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Research Article

Integration of *Tetraselmis chuii* and *Artemia* sp. Culture in Industrial-Scale Salt Production

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Abstract

The common technique of traditional salt production in Indonesia is seawater evaporation technology, which uses sunlight to produce salt crystals from seawater. However, in general, the applied technology only produces salt with about 80% NaCl and water contents >7%, impurities >2%, and other contaminants. This produced salt is not suitable to be used as industrial salt. This study aimed to determine the effects of liquid organic fertilizer Gracilaria sp. addition into the culture media of Tetraselmis chuii on Artemia sp. growth, to identify the profile of length, weight, and survival rate of Artemia sp., and finally attempted to uncover the contribution of Artemia sp. in impurity mineral absorption and NaCl content improvement. The results of the study showed that the density of T. chuii based on the concentration of liquid organic fertilizer Gracilaria sp. was significantly different ($p<0.05$) in each treatment. T. chuii cultivation using liquid organic fertilizer Gracilaria sp. 16 mg N/mL resulted in the highest density on the culture days. However, the studies showed that there was no significant effect ($p>0.05$) on the weight and length gain of Artemia sp. The research conducted can support the downstream use of products from engineering research in the field of technology to increase the added value of salt products and be part of the contribution to the achievement of the 14th SDG on life below water.

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1. Introduction

Traditionally, salt farmers only use evaporation from sea water with the uncertain climate and there is no guarantee of the quality, quantity and the price of salt. Sustainability of salt production is determined by physical and biological factors, in which physical factors are affected by seawater evaporation which is influenced by weather, while biological factors are supported by the presence of various organisms. All of this will determine the quality of the salt that will be produced (Sriwati *et al*, 2022). Based on the contents of NaCl, salt can be classified into industrial salt and edible salt. Edible salt contains 87% NaCl, impurities (sulfate minerals and magnesium) as much as 2%, other contaminants (sands, mud) as much as 1%, and maximum water contents of 7%. On the other hand, industrial salt requires a content of as much as 97% NaCl, 2% impurities, and a very small number of contaminants and water. One of the innovations to produce industrial salt is the use of seawater evaporation technology with the help of sunlight. Other methods, such as evaporation using the heat of evaporator devices, crystallization, and electrochemical separation through electrolysis, are considered costly and unaffordable by traditional salt farmers in Indonesia. Therefore, Indonesia's salt production does not meet the needs of industrial salt with the criteria of water contents <0.5%, NaCl contents > 97%, insoluble components < 0.5%, Ca < 0.06%, Mg < 0.06%, and I > 30 mg/kg (National Standardization Agency of Indonesia, 2016). Data show that Indonesia imports about 2.36 million tons of industrial salt every year. Putri *et al.* (2021) mentioned several factors that affect the quality of salt production and become obstacles in salt production, namely seawater contamination by pollutants and fresh water, high precipitation in salt production areas, the quality of salt products that barely meets the criteria of industrial salt, and unstandardized salt products. Marihati *et al.* (2014) explained that one of the causes of low NaCl contents is the co-precipitation of mineral impurities that crystallize alongside NaCl. Co-precipitation can be prevented by using halophilic bacteria fed with *Luria bertani* during NaCl purification. Korovessis (2009) also stated that halophilic bacteria in salt fields can accelerate evaporation and reproduce by consuming organic matter, which causes turbidity of brine. The composition of the saline soils influences the occurrence of microbial communities.

Further studies have been performed to understand how microorganisms can withstand the stress associated with saline or sodic environments. Electrical conductivity and sodium adsorption ratio have a key role in microbial activities. The growth of non-halophilic microorganisms is affected by high salt concentration; in contrast, halophilic microorganisms, especially strict halophiles, require high salt concentrations for their growth. Even some halophilic microorganisms can accumulate KCl as an osmolyte in their cytoplasm, increasing their salt tolerance (Singh, 2016). It has been determined that the presence of microorganisms in arid soils is significantly correlated with total organic carbon and nitrogen, soil moisture, NO_3^- and total phosphorus. Low availability of those nutrients in arid soils limits the microbial diversity, this was reported for Cuatro Cienegas soils (Pajares *et al*., 2016). Thus, the presence of some ions has a positive impact on microorganisms, particularly halophilic ones. It has been reported that those microorganisms can use different ions, such as Na^+ or Cl^- which are important to their survival. For example, Na^+ can be used for halophilic archaea and bacteria as amino acid transport dependent (Na^+-symport). In the case of *Natronococcus ocultus*, e.g., the acetate and propionate are driven by the Na^+ gradient over the entire membrane (Oren, 2002). On the other hand, anions such as Cl^- participate in the volume increase during cell division and can act as cotransporters with sodium ions using the light-driven primary chloride pump, halorhodopsin (Müller and Oren, 2003). The use of some cations, such as sodium or potassium, has a key role in the antiporter cation/proton system for the cytoplasmic pH of the alkaliphilic bacteria (Grant and Jones, 2016). Other cations like Ca^{2+} act in the transport system, probably as Na^+/Ca^{2+} antiporters (Oren, 2002). In arid and semi-arid areas affected by salts, there is low availability of water, and the salts are concentrated, promoting a dehydration of the microorganisms. However, the halophilic microorganisms can exchange cations and osmolytes when the salt concentrations in the environment are elevated (Chowdhury *et al*., 2011). Meanwhile, *Artemia* sp. is one of the zooplankton that becomes the main nutrition in fish and shrimp larvae feed. One of the main zooplankton used as natural feed for fish and shrimp farming is *Artemia* sp., as it contains high amount of protein and amino acids.

Marihati *et al.*, (2014) also stated that *Artemia* culture in salt fields can also serve as the food source for halophilic bacteria of *Artemia sp.* detritus (carrion and excrements), accelerate the evaporation rate, reduce mineral contaminants (Mg^{2+} and SO_4^{2-}), and improve NaCl purity. The growth stimulation of the naturally occurring halophilic bacterial flora in the culture ponds, as a complementary food source for the Artemia nauplii, is currently bein considered as a viable way to improve Artemia biomass and cyst production. The addition of most tested halophilic bacteria, either as live or dead biomass, to the Artemia culture water, allowed for significantly superior nauplii survival than the corresponding negative control (starvation treatment) (Lopes-dos-Santos *et al*., 2019). Furthermore, significantly higher individual length in comparison to the positive control (a standard marine bacterial diet used in Artemia gnotobiotic tests) was also observed, especially when feeding

the nauplii with live halophilic bacteria biomass. The success at both salinities of the tested halophilic bacteria mono-diets when compared to both controls, clearly denoted that despite having a low nutritional value as far as fatty acids are concerned, they can be an integral part of its diet during its first developmental stages. Although our findings need to be confirmed in field conditions, they are of importance for Artemia pond production as they confirm the potential of these microorganisms of halophilic bacterial flora to be used as a viable dietary source, complementing the present focus on phytoplankton blooms to sustain Artemia density.

