EFFECTS OF TOTAL AMMONIA NITROGEN (TAN) CONCENTRATION ON GROWTH AND CHLOROPHYLL-A CONTENT OF SEAWEED *Ulva* sp. IN A CONTROLLED CULTURE SYSTEM

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ABSTRACT

Seaweed *Ulva* sp. has great potential in sustainable integrated cultivation systems to reduce the impact of exploitation in nature. This research aimed to analyze the effect of Total Ammonia Nitrogen (TAN) concentration on the growth and chlorophyll-a content of seaweed *Ulva* sp. in a controlled culture system. The importance of this research is to develop sustainable cultivation systems without harming natural ecosystems by utilizing available nutrients in the form of TAN. The research method used a Completely Randomized Design (CRD) with 5 TAN concentration treatments: (P0: 0 ppm, P1: 0,010 ppm, P2: 0,025 ppm, P3: 0,050 ppm, P4: 0,075 ppm) with 3 replications. The culture was conducted for 15 days at the Aquaculture Environment Laboratory, University of Mataram. Parameters observed included absolute weight growth, specific weight growth rate, thallus sheet width and length, and *Chlorophyll-a* content. Research results showed that the highest absolute weight growth was achieved in treatment P1 (17,67%), the highest specific growth rate in P3 (3,39%), the highest thallus sheet width growth in P4 (2,83 cm), and the highest thallus length growth in P4 (3,50 cm). The highest *Chlorophyll-a* content was found in the control treatment P0 (8,06 mg/l), but based on the one-way ANOVA test results, there was no significant (*p>0,05)* effect on the growth of *Ulva* sp. However, the 2nd order polynomial regression test revealed a strong relationship between TAN concentration and growth, with R^2 ranging from 88-94%. Water quality during culture remained within normal range. The conclusion of this research indicates that TAN concentrations in the range of 0-0.075 ppm (mg/L) were only able to support the survival of seaweed *Ulva* sp. without providing significant effects on its growth. Further research with higher TAN concentration ranges is recommended to determine the optimal growth point for *Ulva* sp.

Keywords: Total Ammonia Nitrogen, Ulva sp., growth, chlorophyll-a, controlled culture.

1. INTRODUCTION

Ulva sp. seaweed is a type of seaweed that has the ability to sustain itself in extreme environmental conditions (Ardinata & Manguntungi 2020). Additionally, *Ulva* sp. has abundant nutritional content such as protein reaching 20%, fiber, as well as vitamin C and minerals equivalent to high-level vegetables. *Ulva* sp. seaweed has potential uses in cosmetic industries, pharmaceuticals, and even as fertilizer. The benefits of *Ulva* sp. as food and nutrition material include food ingredients (Citra *et al*., 2024). In previous research, *Ulva* seaweed was utilized as compost fertilizer due to its abundant natural stock (Wosnitza & Barrantes, 2006). However, over time, without proper follow-up actions, this practice could lead to increased environmental exploitation, thereby threatening the natural habitat of *Ulva* species.

The increasing interest in seaweed potential causes risks of excessive exploitation. The main problem is the lack of effective cultivation techniques, making production still dependent on nature. Nitrogen (N) plays an important role in chlorophyll formation and photosynthesis processes. Nitrogen in the form of Total Ammonia Nitrogen (TAN) can be obtained through the application of urea fertilizer, which affects protein levels as a component of chlorophyll formation. According to Sigurdarson *et al.,* (2018), the transformation of urea into Total Ammonia Nitrogen (TAN) occurs through hydrolysis by the urease enzyme, initially yielding ammonia (NH3), which subsequently forms ammonium ions when reacting with water. This process is significantly influenced by pH and temperature conditions. The sum of these two compounds—ammonia and ammonium—constitutes the Total Ammonia Nitrogen concentration. Research by Farliani *et al*., (2020) shows the importance of nitrogen requirements as a component of chlorophyll protein in *Ulva* sp. enrichment. Therefore, the development of effective cultivation techniques is essential to reduce exploitation from nature and maintain ecosystem balance.

2. MATERIAL AND METHODS

2.1 Research Material

This research used materials consisting of primary and secondary data. Primary data is the main data in the research and obtained directly during sampling activities throughout the research. The primary data used were water samples and *Ulva* sp. seaweed, while secondary data is supplementary data, and the data used in this research was water quality parameters (temperature, pH, salinity, and light).

