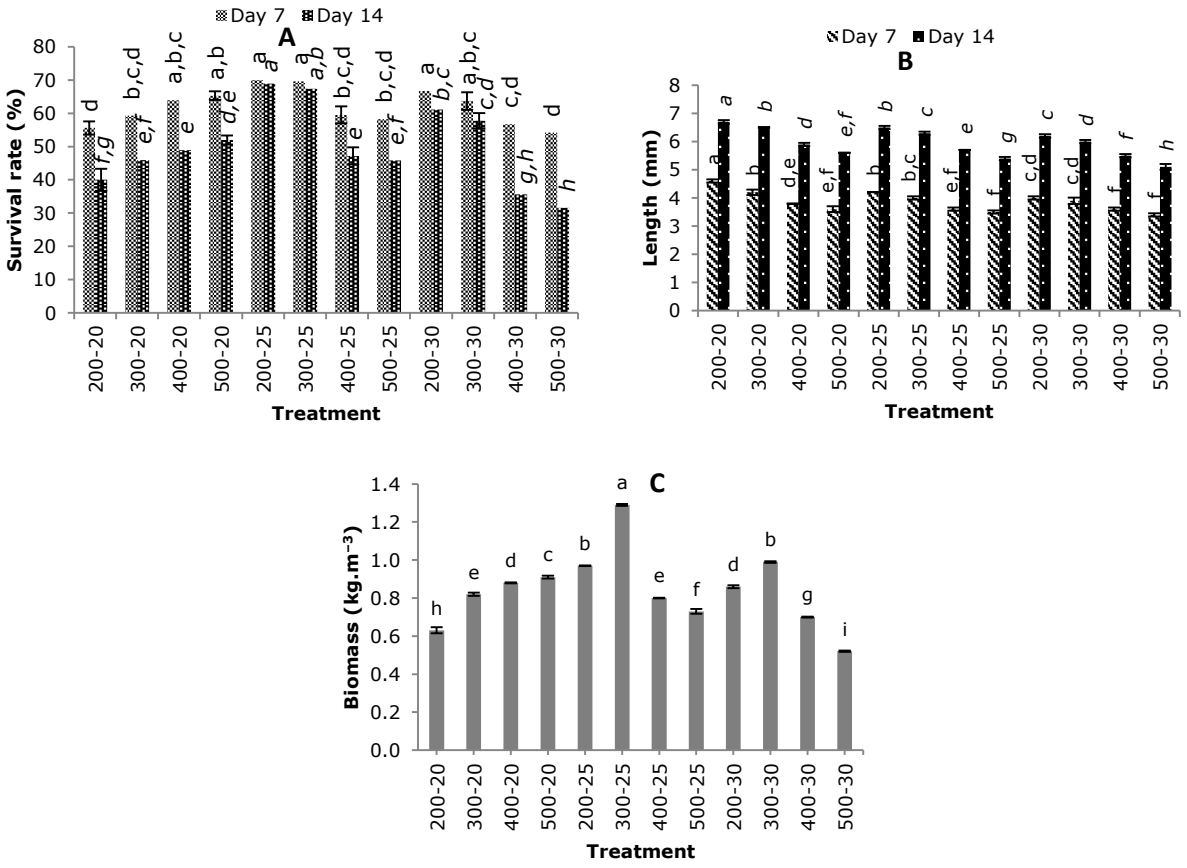



Figure 4. *Artemia* (A) survival rate, (B) growth (length), (C) biomass (kg $m^{-3}$) during the first subtrial. Different superscripts indicate significant differences ($p < 0.05$) among treatments.

### *Effect of different salinity levels*

*Water quality.* No significant difference ($p > 0.05$) was observed in daily water quality, including temperature, pH, and DO, in the morning and afternoon during the experiment. Meanwhile, TAN concentrations were lower under a salinity of 30 g $L^{-1}$. The same trend can be seen in alkalinity. The TN average during the first week was 1.01-1.12 mg $L^{-1}$ and tended to increase to 1.22-1.47 mg $L^{-1}$ in the second week. TP fluctuated between treatments with an average concentration of 0.22-0.39 mg. Regarding TSS, a significant difference ($p < 0.05$) was observed between treatments on day 7. On day 14, a significant difference was observed only under a salinity of 30 g $L^{-1}$ compared to other treatments (Figures 5 and 6).

The same algae species as in the first subtrial were found (Table 3 and Figure 7). The algal composition during the second subtrial was dominated with *Nannochloropsis* sp. from the phylum Chlorophyta in approximately 20% of all treatments.