The protein content of *Artemia sp*. is about 63% of its dry weight (Widodo *et al*., 2016). Léger *et al.* (1987) stated that fish and shrimps are easy to catch and digest *Artemia sp*. because of its characteristics, namely the slow movement and thin exoskeleton. Compared to *Chaetoceros* sp. and *Skeletonema* sp., *T. chuii* has higher protein contents (Firmansyah *et al*., 2013). High protein contents make *T. chuii* a potential feed for brine shrimps, fish, and shrimp larvae (Da Costa *et al*., 2004). The growth of *T. chuii* microalgae highly depends on the availability of macronutrients and micro-minerals. In this sense, *Gracilaria* sp. liquid fertilizer plays an important role in providing the required nutrients. Several information concerning the nutrient composition of *Sargassum* sp. for liquid fertilizer had been reported (Basmal *et al*., 2019) and applied for mustard greens (*Brassica* sp.) (Dewi *et al*., 2019). Some studies have also reported from other seaweed (*Ulvalactuca*) (Suryaningrum and Samsudin, 2020) on mung bean seedlings (Castellanos-Barriga, 2017), as well as *Enteromorpha* sp. (Osman *et al*., 2004). Fertilizer from seaweed is non-toxic compounds and naturally degraded, so, therefore, environmentally friendly (Hernández-Herrera *et al*., 2016). Up to now, the application of alginate waste extracted alginate is very limited, particularly with some relevant application in *Gracilaria* sp. culture. Pigment synthesis is influenced by environmental factors, particularly nutrients such as nitrates and phosphates. Nutrients in the photosynthesis process play a role in the formation of chlorophyll so that the increase of nutrient content in the water will increase the production of chlorophyll a (Yudiati *et al*., 2020). Therefore, in controlled culture media, many nutrient enrichment technologies have been carried out to increase the growth and production of algae *T. chuii* pigments. Media enrichment can be formed as fertilizer. Furthermore, the ability of *T. chuii* to trigger plankton to support the growth and nutrient contents of *Artemia* sp. needs further exploration so that it can improve the production of *Artemia* sp. contributing as nutrients for halophilic bacteria growth that reduces salt impurities and improves NaCl contents.

2. Materials and Methods

2.1 Material

2.1.1 Ethical approval

Research conducted of Integration of *Tetraselmis chuii* and *Artemia* sp. culture in industrial-scale salt production does not require ethical clearance because it is not related to animal clinical trials or harm animal on this session.

2.2 Method

2.1 Materials and equipment preparation

First, the culture equipment was sterilized by washing using detergent and running water to remove dust and dirt that stick to it. The equipment was soaked in a chlorine solution for 24 hours and then washed and dried. Chlorine functioning as a disinfectant was also used to sterilize water in plankton culture media. Seawater and freshwater were sterilized using chlorine and given an aeration system for 24 hours. Then, sodium thiosulfate ($Na_2S_2O_3$) was added to neutralize the chlorine in the water. The dose of chlorine used was 60 ppm and sodium thiosulfate were 30 ppm (Supriyantini, 2013).

2.1.1 Walne fertilizer and vitamin production

A total of 100 g NaNO3, 20 g NaH2PO4, 45 g Na2EDTA, 33.6 g H3BO3, 0.36 g MnCl2, and 1.3 g FeCl3 were dissolved in an Erlenmeyer containing 1000 mL of distilled water that had been boiled on a hotplate. The speed of the magnetic stirrer could be adjusted up to 3000 rpm. Then, the medium was sterilized in the autoclave at 115°C for 30 minutes. Next, the medium was cooled and stored in the refrigerator. Vitamin B1 100 ppm and B12 5 ppm were added as a complement to Walne fertilizer nutrients used in treatment.

2.1.2 Gracilaria sp. *liquid organic fertilizer production*

Gracilaria liquid fertilizer was prepared by washing *Gracilaria* seaweed in fresh water to remove mud, sand, salt, shells, and dirt sticking to it. A total of 400 grams of seaweed was manually chopped, ground, and pulverized. A total of 20 ml of Bacillus sp. starter was added to accelerate the fermentation, followed by the addition of 100 grams of fish powder 1 L distilled water as the solvent and 20 ml of sugar solution (Karim *et al*., 2014) . According to Ratrinia *et al*., (2016) , the fermentation process takes place in a semi-anaerobic condition inside a closed container for approximately seven days.

2.1.3 Tetraselmis chuii culture medium preparation

A sterilized 600 ml plastic bottle used as the medium for *T. chuii* culture was filled with ± 500 ml of culture medium consisting of 70% sterilized seawater and 30% *T. chuii* from the culture stock. The cultivated

starter was continuously aerated so that the microalgae could be continuously fed. *T. chuii* was cultivated at a pH range of 7-8 and light intensity of 4500-8000 lux, inoculation was carried out at room temperature (25°C) under 16-watt 1600-lumen lighting. The measurement of *T. chuii* cellular density was carried out using the big block method due to the fact that *T. chuii* cells are single cells sized 7-12 µm (Taufiq *et al.*, 2012).

2.1.4 Artemia sp. *preparation*

Before cultivation, *Artemia* sp. cysts underwent decapsulation (Léger *et al.*, 1987). A total of 2 grams of *Artemia* cysts were soaked in the decapsulating solution for 15 minutes while the solution was continuously stirred until the color changed. The cysts were filtered and washed with fresh water then drained again and soaked in sodium thiosulfate solution until the chlorine smell disappeared. Nauplii that no longer contained cysts will settle to the bottom while the intact cysts would float to the surface (Choi *et al.*, 2006). Initial spreading of Artemia sp. Nauplii performed when entering the Instar 1 stage (Adhiyatma *et al.*, 2022). The number of *Artemia* sp. nauplii spread was 500 individuals/L with 10 L of seawater. The spreading of *Artemia* sp. nauplii was carried out based on the volumetric method.

The administration of treatment feed of *T. chuii* cultivated in culture media with *Gracilaria* organic fertilizer and Walne fertilizer to *Artemia* culture in each culture medium was carried out since the stocking day. The feed was administered twice a day, at 8 AM and 3 PM. Feed administration was carried out when *Artemia* sp. entered the instar II phase (eight hours after hatching). The instar II phase, called the metanauplii phase, was when *Artemia* started to feed, indicated by the development of digestive tracts. At this phase, *Artemia* started to be able to feed on particles that were smaller than 60 microns (Widiastuti *et al.*, 2012). The digestive tract grew longer at Artemia sp. tails (Norouzitallab *et al.*, 2015). The volume of *T. chuii* feed administration was 22500 cells/individual/day.

2.1.5 Measurement of Artemia sp. *nauplii spread*

A total of 5 mL of *Artemia* sample was collected using a pipette and put into a measuring tube. Then, the sample was moved into a Sedgwick rafter 1 mm^2 slowly 1 mL at a time. The measurement of the number of nauplii spread was carried out under a microscope using a hand counter. The result of measurements of the five repetitions was summed to obtain the total number of *Artemia* in a 5 mL culture medium. The number of *Artemia* in every liter of culture medium was measured based on the following formula:

$$N = \frac{1000}{5} x \sum n \quad \text{....Eq 1}$$

Note
Σn= the number of organisms in/5 mL sampling
N= the number of organisms/L

2.1.6 Measurement of Artemia sp. *length growth*

The measurement of *Artemia* sp. length growth is carried out once in three days of 21 days cultivation period. Dharmawan *et al.* (2020) explained that the measurement of *Artemia* sp. length growth is carried out through random sampling by taking five individuals from each culture medium. The length is measured under a camera microscope at 40 times magnification. The measured length is the distance from the anterior tip to the posterior tip of *Artemia* sp. Taunay *et al.* (2013) described the length growth of *Artemia* sp. can be measured based on the following formula:

$$L=(L_t-L_o) \quad \text{.....Eq 2}$$

Note
L = length growth (mm)
Lt = length at the end of cultivation (mm)
Lo = length at the beginning of cultivation (mm)

