Asian Journal of Aquatic Sciences, August 2024. Vol 7, Issue (2) 284-291
e-issn: 2716-4608
p-issn: 2655-366X

EFFECT OF THE COMBINATION OF SALINITY AND WALNE FERTILIZER ON THE POPULATION GROWTH OF *Nannochloropsis* sp ON LABORATORY SCALE

Atthoriq Habib Harry Aslam[1*], Syafruddin Nasution[1], Irvina Nurrachmi[1]
[1]Department of Marine Science, Faculty of Fisheries and Marine,
Universitas Riau, Pekanbaru, 28293 Indonesia
*atthoriq.habib5046@student.unri.ac.id

ABSTRACT

Activities of phytoplankton culture needed to be supported by physical and chemical parameters. This study aimed to analyze the effect of a combination of salinity and Walne fertilizer on the population growth of *Nannochloropsis* sp on the laboratory scale. The method used in this study is an experimental method using CRD (Completely Randomized Design) experimental design with 2 factors, namely salinity and Walne fertilizer, and each factor is given 3 levels of treatment. The level value of each factor is combined to obtain 9 combinations with 3 replications to obtain a total of 27 experimental units. The results of measuring the water quality parameters show that the media conditions were in good condition: an average temperature of 21°C, an average pH of 7.6, and the LED light used is ±2,000 lux. The results showed no significant effect of the combination of salinity and Walne fertilizer on the population growth of *Nannochloropsis* sp. The best combination value was obtained in the S1W1 treatment with a combination of 15 ppt salinity and 0.5 mL Walne fertilizer dose, resulting in cell population density ($4125x10^3$ cells/mL). The second best was obtained in the S1W2 combination with 15 ppt salinity and 1 mL Walne fertilizer dose ($3775x10^3$ cells/mL).

Keywords: Microalgae, Nutrient, Salt content

1. INTRODUCTION

Phytoplankton is one of the main components that act as natural food to obtain nutrients in aquaculture activities such as zooplankton farming or fish and shrimp hatcheries because the nutrients in natural food cannot be replaced by other feeds[1]. Natural feed must contain sufficient nutrients to grow the cultivated organisms. The one natural food that is often used is *Nannochloropsis* sp. This phytoplankton has a nutritional content of 50-55% protein, 16% carbohydrate, 28.3% fat, and 0.05% chlorophyll-a[2]. Its non-motile and non-flagellated spherical cells have a size of 2-4 µm and are the food of *Brachionus plicatilis* and *Artemia*[3]. *Nannochloropsis* sp. also has a short growth time of 4-6 days[4].

In phytoplankton culture activities, some components must be considered to support the success of culture activities. These include physical and chemical parameters such as salinity, pH, temperature in the culture medium, and nutrients obtained from the fertilizer used. One parameter that can affect the growth of *Nannochloropsis* sp is salinity. The salinity range owned by *Nannochloropsis* sp to be able to live is 0-35 ppt[5]. Lower salinity increased vital growth parameters and lipid productivity in *Nannochloropsis* sp[6]. Salinity is also a parameter that can affect growth rate and feed consumption in fish[7].

Another factor that plays a role in the culture of *Nannochloropsis* sp is fertilizer, which must have sufficient nutrients for phytoplankton to live and develop. The

Received: 21 June 2024
Accepted: 22 July 2024

development of natural food is related to macro and micro components[8]. The use of Walne fertilizer is better for increasing green microalgae growth than other media because of its better nutritional content[9]. Using synthetic fertilizers such as Walne fertilizer for microalgae culture is also considered more practical because of its complete nutrient content compared to organic fertilizers[10]. Walne medium is also a commonly used medium in microalgae culture[11].

The effect of salinity on the growth of other phytoplankton has been done comparing the effect of salinity on population density and chlorophyll-a concentration in *Spirulina* sp and found that an increase in salinity can increase the population density in *Spirulina* sp[12]. Research has also been done comparing the concentration of Walne fertilizer on the growth of *Nannochloropsis* sp. and obtained the results that the dose of Walne fertilizer does not affect the growth of *Nannochloropsis* sp[13]. So, in this study, experiments were conducted by combining salinity values and Walne fertilizer concentrations on the growth of phytoplankton *Nannochloropsis* sp.