*Artemia survival and growth.* A significant difference ($p < 0.05$) was observed in the second week, with the highest survival rate at a salinity of 30 g $L^{-1}$ (68.9%). The growth (mm) of *Artemia* increased during the culture period. On day 7, the total length under salinities of 20, 25, and 30 g $L^{-1}$ was similar but not significantly different ($p > 0.05$) from 15 g $L^{-1}$. A similar trend was observed on day 14; the lower the salinity, the shorter the length of *Artemia* (Figure 8).

*Artemia biomass.* The biomass among treatments was significantly different ($p < 0.05$). The highest biomass was under a salinity of 30 g $L^{-1}$ (1.5 kg $m^{-3}$) (Figure 8).

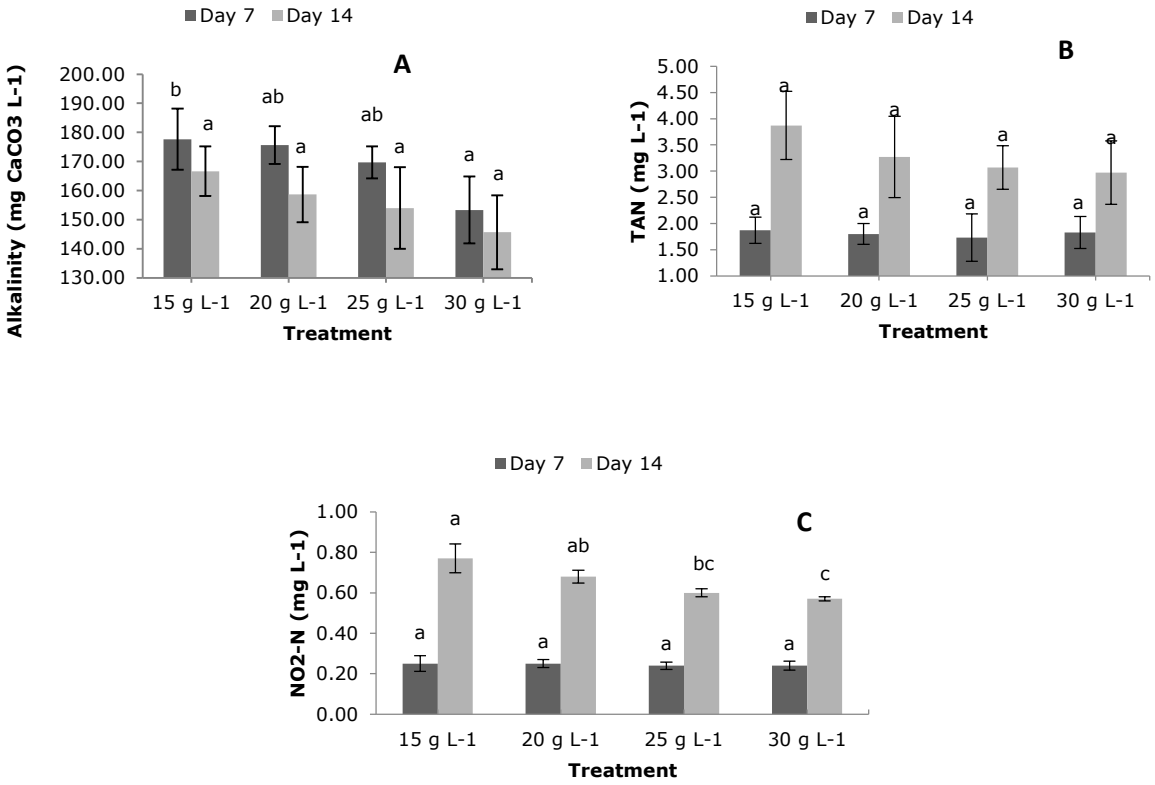



Figure 5. (A) Alkalinity, (B) TAN, (C) $NO_2$–N during the second subtrial. Different superscripts indicate significant differences ($p < 0.05$) among treatments.