2.1.7 Measurement of Artemia sp. *weight growth*

As cited in Ernawati *et al.* (2020) the measurement of *Artemia* sp. weight is carried out by measuring the wet weight of *Artemia* sp. at the beginning and the end of cultivation. The average weight of an individual, *Artemia* sp., is obtained by dividing the total weight an individual gained by the number of individuals in a culture. describes the measurement in the following formula:

$$W_i = \frac{w}{\Sigma t} \quad \text{.....Eq 3}$$

Note:
Wi = weight of *Artemia* sp. individual (mg)
W= weight growth (mg)
Σt = number of individuals in a sample

2.1.8 Analysis of Gracilaria sp. organic liquid fertilizer mineral contents

The determination of total nitrogen contents was carried out using titration based on the Kjeldahl method. Sample destruction was performed by putting 0.5 g of dry sample into a Kjeldahl flask and was added with selenium and concentrated H_2SO4 mixture. The mixture was stirred slowly until the mixture became homogeneous and shimmered. The mixture then was heated until the mixture turned bluish or clear. The next phase was distillation, carried out by diluting the solution of the product of the destruction process (which had cooled down) in 50 ml of distilled water and was moved to a distillation flask. NaOH 40% and some boiling chip granules were added to basify the solution. The

solution underwent distillation, and the distillate was collected inside an Erlenmeyer flask containing 10 mL of H_3BO4 and a few drops of indicator (a mixture of Bromocresol and methyl red). Distillation ended when the volume of distillate was 50 mL. The titration process was carried out by titrating the obtained distillate with H_2SO4 0.005 N until the color of the solution changed from blue to pink. The volume of used H_2SO4 for the blank solution was determined by using distilled water.

The determination of total phosphorus contents also went through a destruction process. The destruction process was carried out by burning 2.5 g of dry sample in a furnace at 400°C for four hours. The cooled ash was diluted in 10 mL HNO_3 and added with distilled water until the volume reached 50 mL. Then, the solution was filtered using Whitman 42 filter papers. The filtrate was diluted to 100 mL volume inside a measuring flask. The determination of color stabilization time was carried out by pipetting 5 mL of solution (concentration 6 ppm) and added with 10 mL of ammonium molybdate-vanadate. The solution was, then, shaken to make it homogeneous and the absorbance was measured every 5-minute intervals for one hour (until the color stability time was obtained) at a wavelength of 395 nm. To determine the optimum wavelength, 5 mL of working solution (6 ppm concentration) was pipetted, and 10 mL of ammonium molybdate-vanadate was added. The solution was then homogenized, and its absorbance was measured after achieving color stability at a wavelength of 380-440 nm for every 5 nm interval. The calibration curve was determined by pipetting 5 mL of working solution (concentrations 2; 4; 6; 8 and 10 ppm) into an Erlenmeyer and 10 mL of ammonium molybdate-vanadate was added. After the solution was homogenized, a measurement of the solution's color stabilizing time and absorbance at the optimum wavelength of 395 nm was performed. A curve of absorbance calibration to concentration was made. The absorbance measurement of the solution was carried out by pipetting 5 mL of solution and adding 10 mL of ammonium molybdate-vanadate. The absorbance was measured using a spectrophotometer at the optimum wavelength after it reached color stabilization. The phosphorus contents were measured by comparing the sample of the absorbance with the standard absorbance in the standard calibration curve.

The determination of total potassium contents (Davies *et al.*, 2015) also started with destruction. The destruction was carried out by burning 2.004 g of dry sample in a furnace at 400°C for four hours. The cooled-down ashes were then diluted with 3 ml of concentrated HNO3 and added with distilled water until the volume of 10 mL. The solution was filtered using Whitman 42 filter paper and the filtrate was diluted in a measurement flask until the volume of 50 mL. The determination of potassium standard curve was carried out by measuring the emission of each standard solution with concentrations of 0 ppm, 2 ppm, and 5 ppm through K filter of a flame photometer. The measurement of the sample solution emission was carried out by pipetting 1 ml of the sample and diluting it in a measurement flask until the volume of 10 mL. The concentration of potassium was measured based on the regression equation of the standard calibration curve.

2.1.9 Analysis of Tetraselmis chuii and Artemia sp. *nutritional contents*

The analysis of protein contents was performed based on the Kjeldahl method. This method consisted of several phases based on (Paez *et al.*, 2016), namely weighing 1 g of ground sample, putting the sample into a Kjeldahl flask, weighing 7 g of potassium sulfate and 0.8 g of copper (II) sulfate, adding the 7 g of potassium sulfate and 0.8 grams copper (II) sulfate into the sample, followed by adding 12 mL H_2SO_4. This process was carried out inside a fume hood. The destruction process was carried out inside the fume hood by heating the sample on an electric stove until the sample turned greenish tosca. The next process was cooling down the Kjeldahl flask by letting it rest for 20 minutes followed by 25 mL distilled water , 50 ml NaOH, and some boiling chips into the flask/. The next phase was the addition of 30 mL H_3BO_3 and 3 drops of BCG-MR indicator to catch the distillate. The distillate was titrated using the HCl 0.1N standard solution until the solution turned pink. The same procedures were applied to determine the percentage for blank solution (change the sample with distilled water).

$$\%N = \frac{HCl\ volume\ (ml)(sample-blank)}{sample\ weight\ (grams)x1000}\ xN\ HCl\ x\ 14.008\ x\ 100\%.$$

.....Eq 4

% rough protein = %N x protein conversion factors

The analysis of fat contents was performed based on the Soxhlet method. The procedures were preparing a boiling flask, drying the flask in an oven at 105°C for an hour, cooling the flask in a desiccator for 15 minutes, and weighing the flask (W1). A total of ± 5 g of ground sample was wrapped in a thimble made of filter papers and weighed (W2). The sample was then put into a Soxhlet extractor and added with hexane solvent enough for 1.5 cycles. The extraction was carried out for ± 6 hours until the solvent dropped into the boiling flask and turned clear. The fats' separation from extraction and hexane was performed using a rotary evaporator (50 rpm, 69°C temperatures). Fats (separated from hexane) were heated in an oven at 105°C for an hour, cooled the flask in a desiccator for 15 minutes, and weighed the flask (W3). The reheating process continued if the difference between the weight of extracted

fats and the weight of the previous measurement was lower than 0.0002 g .

The percentage of fat contents was measured based on the following formula:

$$\%lipids = \frac{W3-W2}{W1} x\ 100\% \quad \text{.....Eq 5}$$

Note: W1 = weight of the sample (grams), W2 =weight of the flask (empty)(grams), W3(weight of the flask and extraction products (grams)

The analysis of carbohydrate contents was conducted by measuring the difference in moisture, protein contents, ash contents, and fat contents. The formula used for measuring carbohydrate contents was carbohydrate contents (%) = 100%- (moisture (%) + ash contents (%) + protein contents (%) + fat contents (%). The objective of ash content analysis was to identify the unevaporated components (inorganic components and mineral salts) remaining after the combustion of organic substances.