2.2 Research Method

This research was conducted for 45 days from September to October 2024 at the Aquatic Environment Laboratory, Aquaculture Study Program, Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Mataram. *Chlorophyll-a* and TAN tests were conducted at the Analytical Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram. The equipment used included glass jars, aeration systems, refractometer, pH meter, lux meter, ruler, analytical scale, scissors, aquades, and urea fertilizer.

The research method was experimental using a Completely Randomized Design (CRD) consisting of 5 treatments and 3 replications, resulting in 15 total experiments. The TAN content in water was derived from commercial fertilizer that underwent laboratory testing.

Table 1. Treatment

Treatment	Concentration
(P0)	Without TAN Concentration
(P1)	TAN Concentration 0,010 mg/l
(P2)	TAN Concentration 0,025 mg/l
(P3)	TAN Concentration 0,050 mg/l
(P4)	TAN Concentration 0,075 mg/l

The culture procedure for *Ulva* sp. seaweed began with the preparation of culture containers using tiered racks equipped with LED lights with an intensity of 1,300,6-4,160,5 Lux (Zunnuraini *et al*., 2023), equipped with oxygen pipeline installations for aeration devices. Artificial seawater was prepared using 150 L of aquades with salinity adjusted to

31-32 ppt using fish salt, then sterilized with 15 ppm chlorine and neutralized through aeration for 2-3 days. *Ulva* sp. seaweed seedlings taken from sea waters were acclimatized for 3 days, weighed at 25-30 g/sample, and sterilized using dishwashing soap for 8-10 minutes, then rinsed with sterile seawater and tied to a substrate. Culture maintenance lasted for 15 days with the addition of pure aquades and daily water quality monitoring including temperature, salinity, pH, and light, as well as the addition of urea fertilizer based on Total Ammonia Nitrogen (TAN) concentration. Growth observations included weight, length, and width of *Ulva* sp. thallus, and *Chlorophyll-a* content was conducted every 15 days, with TAN measurements carried out every 5 days from day 0 to day 15. The following is an illustration of the experimental design of the study:

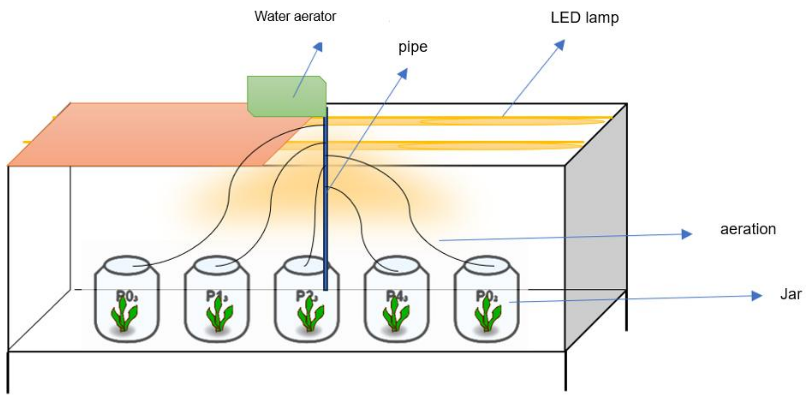


Figure 1. Experimental Design for Research

3. RESULTS AND DISCUSSION

3.1 Absolute Weight Growth of *Ulva* sp. Seaweed

Based on the research results, the absolute weight values of *Ulva* sp. seaweed over 15 days ranged from 13,00 g to 17,67 g. The highest absolute weight growth was in P1 at 17,67 g, followed by P3 with an absolute weight of 17,00 g, then P2 at 16,67 g, P0 at 13,33 g, and the lowest absolute weight growth was in P4 at 13,00 g.

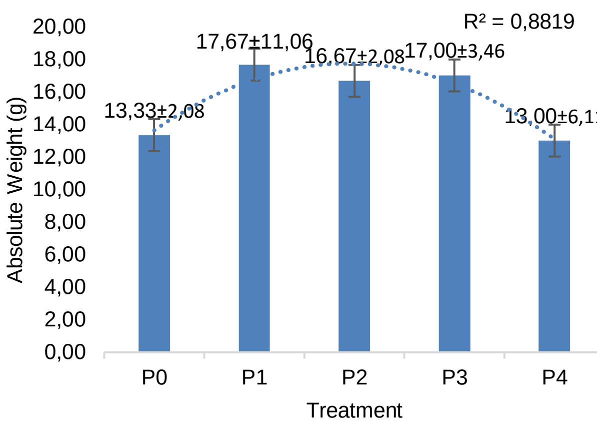
Based on One-Way ANOVA test results *(p=0,05),* TAN concentration in the water media did not significantly affect absolute weight growth. This is due to the range of TAN concentrations in each treatment producing growth that was not significantly *p<0,05* different, as shown in Figure 1. Additionally, according to ammonia quality standards for marine biota <0,3 mg/l, when compared to the range of TAN concentrations used in this study (0-0,075 mg/l), this range still meets quality standards. According to Peraturan Presiden No. 22 (2021), the ammonia value for biota in the ocean is <0,3 mg/l.