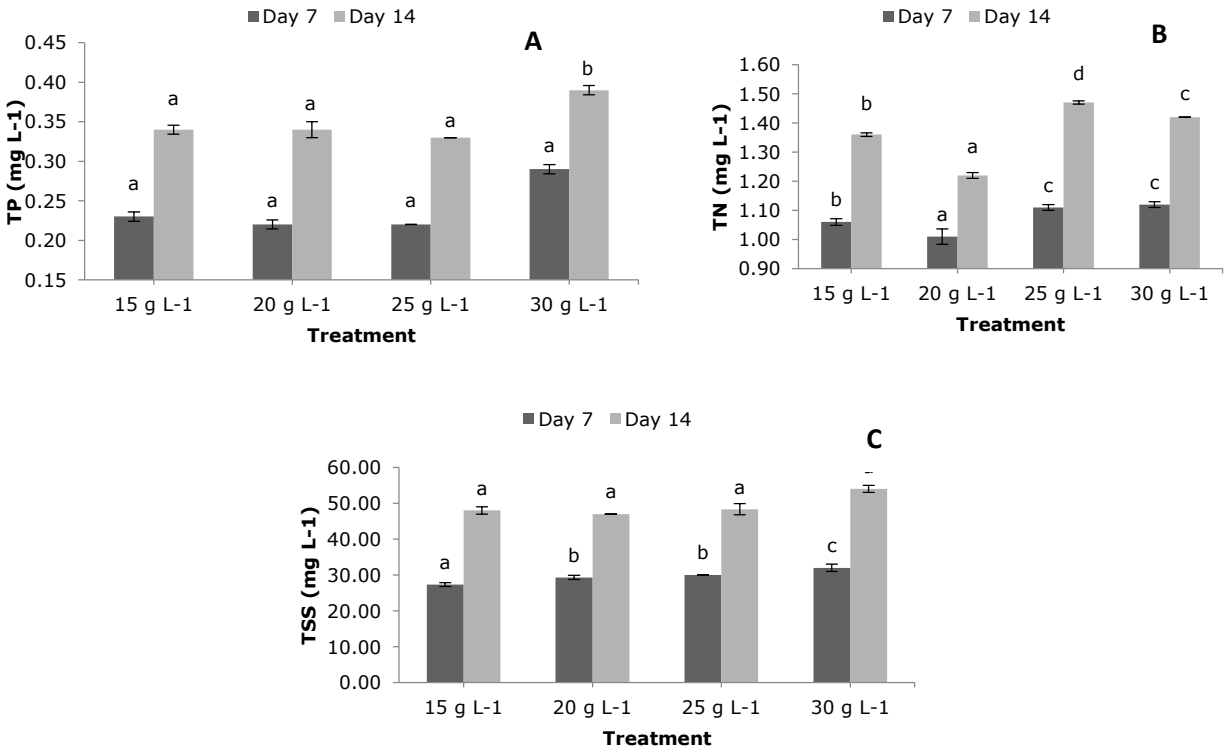



Figure 6. (A) TN, (B) TP, and (C) TSS during the second subtrial. Different superscripts indicate significant differences ($p < 0.05$) among treatments.

Table 2

Algal appearance frequencies during the second subtrial

| *Species* | *Treatment* | | | |
|---|---|---|---|---|
| | *15 g L$^{-1}$* | *20 g L$^{-1}$* | *25 g L$^{-1}$* | *30 g L$^{-1}$* |
| Chlorophyta | | | | |
| *Nannochloropsis* sp. | + | + | + | + |
| *Chlorella* sp. | + | + | + | + |
| *Oocystis* sp. | + | + | + | + |
| *Closterium* sp. | + | + | + | + |
| Cyanophyta | | | | |
| *Oscillatoria* sp. | + | + | + | + |
| *Pseudanabaena* sp. | + | + | + | + |
| *Chroococcus* sp. | + | + | + | + |
| *Trichodesmium lacustre* | + | + | + | + |
| Bacillariophyta | | | | |
| *Cyclotella meneghiniana* | + | + | + | + |
| *Navicula* sp. | + | + | + | + |
| *Nitzschia* sp. | + | + | + | + |
| *Thalassiosira* | + | + | + | + |

Note: +++: high density (> 60%); ++: medium density (30-60%); and +: low density (< 30%).