The analysis of rough fibers was performed through the gravimetric method. Namely by putting 2 g of samples into an Erlenmeyer flask and was added with 200 mL of boiling H2SO4 (0.225N). The suspense was filtered, and the residue was washed with boiling distilled water until the residue on the filter paper became not acidic. The residue was then put into an Erlenmeyer, washed with 200 mL of NaOH (0.313N), and boiled for 30 minutes. The residue was then filtered using a filter paper with constant weights and washed with K_2SO_4 10%. The residue was washed again with boiling distilled water and 15 mL of alcohol 95%. A filter paper was dried in an oven at 110°C, combusted in a furnace at 500°C, and cooled down in a desiccator. The ashes were weighed, and the weighing process was repeated until the weight became constant. The fiber content was measured based on the formula: % fibers = fiber weight/ sample weight x 100% (Yuswi, 2017).

2.1.20 Salt crystallization

The salt crystallization process was conducted through evaporation using a hotplate. The procedures of salt crystallization were a) preparing beaker glass, hotplate, thermometer, clamps, and stand, b) putting 500 mL of seawater sample into the beaker glass, c) evaporating the sample on the hotplate at 100-500°C to produce solid salt crystals, d) moving the salt crystals into a dish with identified weights; e) cooling the salt crystal down in a desiccator, f) weighing the salt crystals in a porcelain dish, and g) measuring the moisture of salt crystal products.

2.1.21 Chemical element analysis

An analysis of mineral contents was carried out on the seawater sample (before and after treatments), *Artemia sp*. (before and after culture), and salt produced through the crystallization process (final product). Mineral contents examined in this study were sodium (Na), chloride (Cl), magnesium (Mg), and sulfate (SO_4) while for salt products there was also an additional examination for NaCl and lead (Pb) contents. The quality and mineral contents of salt in this study referred to the standards for industrial salt of (Herawati and Romadhon, 2020). The analysis of mineral contents for *Artemia* sp. was conducted using SEM-EDX JEOL JSM 6510LA, a scanning electron microscope that could produce an image of a sample by scanning the surface of a sample with electron lights focused on a certain degree of magnification and Energy Dispersive X-ray (EDX) to analyze the chemical contents of a material.

2.1.22 Water quality examination

The examination of water quality was carried out every day. The examined parameters were temperatures, pH, dissolved oxygen (DO) levels, and salinity. The water temperature was measured using a thermometer, pH using a pH meter, oxygen using DO-meter, and salinity using a refractometer.

2.3 Analysis Data

The analysis used in the first phase of this study (*T. chuii* culture) was a randomized complete block design (RCBD) with a single factorial to identify the significant difference among the treatments. The data were analyzed using the Analysis of Variance (ANOVA) continued by Duncan's Multiple Range Test to see if there were significant differences among the treatment (Karim *et al.*, 2014). Meanwhile, the analysis for the latter phases of this study (the cultivation of *Artemia* sp. was carried out using the Kruskal-Wallis' test. The Kruskal-Wallis' test is a non-parametric statistical technique used to identify significant differences between the independent variables and dependent variables (Jamco and Balami, 2022). The provisions that support the Kruskal-Wallis' test are the data are not distributed normally, used to compare two or more population groups with data in the form of ranking, ordinal-scale samples, and each treatment group has the same number of samples. The Kruskal-Wallis' test is a non-parametric examination used to compare two or more sample groups. The result of the examination shows a significant difference if it results in a critical value of $p < 0.05$.

3. Results and Discussion

The result of the first phase of this study is the density of *T. chuii*. (Table 1). This finding is useful to find out the effects of *Gracilaria* sp. liquid organic fertilizer administration as a source of nitrogen that improves the growth of *T. chuii*.

The result of the Duncan test shows that the administration of *Gracilaria* sp. liquid organic fertilizer in different doses provides a significantly different *T. chuii* density in each treatment. On the fifth day, the cultivation of *T. chuii* with 16 mg N/mL *Gracilaria* sp. fertilizer administration (P2) shows the highest population of 5183.87x 10^4 cells/mL while the administration of 16 mg N/mL Walne fertilizer (P0) with 4711.94 x 104 cells/mL; and the administration of 8 mg N/mL *Gracilaria* sp. fertilizer (P1) provides the lowest density of 1550.50 x 10^4 cells/mL. On the seventh day of cultivation, the density of *T. chuii* in each treatment group decreases.

The nutritional analysis of *T. chuii* has been checked by ANOVA statistical analysis and the result showed that there are no significant differences among the treatment groups (Table 2). Manuputty *et al.* (2018) explains the length of the fermentation process increases microbial activities to absorb water and oxygen from the air used to transform carbohydrates and fats to water and CO_2. Thus, the moisture in a fermentation process and its yields increases. According to Nasrun *et al.* (2017), the more probiotic bacteria that are used, the more yield can be gained due to cellular divisions made by the microorganisms. The decrease of *T. chuii* density signifies the decrease of nutrients produced in the fermentation process. Therefore, the nutrients need to be added to maintain the population of *T. chuii*.

The analysis of proteins, carbohydrates, fats, water, ashes, and energy composition in *T. chuii* shows no significant differences between the groups receiving Walne fertilizers and *Gracilaria* sp. liquid organic fertilizers by ANOVA statistical analysis. The production of *Gracilaria* sp. liquid organic fertilizers utilizes waste from *Gracilaria* (seaweed) cultivation ponds in the form of short thallus stems and dull-colored seaweed. The seaweed harvesting waste can be maximally reprocessed to organic fertilizers, which have economic value and can be used as a substitute for Walne fertilizers in microalgae growth. However, seaweed liquid fertilizers require bacteria to bind nitrogen, phosphorus, potassium, and other elements. One of these bacteria is probiotics. Probiotic microorganisms can actively improve nutrients from available organic substances. Organic materials serve as a source of food and energy for microorganisms. Fermentation also enhances microorganisms to grow and develop so that microorganisms need nitrogento survive. This phase is called the log phase. Marihati *et al.* (2014) defined the log phase as a phase when microorganisms start to grow and develop in a logarithmic manner. In this phase, microorganisms undergo the fastest development. When microorganisms have adapted to the new conditions, their cells grow faster and consume nitrogen supplies resulting in decreasing nitrogen levels in the environment. Ratrinia *et al.* (2016) stated that the best condition for fertilizer fermentation is in a semi-anaerobic condition within a closed container and is fermented for about seven days. In this phase, the nitrogen content in the fertilizer is quite high and is related to the number and activity of microbes.

The increasing percentage of nitrogen during the fermentation period is the result of decomposing fermented materials by the microorganisms that change ammonia to nitrite. Nitrogen serves as the source of energy for microorganisms that play an important role in the processes of decomposing organic materials and photosynthesis (Ratna *et al*., 2017). The higher the nitrogen level, the faster organic materials decompose because microorganisms decompose the fermented materials that require nitrogen for their development (Gunawan and Kusmiadi, 2015). This study found that the administration of *Gracilaria* sp. liquid organic fertilizers in different doses resulted in significantly different *T. chuii* density in each treatment because the availability of nutrients in the water affected the growth and development of the microalgae. Nutrients are substances that are used to support the development and survivability of an organism (Erniati *et al*., 2016). The availability of nutrients in water can create an ideal environment that optimally sustains the growth of *T. chuii*.