Table 2. Results of One-Way ANOVA on Absolute Weight Growth

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	33.733	4	8.433	.234	.913
Within Groups	360.667	10	36.067		
Total	394.400	14			

TAN concentrations in this study <0,1 mg/l did not influence absolute weight growth as seen in P1-P4. This is supported by Diamahesa *et al*., (2017) who found that *Ulva* sp. seaweed in the TAN concentration range of 0,0286-0,1394 mg/l did not affect seaweed growth after determining its absorption capacity. Based on research results, the largest absolute weight obtained was 17,67 g in P2 treatment. This value is considered low when compared to Fitri *et al*., (2023) research, which achieved the best absolute weight reaching 45 g.

The second-order polynomial regression test results obtained with R^2 of 0,8819 (88,19%) show a strong relationship between TAN and absolute weight. This is supported by the seaweed age factor that passed the growth phase, so the seaweed's ability to absorb nutrients shows a relationship from the TAN concentration test, although not significantly different. This aligns with Zunnuraini *et al*., (2023) who stated that seaweed growth will be influenced by age, and if too old, it will experience little growth and development because the previous sea lettuce cell production was already optimal.



Treatment	Concentration (mg/l)	Weight (g)	SD
P0	0 (no treatment)	13,33	2.08
P1	0,010	17,67	11,06
P2	0,025	16,67	2,08
P3	0,050	17,00	3,46
P4	0,075	13,00	6,11

(P0) No Treatment; (P1) 0,010 mg/l; (P2) 0,025 mg/l; (P3) 0,050 mg/l; (P4)0,075 mg/l

Figure 2. Absolute Weight Growth of *Ulva* sp. Seaweed at Different TAN Concentrations.

The effect of TAN on absolute weight growth was not significant, p>0,05

3.2 Specific Growth Rate

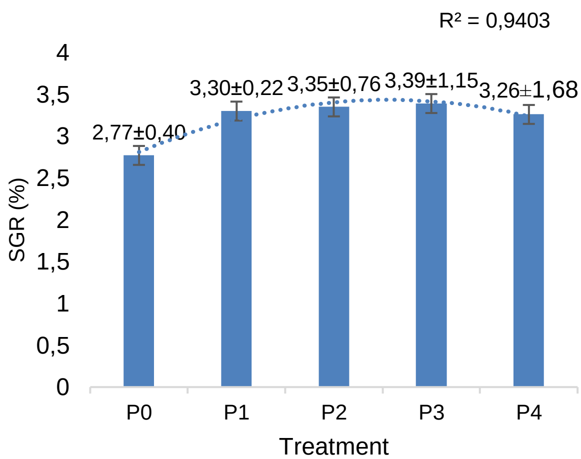
Based on the research results, the specific weight values of *Ulva* sp. seaweed obtained over 15 days ranged from 2,77% to 3.39%. The highest specific weight growth rate was in P3 with an average of 3.39%, followed by P2 with an average of 3,35%, then P1 with an average of 3,30%, P4 with an average of 3,26%, and the lowest was P0 with an average of 2,77%. Based on One-Way ANOVA test results at the 0,05 level, different TAN concentrations in the test media did not significantly affect the specific growth rate of *Ulva* sp. seaweed. The value obtained from the second-order polynomial regression test was R^2 of 0,9403 (94,03%), indicating a strong relationship between specific weight and TAN concentration.

Table 3. Results of One-Way ANOVA on Specific Growth Rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7791.333	4	1947.833	.208	.928
Within Groups	93492.000	10	9349.200		
Total	101283.333	14			

Based on One-Way ANOVA test results at the 0,05 level, specific growth rate results were not significant (*p>0,05)*. This is due to the range of concentrations given not being significantly different, so *Ulva* sp. seaweed did not experience significant growth as seen from P1-P4 results, and this condition is similar to the previous absolute weight growth results. Seaweed has the ability to utilize low nutrient availability for survival. This result is consistent with Hasni *et al*., (2023) who stated that seaweed can still survive without growth by utilizing nutrient reserves stored in the thallus for survival.