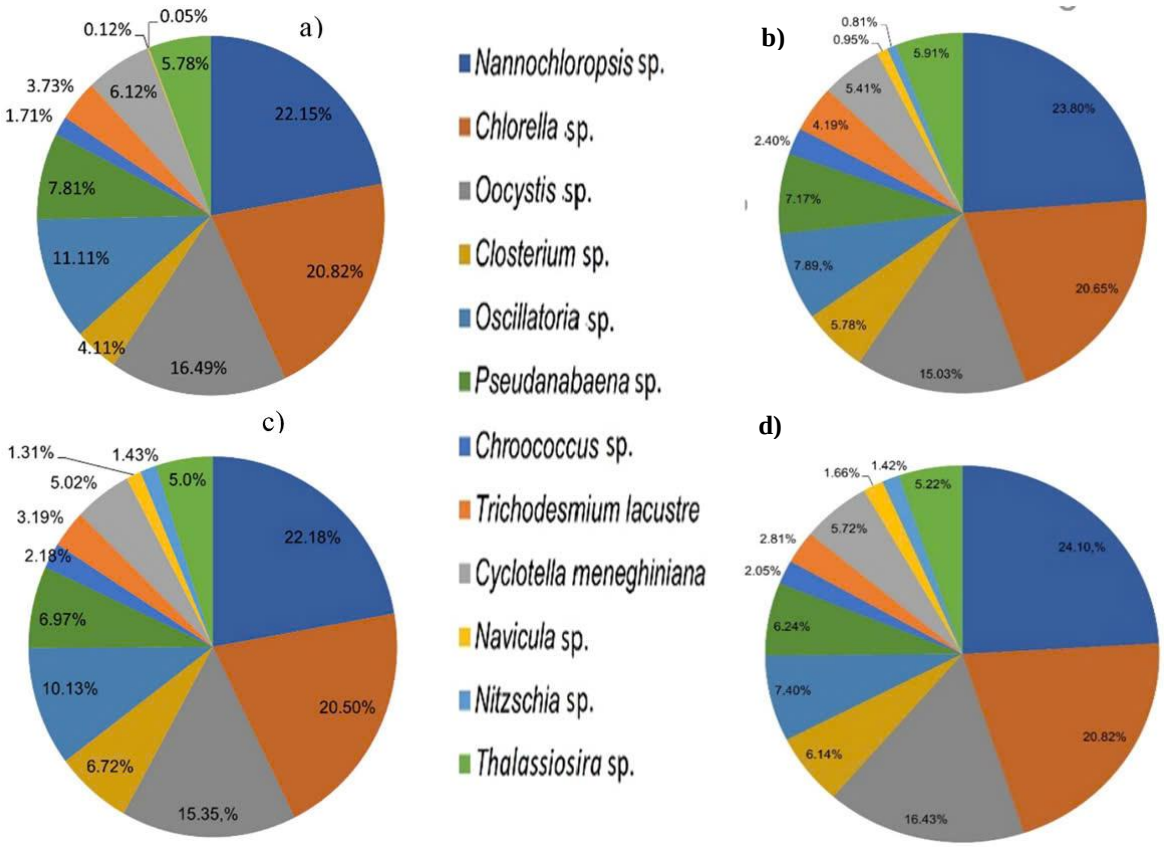



Figure 7. Algal composition in the second subtrial of *Artemia* culture: a) 15 g L$^{-1}$, b) 20 g L$^{-1}$, c) 25 g L$^{-1}$, and d) 30 g L$^{-1}$.