This study aimed to analyze the effect of the combination of salinity and Walne fertilizer on *Nannochloropsis* sp and analyze the best combination value for the growth of phytoplankton *Nannochloropsis* sp.

2. RESEARCH METHOD

Time and Place

This research was conducted from December 2023 to January 2024. Culture analysis and cell counts were conducted at the laboratory of UPTD Balai Perikanan Budidaya Air Laut dan Payau (BPBALP) Teluk Buo, Padang, Sumatera Barat.

Method

The method used in this study is an experimental method with RAL 2 factors, each having 3 treatment levels. The first factor is the difference in salinity (S), and the second is the Walne fertilizer dose (W). The value of the salinity treatment level is taken based on the level value that can be tolerated by *Nannochloropsis* sp, which is 0-35 ppt, then divided into 3 levels of low salinity (15 ppt), medium (25 ppt), and high (35 ppt). The concentration level of Walne fertilizer used was taken from the commonly used concentration value (1.0 mL), then decreased (0.5 mL) and increased (1.5 mL) to see the effect of each combination. Each level was combined to obtain 9 treatment combinations, and then 3 repetitions were carried out to get 27 experimental units. After that, the layout was randomized using a lottery system to determine the location of each experimental unit.

Procedures

This research was conducted in a controlled room with an average temperature of ±21℃. The procedures carried out in this research are the preparation of tools, culture rooms, and media, preparation of seeds, making media, applying fertilizers, inoculating seeds, control, then observation and calculation of density. The preparation of tools includes sterilization by cleaning tools using 70% alcohol. Sterilization of culture containers is done by washing the container using anti-bacterial soap and then drying it in the sun for about 4-5 hours until the container is completely dry. Room sterilization is done by cleaning the room and culture rack from dust and other impurities. Sterilization of culture media is done by filtering the water first using a filter bag and then boiling it until it boils, then cooling it down to normal temperature so that the water used has avoided the presence of bacteria that can cause contamination of the culture object.

The seeds used were pure culture stocks of *Nannochloropsis* sp with a density of $3{,}500 \times 10^3$ cells/mL at UPTD BPBALP Teluk Buo, making culture media using the dilution formula:

$$M1.V1 = M2.V2$$

Descriptions:

M_1 : Initial of solution molarity (g/L)

V_1 : Initial of solution volume (mL)

M_2 : Final of solution molarity (g/L)
V_2 : Final of solution volume (mL)

Then the media was put into the container according to the treatment given 15 ppt (S1), 25 ppt (S2), and 35 ppt (S3) as much as 700ml, after which Walne fertilizer was added according to the dose of 0.5 mL (W1), 1.0 mL (W2), 1.5 mL (W3) using a drop pipette, then stirred until the fertilizer was evenly mixed. The seedlings were inoculated, and aerators were installed. Calculation of initial seedling density using the formula[14]:

$$N_2 = \frac{V1 \; x \; N1}{V2}$$

Descriptions:
V1 : Initial of seedling stocking volume (mL)
V2 : The volume of culture medium used (mL)
N1 : Seedlings stock (cell/mL)
N2 : Desired *Nannochloropsis* sp seedling density (cell/mL)

Population growth calculations were carried out every day for 6 days starting the day after the seedlings were inoculated by checking WQP (Water Quality Parameters) in the form of pH and temperature using a pH meter and digital thermometer, after which observations were made using a 40x magnification microscope and Haemacytometer, the results of observations were then photographed and then calculated using the density formula[15]:

$$D = \frac{n1+n2+\cdots.+Nx}{x} \; x \; 25 \; x10^4 \text{ cell/mL}$$

Descriptions:
D : Phytoplankton density (cell/mL)
n : Number of phytoplankton in the squares
x : Number of squares

Absolute Growth

Absolute growth is calculated using the formula[16]:

$$G = Wt - Wo$$

Descriptions:
G : Absolute Growth (cell/mL)
Wo : Initial cell density (cell/mL)
Wt : Final cells density (cell/mL)

Relative Growth Rate

Relative growth is determined using the formula[16]:

RGR = ((Ct - C0) / C0) x 100%

Descriptions:
RGR: Relative growth rate (%)
C0 : Cell population density (cell/mL) in the first period
Ct : Cell population density (cell/mL) in the last period

Spesific Growth Rate (*SGR*)

The specific growth rate is determined using the formula[17]:

$$SGR = \frac{LnNt - LnNo}{t} \; x \; 100\%$$

Descriptions:
SGR : Spesific growth rate (%/day)
No : Initial population density (cell/mL)
Nt : Final population density (cell/mL)
t : Time (day)

Data Analysis

The population growth data obtained in this study are presented in tables and graphs to compare the growth of *Nannochloropsis* sp. Each treatment combination was analyzed using a two-way Analysis of Variance (two-way ANOVA). Furthermore, the Least Significant Difference (LSD) further test was conducted.

3. RESULT AND DISCUSSION

Population Growth

Observations were made at 10:00 a.m. until completion at the Laboratory of Balai Perikanan Budidaya Air Laut dan Payau (BPBALP) Teluk Buo. The H0 value or initial density is calculated using the initial density formula. The seeds used in each treatment are the same, so it can be said that the initial density value of each treatment is the same. The stock density value of the *Nannochloropsis* sp culture used was 3,500x10^3 cells/mL, so the initial cell population density value was 1,050x103 cells/mL, presented in Figure 1.

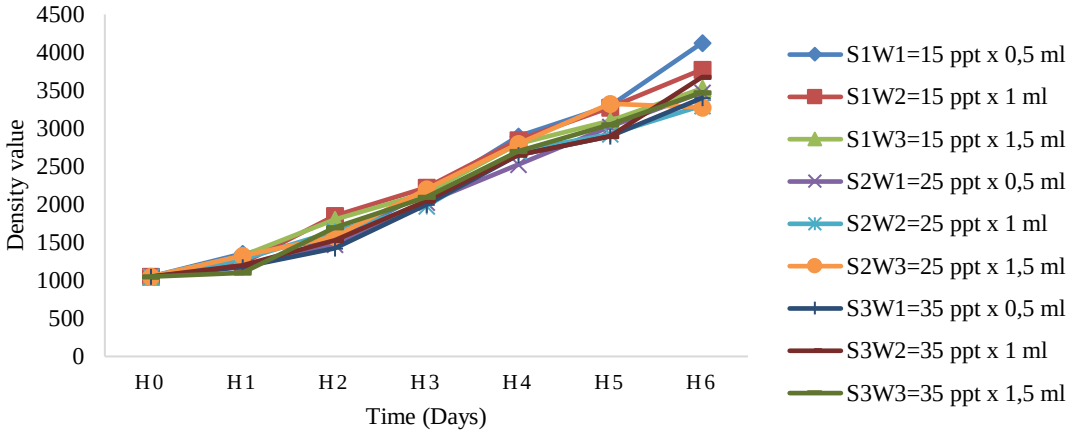

Figure 1. Average population growth of *Nannochloropsis* sp. during the 6-day culture period

The results of the calculations that have been carried out show that the cell growth of *Nannochloropsis* sp has increased every day. However, in the two-way ANOVA analysis test that combines the salinity and walne fertilizer factors, the results of $p>0.05$ significance on each day so that there is no effect of the combination of salinity and walne fertilizer on the growth of *Nannochloropsis* sp.

On day 1, the growth of *Nannochloropsis* sp. is in the lag or adaptation phase because microalgae cells are still adapting to the culture media and are still not actively reproducing. In the adaptation or lag phase, cells adjust to the culture media[18]. On day 2, the highest population density was obtained by treatment S1W2 with a density of $1850x10^3$ cells/mL, and the lowest was found in treatment S3W1, which amounted to $1425x10^3$ cells/mL. Although still in the adaptation phase, there are several treatments whose population has increased by a drastic amount, and this can occur because the difference in daily growth of each treatment is caused by the ability of cells to absorb nutrients contained in the culture medium[19].