In this study, the best specific growth rate was obtained in P3 treatment at 3,39%. According to Zainuddin & Rusdani (2018), specific growth rates reaching 3-5% in cultivation will be profitable. However, in Ale *et al*., (2011) research, *Ulva* sp. seaweed can be profitable at specific growth rates reaching 6-9%. The regression test results for specific weight growth rate have a strong relationship with TAN concentration with R^2 of 94,03 %. This is due to the ability of seaweed thallus to absorb nutrients despite low nutrient availability. This research is supported by Risnawati *et al*., (2018) that seaweed growth rate can be influenced by the number of thallus sheets it has, enabling it to absorb nutrients even at low concentrations.



Treatment	Concentration (mg/l)	SGR (%)	SD
P0	0 (no treatment)	2,77	0,40
P1	0,010	3,30	0,22
P2	0,025	3,35	0,76
P3	0,050	3,39	1,15
P4	0,075	3,26	1,68

(P0) No Treatment; (P1) 0,010 mg/l; (P2) 0,025 mg/l; (P3) 0,050 mg/l; (P4) 0,075 mg/l

Figure 3. Specific Growth Rate of *Ulva* sp. Seaweed at Different TAN Concentrations.
The effect of TAN on specific growth rate was not significant, p>0,05

3.3 Thallus Sheet Width

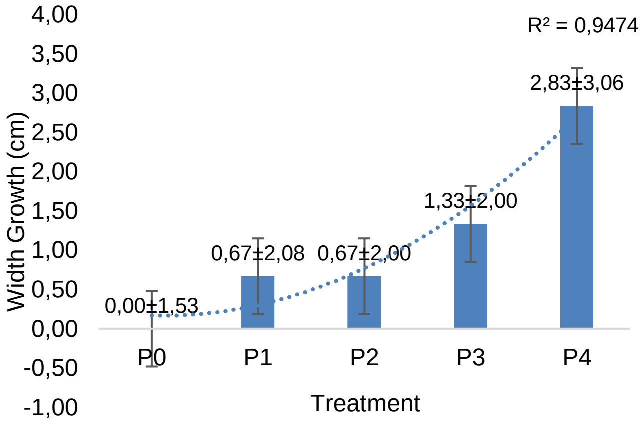
From the research results, the highest thallus width growth value in *Ulva* sp. seaweed, commonly known as broad-leaf seaweed, over 15 days was obtained in P4 with an increase of 2.83 cm, and the lowest was P0 at 0,00 cm. Based on ANOVA value, it was not significant (*p>0,05)* for thallus sheet width growth. The regression test results with R^2 obtained was 0,9474 (94,74%) and classified as a strong relationship between TAN concentration and thallus sheet width.

Table 4. Results of One-Way ANOVA on Thallus Sheet Width

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.433	4	2.358	.215	.924
Within Groups	109.667	10	10.967		
Total	119.100	14			

Based on One-Way ANOVA test results at the 0,05 level, thallus width growth did not show significant (*p>0,05)* differences between treatments due to large growth variations in each treatment, indicating that some treatments experienced faster growth and others slower. In P0, P1, and P2, seaweed conditions experienced much lower growth compared to P3 and P4 because the total ammonia concentrations given at 0-0,025 mg/l did not cause changes in growth, while P3 (0,050 mg/l) and P4 (0,075 mg/l) are concentrations that showed an increase in thallus width though not significantly different. This is consistent with Neisila *et al*., (2020) research that ammonia values >0,04 mg/l can provide growth in *Ulva* sp. seaweed, while in Huo *et al*., (2024) research, *Ulva* sp. seaweed was able to experience good growth at TAN concentrations of 0,11-0,18 mg/l.

The increase in TAN content concentration can contribute to thallus growth due to the ability of *Ulva* sp. seaweed to absorb and utilize nutrients as the main source. According to Maylanda *et al*., (2023), if other nutrients are available, high N concentrations can promote growth and development of aquatic organisms.