The highest density of *T. chuii* resulted from a treatment receiving the dose of 16 N mg/L *Gracilaria* sp. liquid organic fertilizer with > 5 x 10^6 cells/mL. This finding indicates that the administration of 16 N mg/L *Gracilaria* liquid organic fertilizer into the culture medium provided a source of nitrogen and phosphorus for the metabolism of *T. chuii*. The significant growth of microalgae after the administration of 16 N mg/mL has convinced us that *Gracilaria* sp. liquid organic fertilizer can be used as an alternative to substitute Walne fertilizers.

The result of the statistical analysis of the types of organic feed administered to *Artemia* sp. through the Kruskal-Wallis' test shows there is no significant difference ($p>0.05$). The result of the analysis concerning thence ($p>0.05$). The result of the analysis concerning the effect of different types and doses of fertilizer administered to *T. chuii* on the nutritional contents of *Artemia* sp. also shows a similar trend. The data of *Artemia* sp. nutritional contents (Table 4) indicate there is no significant difference among the treatments in terms of protein, carbohydrate, fat, water, ash, and energy compositions in *Artemia* sp. The length and weight growth of *Artemia* sp. fed with *T. chuii* in Walne fertilizer are not significantly different from the growth of *Artemia* sp. receiving *T. chuii* with *Gracilaria* sp. organic fertilizer. This is because the administrations of Walne fertilizer and *Gracilaria* sp. organic fertilizer to *T. chuii* have sufficed the optimum length and weight growth of *Artemia* sp. (Table 3).

Table 1. Data of *Tetrachelmis chuii* density after eight days of culture

Day	Population Density of *Tetraselmis chuii* (x 10^4 cell/mL)				
	P_0	P_1	P_2	P_3	P_4
1	$49.44^b \pm 3.57$	$34.75^a \pm 2.08$	$58.87^c \pm 5.13$	$35.45^a \pm 4.38$	$33.72^a \pm 2.60$
2	$199.44^c \pm 10.24$	$134.75^a \pm 4.63$	$171.37^b \pm 9.02$	$172.95^b \pm 4.69$	$170.23^b \pm 3.93$
3	$1661.94^d \pm 42.23$	$455.25^a \pm 30.72$	$2271.37^e \pm 62.26$	$1235.45^c \pm 26.82$	$817.13^b \pm 52.26$
4	$2936.94^d \pm 56.02$	$992.75^a \pm 88.99$	$3233.87^e \pm 79.91$	$2435.70^c \pm 238.14$	$2017.12^b \pm 115.62$
5	$4711.94^d \pm 251.30$	$1550.50^a \pm 135.68$	$5183.87^e \pm 178.96$	$3860.45^c \pm 430.94$	$3442.13^b \pm 191.13$
6	$4599.44^d \pm 238.92$	$1988.00^a \pm 147.83$	$4533.87^d \pm 376.93$	$3385.45^c \pm 95.95$	$2967.13^b \pm 173.27$
7	$3255.44^d \pm 280.88$	$1072.75^a \pm 90.60$	$3296.37^d \pm 451.21$	$2435.45^c \pm 233.78$	$2017.13^b \pm 81.07$
8	$2299.44^d \pm 150.74$	$635.25^a \pm 26.96$	$2083.87^d \pm 202.81$	$1472.95^c \pm 250.65$	$1070.38^b \pm 80.26$

Note:
P0: 16 mg N/mL Walne fertilizer administration (control group)
P1: 8 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration
P2: 16 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration
P3: 24 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration
P4: 32 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration
Different superscripts indicate significant differences.

Table 2. Data of *Tetraselmis chuii* nutritional composition

Treatment	Protein (%)	Carbohydrate (%)	Fat (%)	Water (%)	Ash (%)	Energy (Kcal/kg)
P_0	26.2	25.4	3.8	19.8	24.9	2868.9
P_1	26.1	25.2	3.8	19.4	24.9	2832.7
P_2	26.7	25.4	3.9	19.6	25.1	2887.3
P_3	26.2	25.4	3.8	19.7	25.1	2863.9
P_4	26.7	25.3	3.8	19.7	24.9	2844.6

Note:
P0: 16 mg N/mL Walne fertilizer administration (control group)
P1: 8 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration
P2: 16 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration
P3: 24 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration
P4: 32 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration

The nutrient contents in the culture medium play an important role in phytoplankton growth and reproduction. Nutrients in water are classified into macronutrients and micronutrients. Macronutrients consist of carbon (C), hydrogen (H), oxygen (O), nitrogen (N), sulfur (S), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), and chloride (Cl). The micronutrients are irons (Fe), cobalt (Co), zinc (Zn), boron (B), silicate (Si), manganese (Mn), and copper (Cu). The most essential elements for aquatic organisms are carbon, nitrogen, and phosphorus (Erniati *et al*., 2016). Chrismadha *et al.* (2006) strengthens this by stating that Nitrogen and Phosphorus play a vital role in composing protein compounds in cells. Deficiencies of these two elements may reduce the protein level in algae cells, which is generally followed by degrading the components of cells that are related to protein synthesis. Low nitrogen and phosphorus concentrations may inhibit the synthesis of protein and carbohydrates. The dose of *Gracilaria* sp. organic fertilizer and Walne fertilizer administered is 16 mg N/mL while the amount of nitrogen needed by microalgae is 0.14-0.7 mg. Therefore, the amount of nitrogen obtained from administered fertilizers has sufficed the nitrogen needs of *T. chuii*.

Nitrogen, phosphate, and carbon are essential elements that affect the availability of nutrients in the aquatic ecosystem (Warman, 2015). These elements play an important role in phytoplankton composition and biomasses that determine primary marine productivity. Nitrogen compounds originate from the metabolism of water organisms and the decomposition of organic materials by bacteria (Stone and Thomforde, 2004). Nitrogen composes proteins absorbed by organisms in the form of ammonia and nitrate. The availability of nitrogen affects variations in species and nutritional contents and abundance in an organism (Davies *et al*., 2015).

Phosphorus is very important for the life of aquatic organisms because it functions in the storage and transfer of energy in cells and functions in the genetic system (Korovessis, 2009). Phosphorus functions as a bone builder, organic compounds, energy metabolism, carbohydrates, amino acids and fats, fatty acid transportation, and coenzyme parts. Phosphorus as phosphate plays an important role in the structure and function of living cells (Widodo *et al*., 2016). Phosphorus content in fertilizer doses of *Gracilaria* sp. in the P2 treatment was 1.4 mg P/mL and the phosphorus content at Walne fertilizer dose was 0.004 mg P/mL, while the need for phosphorus for microalgae in culture was 0.0154-0.6194 mg P/mL (Eyster *et al*., 2018). The phosphorus content of liquid fertilizer *Gracilaria* sp. can meet the needs of phosphorus *T. chuii* and is higher than Walne fertilizer.

Another mineral that also plays a role in supporting the growth process of microalgae is potassium, especially in the photosynthetic reactions through the translocation of sugars and activating the work of enzymes for the chemical sequence of photosynthesis and respiration. The availability of potassium is also useful in regulating the balance of nitrogen and phosphorus elements (Herawati and Romadhon, 2020) he advantages of natural feed when compared to artificial feed as feed, namely having a more complete and high nutritional content, dense cell contents, and thin cell walls so that they are easily absorbed and digested (Chahyaningrum *et al*., 2015). . Important nutritional values in the form of protein, fat, and carbohydrate content affect the growth rate of *Artemia* sp. because each nutrient in the feed given has a different function and complements each other for growth.