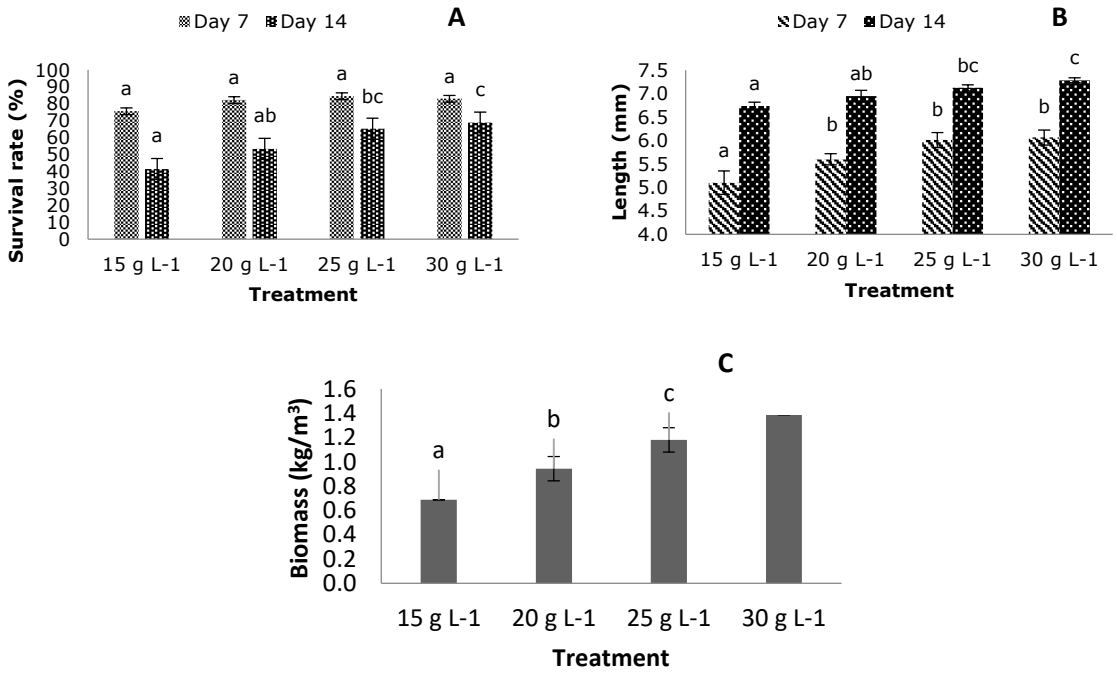



Figure 8. (A) Survival rate, (B) growth (length), and (C) biomass (kg $m^{-3}$) during the second subtrial. Different superscripts indicate significant differences ($p < 0.05$) among treatments.

## Discussion

***Physico-chemical parameters***. Temperature, pH, and DO were stable throughout the study culture period. In the first subtrial, most water quality parameters (alkalinity, TAN, $NO_2$-N, TN, and TSS) were significantly different, except for TP in the first week, which was only affected by turbidity. The interaction between stocking density and turbidity had an antagonistic effect on all water quality parameters.

In the second subtrial, no significant difference was observed in water parameters among the different treatments, except for TAN. Increased concentrations in water parameters (TAN, $NO_2$-N, TN, TP, and TSS) at the end of the second week were probably due to accumulation, *Artemia* mortality, metabolism, nitrogen, and the presence of heterotrophic bacteria (Tunvilai 1991; Ronald 2010; Wang et al 2019). Yet, the conditions remained optimum for *Artemia* culture.

Three phyla were identified: Chlorophyta, Cyanophyta, and Bacillariophyta. The dominant genera were *Chlorella* sp. and *Oocystis* sp. from the phylum Chlorophyta. According to Hoa & Hong (2019), *Artemia* can filter green algae but not digest them because of their thick cell wall (e.g., *Nannochloropsis*).

Furthermore, wild algae include various genera/species, but not all of them are suitable species for *Artemia* feeding. Some well-documented microalgae are suitable for *Artemia* growth: *Dunaliella*, *Tetraselmis*, *Nitzchia*, *Skeletonema*, and *Chaetoceros* (Fábregas et al 1996; Thinh et al 1999; Marques et al 2005; Herawati et al 2014).

Knowledge of the algal composition that can help induce *Artemia* survival and development is limited. According to Hoa et al (2020), various species composition and nutrient availability or deficiency do not always result in an optimal feeding regime. The *Artemia* population can decline due to poor nutrition availability. These observations illustrate the community-level transitions that can occur due to multiple trophic-level interactions in response to alterations in salinity, nutrient composition, and bioavailability (Marden et al 2020).

During the culture period, the phylum composition varied. Muylaert et al (2000) stated that salinity affects growth rate, which is essential for phytoplankton competition and can lead to changes in algal composition. Additionally, Larson & Belovsky (2013) discovered that salinity is a strong determinant of phytoplankton diversity. Thus, different

algal compositions between the first and second subtrials can occur because the salinity differs. Yet, no difference was observed in the composition and frequency of algae between the large-scale experiment and the second subtrial.

THB decreased during the large-scale experiment. A decrease in bacteria is possible since *Artemia* could filter bacteria efficiently (Toi et al 2013). Moreover, bacteria can be an alternative feed during the first development stages, although in practice most of the time phytoplankton is considered as a diet for *Artemia* (Lopes-dos-Santos et al 2019). Tkavc et al (2011) stated that the structure of bacterial communities in *Artemia* was highly diverse between developmental stages and strongly influenced by the environment. Many microorganisms can be considered as potential sources of nutrients for *Artemia*, depending on nutritional requirements and accessibility of the nutrient (i.e., thickness or smoothness of the cell wall) (Marques et al 2005).

Meanwhile, average colonies of *Vibrio* spp. increased to 4.1 x $10^2$ CFU $mL^{-1}$ in the second week of the experiment. Presumptive *Vibrio* were counted as yellow and green colonies (Moriarty 1998). According to Salvesen et al (2000), algal cells may carry bacteria, including *Vibrio* spp., which occurs in much higher numbers during the culture's stationary phase.