On day 3, the highest density peak was obtained by treatment S1W2 with an abundance of $2225x10^3$ cells/mL and the lowest was obtained by treatment S2W2 with an abundance of $1975x10^3$ cells/mL. On day 3, the abundance obtained by each treatment had almost the same value, whereas the *Nannochloropsis* sp cells had nearly entered the exponential growth phase on day 4; when the cells entered the exponential phase, the highest density was in treatment S1W1 with a density of $2900x10^3$ cells/mL and the lowest was obtained by treatment S2W1 with a density of $2525x10^3$ cells/mL. When entering the exponential phase, phytoplankton cells begin to absorb nutrients quickly and experience cell division so that they tend to increase the number of cells[20]. The growth rate on day 4 in each treatment also tended to be higher than the other days.

On the 5th day, *Nannochloropsis* sp. cells still increased where the peak density was obtained by S2W3 treatment with a total density of $3325x10^3$ cells/mL and the lowest in S3W1 and S3W2 treatments with a total density of $2900x10^3$ cells/mL on the 5th day, *Nannochloropsis* sp cells were still in the exponential growth phase so that cell density continued to increase. On the 6th day, S2W3 treatment experienced a slight decrease in cell density to $3275x10^3$ cells/mL, and this indicates that *Nannochloropsis* sp cells have entered the stationary phase where the rate of phytoplankton death is relatively the same as the growth rate, so that the density of phytoplankton in this phase is relatively constant.

However, on day 6, other treatments still experienced growth characterized by an increase in the number of cell densities

where the highest density was obtained by the S1W1 treatment as well as the highest density value in this study for 6 days of culturing, which amounted to 4125x103 cells/ml and the lowest density value on day 6 was obtained by the S2W3 treatment with a cell density value of 3275x10^3 cells/mL.

Absolute Growth

The highest absolute growth value in this study was found in the S1W1 treatment in the H5-H6 period, with a growth value of 825x10^3 cells/mL. The lowest value was found in the S2W3 treatment in the same period, H5-H6, where the growth value reached -50x10^3 cells/mL, which means that on day 6, the average cell population in the S2W3 treatment decreased compared to day 5.

The absolute growth that occurred in each treatment had different patterns in each period; in the H1-H2 period, some treatments experienced an increase in absolute growth, but several treatments experienced a decrease in absolute growth, but the treatment that experienced a reduction of absolute growth in the H1-H2 period experienced an increase in absolute growth in the next period on the contrary, the treatment that experienced a rise in absolute growth in the H1-H2 period experienced a decrease in absolute growth in the H2-H3 period. In the H3-H4 period, the treatment tended to increase due to cells entering the exponential phase, with the highest increase obtained by treatment S1W1 with a value of 750x10^3 cells/mL. In the H4-H5 period, the absolute growth in each treatment decreased and increased again in the H5-H6 period where the cells were still in the exponential phase, as presented in Figure 2.

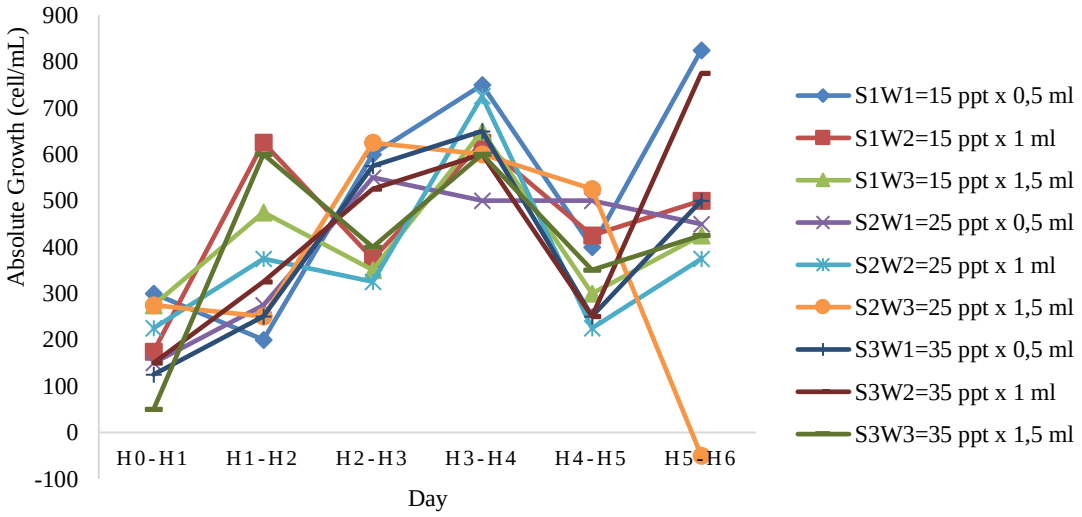

Figure 2. Absolute growth of *Nannochloropsis* sp. over a period of 1 day (24 hours)