Table 3. The results of *Artemia* sp. weight, length, and survivability rate measurements.

Treatment	Length (mm) ± SD	Weight (mg) ± SD	SR (%) ± SD
P_0	13.69 ± 0.6	1.81 ± 0.001	69.53 ± 1.31
P_1	13.54 ± 0.7	1.80 ± 0.002	68.91 ± 1.17
P_2	13.90 ± 0.6	1.83 ± 0.001	70.62 ± 1.05
P_3	13.63 ± 0.4	1.82 ± 0.000	69.02 ± 1.13
P_4	13.47 ± 0.4	1.79 ± 0.001	68.97 ± 1.09

Note:

P0: 16 mg N/mL Walne fertilizer administration (control group)

P1: 8 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration

P2: 16 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration

P3: 24 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration

P4: 32 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration

Different superscripts indicate significant differences.

Nasrun *et al*. (2017) explained that the functions of protein include maintaining damaged body tissues, growth, forming enzyme systems in the digestive system, and as a source of energy in egg formation and reproduction. The function of fat is as a source of energy, helping the absorption of minerals and fat-soluble vitamins (vitamins A, D, E, and K). Fauzi and Sari (2018) stated that carbohydrates are a source of energy for growth and metabolism.

Growth is an important factor in successful cultivation (Taunay *et al*., 2013). It is defined as the increasing size and number of cells and intracellular tissue in a

period so that it can be expressed in units of length and weight. Feeding is one of the important factors that affect *Artemia* sp. culture growth. In taking nutrients from its environment, *Artemia* sp. is a non-selective filterer. Everything that enters its mouth can become its food. The nutrient contents of *Artemia* sp. are highly affected by the quality of foods available in water or culture media (Supriyantini, 2013). The availability of nutrients influences the growth rate, so the amount and quality of foods are the main factors that supply the nutrients needed by *Artemia* sp. to grow optimally. The examination of mineral contents (Na, Cl, Mg, and SO4) was carried out before and after the cultivation of *Artemia* sp. in each treatment (Table 5).

The results of the Kruskall-Wallis' test with a 95% confidence interval on the parameters of the mineral content of Na^+ and Cl^- showed P> 0.05. This means H_0 was accepted and there was no significant difference between the A0, A1, and A2 treatments on the mineral content of Na^+ and Cl^- in seawater samples resulting from the administration of *Artemia sp*. in different densities. However, there was a significant difference in the parameters of Mg^{2+} and SO_4^{2-} ($p< 0.05$ and H0 is rejected), indicating that A0, A1, and A2 treatments had significant effects on Mg^{2+} and SO_4^{2-} contents depending on different densities.

The results of the mineral content test on *Artemia* sp. show the largest percentage change in $Mg^{2+} > SO_4^{2-} > Cl^- > Na^+$. The densities of *Artemia* sp. 1000 individuals/L and 2000 individuals/L showed a positive trend of increasing levels of Mg^{2+} and SO_4^{2-}, which coincided with decreasing levels of Mg^{2+} and SO_4^{2-} in seawater media (Table 5 and Table 6).

Table 4. Data of *Artemia* sp. compositions

Treatment	Protein (%)	Carbohydrate (%)	Fat (%)	Water (%)	Ash (%)	Energy (Kcal/kg)
P_0	24.8	22.9	4.5	18.8	27.9	2836.5
P_1	24.9	22.7	4.2	18.9	27.9	2793.4
P_2	25.9	23.6	4.9	18.4	27.5	2863.9
P_3	25.4	23.4	4.5	18.4	27.5	2811.4
P_4	25.1	22.9	4.5	18.4	27.6	2825.7

Note:
P0: 16 mg N/mL Walne fertilizer administration (control group)
P1: 8 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration
P2: 16 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration
P3: 24 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration
P4: 32 mg N/mL *Gracilaria* sp. liquid organic fertilizer administration
Different superscripts indicate significant differences.

Table 5. The analysis of seawater mineral contents

Treatment	Mineral Content (%) ± SD			
	Na^+	Cl^-	Mg^{2+}	SO_4^{2-}
A_0	0.62 ± 0.10	0.23 ± 0.06	0.21^b ± 0.07	0.98^b ± 0.13
A_1	0.64 ± 0.12	0.26 ± 0.05	0.06^a ± 0.01	0.27^a ± 0.14
A_2	0.67 ± 0.19	0.28 ± 0.01	0.06^a ± 0.01	0.27^a ± 0.19

Note: A0= without administrations of *Artemia* sp.; A1 = 1000 individuals/L *Artemia* sp. administered; A2 = 2000 individuals/L *Artemia* sp. administered. Different superscripts indicate significant differences.

The results of SEM-EDX show that on the 21st day of treatment the composition of carbon dominates other minerals (>50%) (Table 8) This indicates a significant growth improvement and the development of *Artemia* sp. exoskeleton tissues. Choi *et al.* (2006) explained that carbon plays an important role in morphogenesis changes and molting of *Artemia* sp. exoskeletons. Carbon is one of the minerals that compose carbohydrates, which serve as the key source of energy. Marihati *et al.* (2014) added that carbon also plays an important role in halophilic bacteria regeneration.

Proteins, which can be used by the body as a support for cell growth and repair processes as well as a buffer that regulates various body processes both directly and indirectly, also contain carbon. Similarly, fats, which function as energy storage, insulation, and protection also contain carbon. If the levels of carbohydrates and fats are adequate, proteins will be used for growth. However, if the availability of fat and carbohydrates is not met, protein will be used as an energy source and growth will be stunted. The nutritional content of Artemia sp. according to Marihati *et al.* (2014) contains 52.76% protein, 4.87% fat, 15.40% carbohydrates, 4.86% fiber, 11.26% ash, and 10.85% water. The high nutritional content of *Artemia* sp. can also be used as nutrition for halophilic microorganisms with the mechanism that occurs is *Artemia sp*. will eat plankton and will be released again as excrements wrapped in chorion / thin membrane that is not easily destroyed and settles to the bottom of the pond. The excrement sedimentation will cause the brine to become clear, and the evaporation process will run smoothly. *Artemia* sp. excrements and remains can be used as nutrition for halophilic bacteria to catalyze the purification of salt (Marihati *et al*., 2014).

A good body metabolism will transform the obtained energy into recovered energy used in growth. Growth occurs when there is an excess of energy from body-maintaining processes, basal metabolisms, and activities (Road, 1989). Proteins are complex organic compounds with high molecular weights, a polymer of amino acids monomers bound by peptides. The optimal needs of protein are highly affected by the use of proteins as energy, the composition of amino acids, and food digestibility. If the level of proteins in foods is too high, only half of it will be used to form and repair body cells while the rest will be used as energy (Kardana, 2012) .

An examination of *Artemia* sp. nutritional contents shows different values of proteins, carbohydrates, and fats contents. The level of proteins in *Artemia sp*. fed with *T. chuii* receiving *Gracilaria* organic fertilizer (P2) is higher than those fed with *T. chuii* receiving Walne fertilizer (P0), or P1, P3, and P4 treatments. This indicates a proportional correlation between the nutrition administered and the level of proteins in *Artemia* sp. Carbohydrates cannot be directly absorbed by cells in the body but must be broken down into even simpler molecules, namely monosaccharides, especially in the form of glucose, and then, glucose metabolism will occur at the cellular level (cellular respiration). The breakdown of carbohydrates is carried out by the process of digestion in the digestive tract (Vinet and Zhedanov, 2011) from the form of polysaccharides broken down into disaccharides and monosaccharides. The function of carbohydrates is to provide the body's energy needs. Moreover, carbohydrates are also needed for the continuity of fat metabolism and to conserve protein (Cruz-Suarez *et al*., 2008).