***Artemia living performance***. Survival rates, growth, and biomass in this study significantly differed among treatments. *Artemia* survival rates and growth in the first subtrial decreased from the first to the second week of the culture period. The survival rate ranged between 31.6 and 70.0%, while the average growth ranged between 3.4 and 4.6 mm on day 7 and between 5.1 and 6.7 mm on day 14. Gharibi et al (2021) observed a survival rate of 84-95.2% on day 8 and 72.2-84% on day 14, with different concentrations of trout effluent water with *Dunaliella* as food and a stocking density of 250 individuals $L^{-1}$. Rosowski (1989) studied the growth of *Artemia* with *Chlorella* as the sole food and observed a mean length of 6-8 mm on days 11-16, with a density of 0.26-2.18 mill. cells $mL^{-1}$. They also observed a mean size of 7.0 mm on day 9 at a density of 0.34 mill. cells $mL^{-1}$ although the survival rates were not mentioned. Another study observed a length of 5.81 mm for *Artemia* with wastewater from whiteleg shrimp without circulation and aeration supplies (Tunvilai 1991). Under a turbidity of 20 cm, the survival rate increased with the stocking density. At lower stocking densities, a high algal density and overfeeding might make *Artemia* thoracopod stuck and slow their digestion rate as the algae pass quickly through the gut, resulting in a decline in *Artemia* survival and growth (Hoa et al 2011).

As stated by Fernández (2001), the food concentration and animal density are necessary to define the range of sizes ingested, which will affect *Artemia* biomass production.

According to Hoa & Hong (2019), at high turbidity (> 25 cm), the stocking density should be reduced. Moreover, a turbidity of 20-25 cm is appropriate to culture *Artemia* in the tank. The biomass during the first subtrial ranged between 0.52 and 1.3 kg $m^{-3}$. *Artemia* density gradually decreased with the increased biomass (Islam et al 2019). A significant difference ($p < 0.05$) in survival was observed in the second subtrial. Generally, the threshold is determined by the tolerance of its predators in the area, and abundant *Artemia* populations are only found at high salinities due to the osmoregulatory capacity and synthesis of highly efficient hemoglobin (Dhont et al 2013).

Furthermore, at salinities of 10-50 g $L^{-1}$, *Artemia* lifespan and survival become lower than at a high salinity of 80 g $L^{-1}$ (Van & Toi 2017; Toi et al 2021). D'Agostino & Provasoli (1968) mentioned that *Artemia* nauplii survived a sudden salinity shift but not adult *Artemia*. A study by Soundarapandian & Saravanakumar (2009) proved that the survival and growth of *Artemia* increased with the salinity (28-33 g $L^{-1}$) to a length of 0.9 cm. *Artemia* was fed with *Chlorella* without any precision regarding the density.

From the second subtrial, the best result remained in the large-scale experiment: a turbidity of 25 cm, stocking density of 300 individuals $L^{-1}$, and a salinity of 30 g $L^{-1}$. The average survival rate in the first week was 81.4% and decreased to 68.2% in the second week.

The average length of *Artemia* of 6.0 mm during the first week of culture increased to 7.2 mm in the second week. According to Van & Toi (2017), low salinity only affects the survival of *Artemia,* not its growth. *Artemia* survival was lower than the best treatment of the second subtrial. This observation might be due to the high temperatures (highest 34.6°C) recorded during the large-scale experiment. As stated by Saygı & Demirkalp (2002), the survival of *Artemia* decreased as the temperature increased. According to Hoa et al (2011), *Artemia* fed with wild algae can only survive until day 22.

**Conclusions**. Utilizing effluent water from whiteleg shrimp intensive culture as food for *Artemia* biomass culture has big potential. The present study demonstrated that a stocking density of 300 individuals $L^{-1}$, turbidity of 25 cm, and a salinity of 30 g $L^{-1}$ gave the best biomass production of 1.5 kg $m^{-3}$ (wet weight). This study presents the potential for using shrimp effluent to culture wild microalgae and *Artemia* on the site. Furthermore, the experiment indicated that indigenous microalga species from the effluent could affect *Artemia* survival and growth during the culture. The study's limitations were the uncontrolled environment conditions; however, the conditions remained optimal for *Artemia* culture. The biomass produced should be further evaluated in terms of nutritional quality and virus/bacteria infection to confirm it could be fed directly to the shrimp in the same system. Additionally, the equipment conditions in the field laboratory to identify the algae must be further improved to meet the needs for algal classification.

**Conflict of interest**. The authors declare that there is no conflict of interest.

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