Relative Growth Rate (*RGR*)

This study's relative growth rate pattern showed a diverse pattern in several periods. In the H0-H1 period, there was S1W1 treatment with the highest RGR value of 28.6%, and the lowest was obtained by S3W3 treatment with an RGR value of only 4.8%. In the following period (H1-H2), the value of S3W3 experienced a drastic increase of 54.5% and became the treatment that obtained the highest value. The H2-H3 period showed an excellent value in each treatment even though, at this stage, the phytoplankton was still in the adaptation phase, but the RGR value showed a good number where the lowest value in this period was obtained by the S1W3 treatment with a value of 19.4%. In the H3-H4 period, where phytoplankton has entered the exponential phase, the value obtained by each treatment is relatively the same, which only ranges between 24- 35%, so it can be concluded that the growth that occurs in this period is relatively the same. The value in each

treatment decreased in the next period (H4-H5), whereas the value obtained in this period only ranged from 8% to 20%. Finally, in the H5-H6 period, the value obtained by each treatment has a diverse pattern; several treatments have decreased, and several others have increased, and there is a treatment that has a negative value in this period, namely the S2W3 treatment with a value of -1.5%, presented in Figure 3.

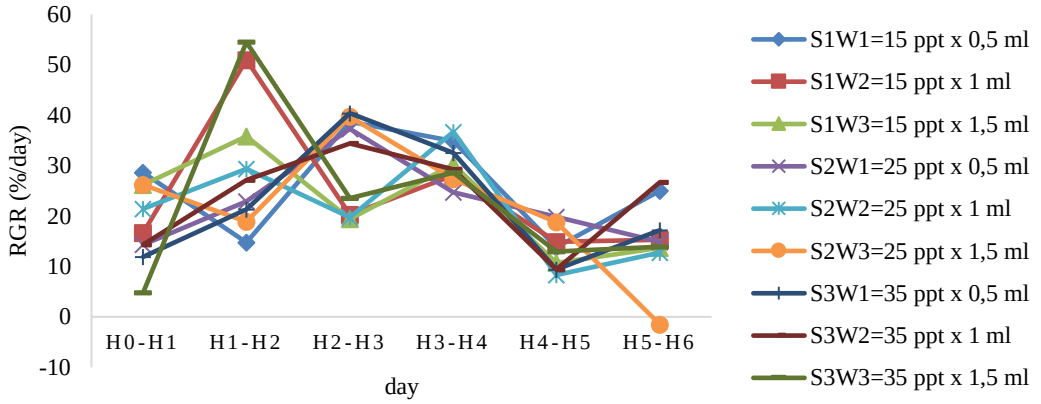

Figure 3. Relative growth rate (RGR) of *Nannochloropsis* sp in 1 day (24 hours) period