Fat serves as a source of energy and essential fatty acids, maintains the shape and function of cell membranes or tissues that are important for certain organs of the body, assists in the absorption of fat-soluble vitamins, and maintains the body's buoyancy. One unit

Table 6. The analysis of mineral contents in *Artemia* sp.

Treatment	Mineral	Initial Concentration (%)	Final Concentration (%)	% Change
A1	Na^+	0.000268	0.004527	1.59
	Cl^-	0.000332	0.00932	2.71
	Mg^{2+}	0.000167	0.021933	13.03
	SO_4^{2-}	0.001158	0.051602	4.36
A2	Na^+	0.000268	0.005009	1.77
	Cl^-	0.000332	0.0098	2.85
	Mg^{2+}	0.000167	0.02883	17.16
	SO_4^{2-}	0.001158	0.052162	4.4

Note: A0= without administrations of *Artemia* sp.; A1 = 1000 individuals/L *Artemia* sp. administered; A2 = 2000 individuals/L *Artemia* sp. administered.

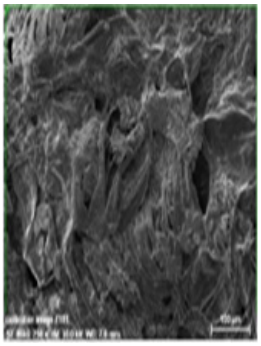
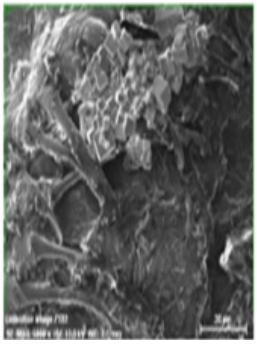
Table 7. Analysis of salt quality in *Artemia* sp. culture water

Sample	NaCl contents (%)	Water contents (%)	Iodine contents (%)	Insoluble (%)
Seawater non-*Artemia* sp. culture	83	0.56	11.31	0.87
Artemia sp. culture water	97	0.51	11.84	0.87

of fat contains twice as much energy as protein and carbohydrates. Fat in feed can provide energy to maintain metabolism so that most of the protein consumed can be used by the body for growth and not used as an energy source (Naylor *et al*., 2009). Fat content in the treatment of *Artemia* sp. fed *T. chuii* with the addition of liquid organic fertilizer *Gracilaria* sp. (P2) was higher than *Artemia* sp. fed *T. chuii* with the addition of Walne (P0) fertilizer. The performance of feeding *T. chuii* microalgae with higher fat content is also directly proportional to the protein content of *Artemia* sp.

Changes in sulfate and magnesium weight percentage on the 7th day and the 21st day were found on *Artemia* sp. samples. On the 21st day, sulfate and magnesium contents were the highest. This condition indicates *Artemia sp*.'s contribution to absorbing sulfate and magnesium in an aquatic environment. Marihati *et al*. (2014) explained that contaminating compounds, such as $CaSO_4$, $MgSO_4$, and $MgCl_2$ might affect the quality of salt crystals in addition to the purity of salt crystals themselves. The low quality of the salt can also be caused by coprecipitation (simultaneous deposition of crystals) by the appearance of NaCl salt crystals and the attachment of the mother liquor to the salt crystals. Coprecipitation is caused by Fe, Ca, Mg, K, and S salts which crystallize together with NaCl salt crystals. According to SNI 4435 (2017), the mineral contaminants that cause impurities in edible salts and industrial salts are sulfate, magnesium, and calcium components which are higher than 2%. The utilization of *Artemia* sp. which can absorb magnesium and sulfate in salt production areas will support the purification of salt products.

Table 8. *Artemia* sp. structure and mineral contents

***Artemia* sp.**	**Structure**	**Mineral Content**						
Treatment on 7 days		El	AN	Series	unn. [wt.%]	C norm. [wt.%]	C Atom [at.%]	C Error [%]
		O	8	K-series	51.15	51.15	48.49	47.2
		C	6	K-series	35.74	35.74	45.13	13.8
		Cl	17	K-series	6.69	6.69	2.95	0.2
		Na	11	K-series	2.51	2.51	1.66	0.2
		S	16	K-series	1.29	1.29	0.59	0.1
		Mg	12	K-series	0.9	0.9	0.54	0.1
		Ca	20	K-series	0.78	0.78	0.30	0.1
		K	19	K-series	0.57	0.57	0.22	0.1
		Ti	22	K-series	0.35	0.35	0.11	0.1
		Si	14	K-series	0.02	0.02	0.01	0.0
		Al	13	K-series	0.00	0.00	0.00	0.0
				Total	100.00	100.00	100.00	
Treatment on 21 days		El	AN	Series	unn. [wt.%]	C norm. [wt.%]	C Atom [at.%]	C Error [%]
		C	6	K-series	51.18	51.18	62.36	18.7
		O	8	K-series	32.56	32.56	29.78	33.1
		Cl	17	K-series	7.54	7.54	3.01	0.3
		Na	11	K-series	4.41	4.41	2.81	0.3
		S	16	K-series	2.35	2.35	1.11	0.1
		Mg	12	K-series	0.92	0.92	0.57	0.1
		Ti	22	K-series	0.40	0.40	0.12	0.1
		Ca	20	K-series	0.33	0.33	0.12	0.1
		K	19	K-series	0.29	0.29	0.11	0.0
		Si	14	K-series	0.02	0.02	0.01	0.0
		Al	13	K-series	0.00	0.00	0.00	0.0
				Total	100.00	100.00	100.00	

The contribution of *Salinabacter* halophilic bacteria in the salt purification process is very important because the bacteria are microorganisms that can survive in an environment with high salinity by maintaining their osmotic pressure (Oren, 2010). The existence of halophilic bacteria on the crystallization table accelerates evaporation and improves the quality of salt products because these bacteria consume organic materials that pollute the brine and absorb the heat of sunlight. Based on these functions, the halophilic bacteria are called a bio-catalyst in salt purification (Marihati *et al*., 2014) mentioned several species of halophilic bacteria. Among them are *Halobacterium salinarum*, *Haloarcula marismortui*, *Haloquadratum walsbyi,* and *haloalkaliphile Natronomonas pharaonis*. These bacteria have specific nutritional needs and abilities to degrade different organic compounds, such as glycerol, pentose, and folic.

The analysis of mineral contents (Na, Cl, Mg, and SO_4) in seawater samples and *Artemia* sp. showed differences in each treatment. The result of the analysis of variance indicated different sulfate contents between the seawater sample and each treatment of *Artemia* sp. because of *Artemia* sp.'s ability to reduce sulfate contents in seawater. As a result, the higher *Artemia* sp. population spread, the fewer sulfate contents in seawater. This finding confirms a statement made by (Islamiyah *et al*., 2022) stating that, the higher the *Artemia* populations, the higher the sulfate reductions. The reduction of sulfate in seawater is high because of *Artemia* sp.' ability to absorb sulfate into its body. This phenomenon occurs because *Artemia* sp. absorbs sulfate in the medium. Sulfate ions will be absorbed into the epithelia. Sulfate is important in *Artemia* sp. metabolism, especially in xenobiotic metabolism that happened in marine invertebrates.