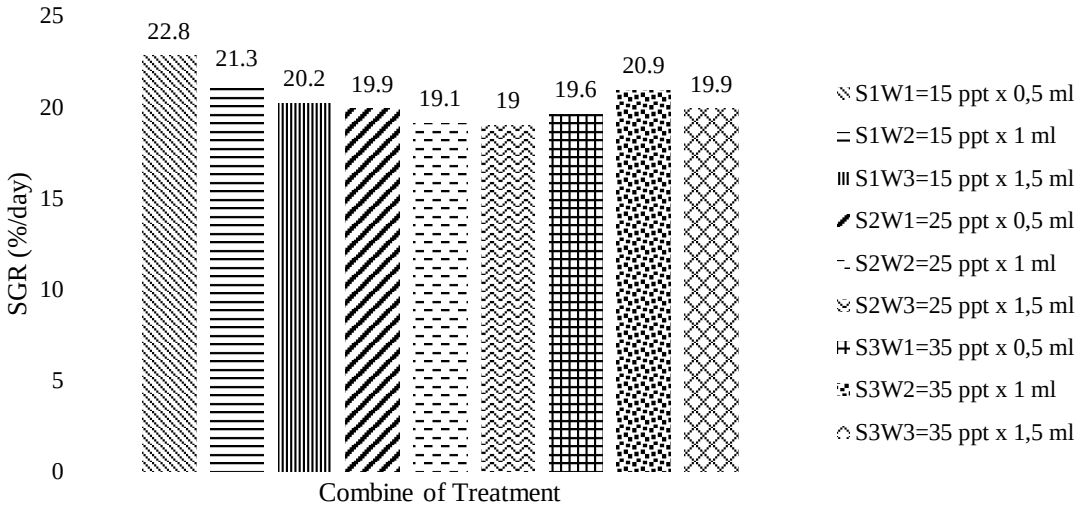

Figure 4. Specific growth rate (SGR) of *Nannochloropsis* sp

Specific Growth Rate

The value of SGR is the calculation of the daily cell population growth of *Nannochloropsis* sp. for 6 days of culturing. The results showed that the highest SGR value was found in the S1W1 treatment with a value of 22.8%/day, and the lowest value was obtained in the S2W3 treatment with a value of 19%/day. It can be seen that the value of each treatment is not too much different. The distance between the lowest and highest values is only 3.8% daily. The value of the growth rate can be used as a benchmark to determine the carrying capacity of the media to the growth of *Nannochloropsis* sp. The higher the growth rate, the better the carrying capacity of the media. It can be seen that all treatments show excellent value in supporting the growth of these phytoplankton cells (Figure 4).

4. CONCLUSION

In this study, it can be concluded that the combination of salinity and Walne fertilizer does not significantly affect the growth of *Nannochloropsis* sp. The best combination in this study was obtained by S1W1 treatment with a combination of 15

ppt salinity and 0.5 mL Walne fertilizer dose, with a population density value of $4125x10^3$ cells/mL. The second best was obtained by an S1W2 combination of 15 ppt salinity and 1 mL Walne fertilizer dose, with a population density value of $3775x10^3$ cells/mL.

REFERENCES

1. Sutomo, R., Komala, E.T., Wahyuni, M.G.L. Pengaruh Jenis Pakan Mikroalga yang Berbeda terhadap Pertumbuhan Populasi Rotifer, *Branchionus rotundifoemis*. *Oseanologi dan Limnologi*, 2007; 33: 159-176.
2. Paes, C.R., Faria, R.G., Tinoco, N.A.B., Castro, D., Barbarino, E., Lourenço, S.O. Growth, Nutrient Uptake and Chemical Composition of *Chlorella* sp and *Nannochloropsis oculata* under Nitrogen Starvation. *Latin American Journal of Aquatic Research*, 2016; 44(2): 275-292.
3. Fachrullah, M.R. *Laju Pertumbuhan Mikroalga Penghasil Biofuel Jenis Chlorella sp dan Nannochloropsis sp yang Dikultivasi Menggunakan Air Limbah Hasil Penambangan Timah di Pulau Bangka.* Institut Pertanian Bogor. Bogor, 2011.
4. Maria, G.S., Angelo, F., Schinas, P., Mamma, D., Kekos, D., Histrouforo, H., Kolisis, F.N. Magnetic Immobilization and Growth of *Nannochloropsis* oceanica and Scenedasmus almeriensis, *Plants*, 2021; 11(1): 21-23.
5. Khotimah, K. Teknik Kultur *Nannochloropsis* sp sebagai Pakan Alami Larva Udang Vaname (*Litopenaeus Vannamei*) di PT. Suri Tani Pemuka (Japfa) Unit Hatchery Makassar Kabupaten Barru. *Jurnal Politeknik Pertanian Negeri Pangkajene Kepulauan Pangkep*, 2019.
6. Pal, D., Khozin-Goldberg, I., Cohen, Z., Boussiba, S. Pengaruh Cahaya, Salinitas, dan Ketersediaan Nitrogen terhadap Produksi Lipid oleh *Nannochloropsis* sp. *Mikrobiologi dan Bioteknologi Terapan*, 2011; 90: 1429-1441.
7. Setiawati, M., Suprayudi, A.M. Pertumbuhan dan Efisiensi Pakan Ikan Nila Merah yang dipelihara pada Media Bersalinitas. *Jurnal Jurusan Budidaya Perairan. Fakultas Perikanan dan Ilmu Kelautan*, 2003.
8. Jadid, R., Dewiyanti, I., Nurfadillah, N. Penambahan Air Kelapa pada Media Pertumbuhan Populasi *Nannochloropsis* sp. *Jurnal Ilmiah Mahasiswa Kelautan Perikanan Unsyiah*, 2017; 2(1): 187053.
9. Putri, D.S., Alaa S. The Growth Comparison of *Haematococcus pluvialis* in Two Different Medium. *Biota*: *Biologi dan Pendidikan Biologi*, 2019; 12(2): 90-97.
10. Budiono, R., Juahir, H., Mamat, M. Modelling Interaction of CO_2 Concentration and the Biomass Algae Due to Reduction of Anthropogenic Carbon Based on Predator-Prey Model. *International Journal of Applied Environmental Sciences*, 2018; 13(1): 27-38.
11. Nabila, M. Pengaruh Jenis Media Kultur terhadap Konsentrasi Biomassa *Nannochloropsis* sp. *Pasundan Food Technology Journal* (*PFTJ*), 2020; 7(3): 97-101
12. Angeraini, A., Utami, E., Mahardika, R.G. Pengaruh Salinitas terhadap Kepadatan Populasi dan Konsentrasi Klorofil-a *Spirulina* pada Media Kultur Modifikasi Walne dan Air Limbah Budidaya Ikan. *EKOTONIA*: *Jurnal Penelitian Biologi, Botani, Zoologi dan Mikrobiologi*, 2022; 7(2): 112-120.
13. Wardani, N.K., Supriyantini, E., Santosa, G.W. Pengaruh Konsentrasi Pupuk Walne Terhadap Laju Pertumbuhan dan Kandungan Klorofil-a *Tetraselmis chuii*, *Journal of Marine Research*, 2022; 11(1): 77-85.
14. Edhy, E., Muchlisin, R., Abidin, Z. Pengaruh Konsentrasi Pupuk Amonium Sulfat Terhadap Pertumbuhan Populasi Sel *Nannochloropsis* sp. *Jurnal Biowallacea*, 2003; 3(3):149-155

15. Mudjiman, M., Mukhlis, A., Abidin, Z., Rahman, I. Pengaruh Konsentrasi Pipuk Ammonium Sulfat terhadap Pertumbuhan Populasi Sel *Nannochloropsis* sp. *Jurnal Ilmiah Ilmu Biologi*, 2008; 3(3): 149-155
16. Sopian, S., Hirata, H., Andarias, H.I., Yamasaki, S. Effect of Salinity Temperature on the growth of the Marine Phytoplankton *Chlorella saccharophila*. *Mem. Fac. Fish*. Kaghosima Univ, 2019; 30(9): 257-262.
17. Becker, E.W. *Microalgae: Biotechnology and Microbiology*. Cambridge University Press, 1994.
18. Utami, W., Indrayanto, G., Sondakh, R., Syahrani, A. XX *Solanum mammosum* L. (Terong Susu): In Vitro. *Medicinal and Aromatic Plants*, 2012; (10) 41: 394.
19. Arfah, Y., Cokrowati, N., Mukhlis, A. Pengaruh Konsentrasi Pupuk Urea terhadap Pertumbuhan Populasi Sel *Nannochloropsis* sp. *Jurnal Kelautan: Indonesian Journal of Marine Science and Technology*, 2019; 12(1): 45-51.
20. Susilowati, T. The Improvement of the Survival, Growth and Production of Vaname Shrimp *Litopenaeus vannamei* and *Seaweed Gracilaria verucosa* based on Polyculture Cultivation. *International Journal of Marine and Aquatic Resource Conservation and Co-existence*, 2014; 1(1): 6-11.