Artemia sp. can absorb magnesium and calcium for growth, form exoskeletons, and carry out metabolism (Islamiyah *et al*., 2022). This ability can be utilized in salt ponds to reduce magnesium and calcium content in seawater. Magnesium plays a very important role in the growth and survival of *Artemia* sp. Magnesium also has an important role in lipid, protein, and carbohydrate metabolism as a cofactor in enzymatic and metabolic reactions. Low concentrations of magnesium ions can cause high respiration rates. High respiration rates at low magnesium concentrations lead to stress and decreased survival rates. Magnesium also contributes to energy activation through the hemolymph absorbance and secretion to activate energy in *Artemia*.

Analysis of the mineral content (Na, Cl, Mg, and SO_4) in the salt samples showed that there were differences in each treatment. The results of the analysis of variance showed an increase in chloride (Cl) levels followed by an increase in magnesium (Mg) and sulfate (SO_4) levels. The salt crystallization process causes salt and minerals to settle to the bottom so that only the water content evaporates. The solubility level of each mineral determines whether the salt crystallizes easily. This phenomenon confirms the statements of Islamiyah *et al*. (2022) stating that, the higher the solubility rate, the easier salt crystallizes. In the seawater evaporation process, calcium compounds crystallize earlier, followed by NaCl salts, potassium chloride, and finally magnesium salts.

Increase in magnesium levels is slower than sodium, where sodium binds to chloride to form NaCl, while magnesium binds to residual chloride and sulfate so that, during the concentration process, there is a precipitating element of magnesium (Sulistiyono and Shofi, 2017). Water evaporation is a significant factor in increasing sodium levels during summer. Like sodium, potassium is a natural element, but its concentration is lower than sodium and calcium. In salt crystallization, the ideal concentration for bittern is 25-29 °Be. If the bittern concentration is below 25°Be, calcium sulfate sedimentation will appear. Meanwhile, if the concentration of bittern is higher than 29°Be magnesium sedimentation will appear (Sartono *et al*., 2013).

The analysis of magnesium and sulfate contents is one of the factors that determine the quality of salt products. The higher the concentration of contaminants, the lower the concentration of salt contents. The appropriate concentration of magnesium and sulfate for industrial salt according to Indonesian National Standards is 0.06% (Mg^{2+}) and 0.2% (SO_4^{2-}). The high concentration of contaminants in salt is caused by the crystallization of Mg^{2+} and SO_4^{2-}. This finding is in line with the statement of Marihati *et al*. (2014) finding that the high level of impurities in salts is caused by crystallizing Mg2+ and Ca2+ together with NaCl. Karim *et al*. (2014) stated that Ca^{2+}, Mg^{2+}, SO_4^{2-}, and K^+ are elements that can reduce the quality of salt in seawater. If these ions bond with each other, they will produce salt with low salt content, brown in color, and bitter taste.

The results of NaCl content measurement in water with *Artemia sp*. culture integration show salt with excellent quality of 97% (Table 7) that met the criteria of industrial salt (Taunay *et al*., 2013). The contribution of *Artemia* sp. to improving the quality of salt products is interesting and has the potential to serve as an alternative to the traditional salt-farming system that is currently practiced.

One of the causes of low-quality salt products is the coprecipitation of magnesium, sulfate, and calcium salts that crystallize with NaCl called impurities. Coprecipitation can be prevented through the utilization of halophilic bacteria fed with *Luria bertani* nutrition in

the salt purification process. However, the application of *Luria bertani* in salt ponds will increase the total cost of salt production. *Artemia sp*. biomass culture is an alternative that can reduce magnesium and sulfate coprecipitation with more affordable costs (Marihati *et al*., 2014) he salts crystallization process is also affected by several environmental factors, such as air temperature, moisture, light intensity, water temperature, and pH.

The measurement of water quality conducted during the culture period shows optimum results to support microalgae and *Artemia sp*. growth. Schenk *et al.* (2008) defined the optimum temperature for microalgae growth as between 20-30°C. In higher temperatures, microalgae tend to maintain their survivability rather than regenerating new cells. Ernawati *et al.* (2020) also defined the optimum salinity and pH to support microalgae growth are 25-35 ppt and pH 7.8. *Artemia* sp. lives in water with high salinity and has planktonic characteristics. The results of water quality measurement during this study show an average temperature of 25-27°C, a pH range of 7.5-9, a salinity of 30-40 ppt, and a dissolved oxygen level of 2.4-2.7 mg/L. Libralato (2014) mentioned the factors that may affect *Artemia* sp. population are temperature, salinity, and the availability of foods in the environment. *Artemia* sp. has a high range of temperature tolerance between 6-35°C (Widiastuti *et al*., 2012). Atmoko and Ma'ruf (2009) mentioned the suitable temperature for the survival of *Artemia* sp. ranges from 26 - 31°C. According to Saravanakumar *et al.* (2014), seawater salinity of 35-55‰ provides a high survival rate for Artemia sp. This range is in line with the findings of a study conducted by (Bahr *et al*., 2021). He finds that *Artemia* sp. can live in an aquatic environment with a salinity tolerance of 5-200 ‰ while the optimum salinity for biomass growth is 30-50 ‰ and the optimum pH for growth and reproduction ranges between 7.5-8. Purba (2012) mentioned the optimum pH of seawater media for optimal growth ranges of 7-8.5. Artemia sp. is a creature that can efficiently synthesize hemoglobin so that it can live in an environment with very low dissolved oxygen levels, even in DO levels of 1 mg/L. However, the optimum oxygen level to sustain a normal life is 2-7 mg/L (Léger *et al*., 1987). The dissolved oxygen level examined in this study is still suitable with the findings of Swingle (1966) who found the minimum dissolved oxygen level was 2 ppm in normal conditions and not contaminated by toxic substances. This minimum dissolved oxygen level can sustain the life of organisms.

4. Conclusion

The administration of 16 mg N/mL *Gracilaria* sp. liquid organic fertilizer to *T. chuii* culture medium provides the best effect on *Artemia* sp. length and weight growth. It also contributes to the nutritional contents (proteins, carbohydrates, fats, water, and ashes) of *Artemia* sp. Therefore, this organic fertilizer can serve as an alternative to Walne fertilizer. The good growth of *Artemia* sp. also contributes to the absorption of Mg2+ and SO42- contaminating minerals that are utilized in growth, metabolisms, and the formation of exoskeletons in *Artemia* sp. (indicated by high carbon contents. *Artemia* sp. growth is also able to reduce coprecipitation in salt crystals and improve NaCl purity rate up to 97%, the standard for industrial salts based on industrial salt quality requirements.

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Authors' Contributions

Mochammad Amin Alamsjah, Dwi Yuli Pujiastuti, Adibi Rahiman Bin Md Nor were contributing in arranged, performed and analyzed of research experiment, whereas Himna Sayyidatul Islamiyah, Fadhilah Atika Putri, Rizka Sandra Amalia, Putranti Hikmah Triningtyas were contributing in supported and collected data.

Conflict of Interest

The authors declare that they have no conflict of interests.

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