Treatment	Concentration (mg/l)	Width (cm)	SD
P0	0 (no treatment)	0,00	1,53
P1	0,010	0,67	2,08
P2	0,025	0,67	2,00
P3	0,050	1,33	2,00
P4	0,075	2,83	3,06

(P0) No Treatment; (P1) 0,010 mg/l; (P2) 0,025 mg/l; (P3) 0,050 mg/l; (P4) 0,075 mg/l

Figure 4. Thallus Sheet Width Growth of *Ulva* sp. Seaweed at Different TAN Concentrations.

The effect of TAN on thallus sheet width was not significant, p<0,05

3.4 Thallus Sheet Length

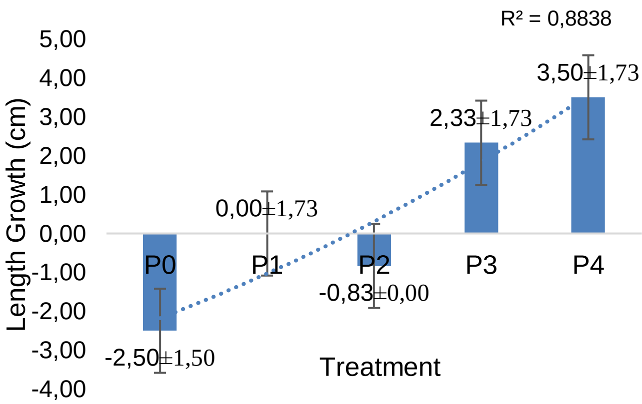
From the research conducted over 15 days, the thallus sheet length growth value of *Ulva* sp. seaweed was highest in P4 with growth up to 3,50 cm, followed by P3 at 2,33 cm, then P1 at 0,00 cm meaning no growth occurred, followed by P2 with a decrease of -0,83 cm, and a decrease in P0 of -2,50 cm. Based on ANOVA test results at the 0,05 level, the results were not significant (*p>0,05)* for thallus sheet length growth, thus showing an increase and decrease in thallus length growth. Second-order polynomial regression test results obtained an R^2 of 0,8838 (88,38%) and included in the category of strong relationship between TAN and thallus sheet length.

Table 5. Results of One-Way ANOVA on Thallus sheet length

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	70.167	4	17.542	2.625	.098
Within Groups	66.833	10	6.683		
Total	137.000	14			

Based on One-Way ANOVA test results at the *p= 0,05* level, thallus sheet length growth of seaweed at TAN concentrations yielded results that were not significant (*p>0,05)* in the treatments. This is due to large data variations despite increases or decreases in thallus length growth in treatments caused by the seaweed's ability to absorb nutrients. Additionally, at low and medium concentrations (P0-P2), growth tended to be stagnant and experienced decreases, while increases in thallus length were clearly visible in P3 and P4. In this study, the seaweed sheet length obtained was 3,50 cm, which is relatively low compared to Fitri *et al*., (2023) research where *Ulva* sp. seaweed was able to grow up to 5-7 cm.

The regression test results show a high R^2 88,38 % value, meaning the relationship between TAN concentration and thallus sheet length growth is strong. One of the reasons suspected to support this strength is the seaweed growth phase that is able to absorb nutrients, namely TAN utilized by the thallus to support its growth. This can be seen in the increase in thallus sheet length in P3 and P4, although statistically not significant due to high data variation. According to Togatorop *et al*., (2017), the early growth period of seaweed is called the adaptation phase where growth is inhibited because part of the energy is used for survival. This is suspected to cause changes in physiological habits such as photosynthesis and nutrient absorption. The logarithmic phase is when there is an increase in thallus length growth, while the linear phase is when seaweed growth has generally reached maximum conditions for some time and does not increase until it enters the aging phase. In this study, a decrease occurred in P0, P1, and P2, marked by the shedding of some thallus. Basically, nitrogen content in waters is needed to stimulate algae growth; if there is a nitrogen deficiency, it will affect growth (Fitri *et al*., 2023).



Treatment	Concentration (mg/l)	Length (cm)	SD
P0	0 (no treatment)	-2,50	1,50
P1	0,010	0,00	1,73
P2	0,025	-0,83	0,00
P3	0,050	2,33	1,73
P4	0,075	3,50	1,73

(P0) No Treatment; (P1) 0,010 mg/l; (P2) 0,025 mg/l; (P3) 0,050 mg/l; (P4) 0,075 mg/l

Figure 5. Thallus Sheet Length Growth of *Ulva* sp. Seaweed at Different TAN Concentrations.

The effect of TAN on thallus sheet length growth was not significant, p>0,05

3.5 *Chlorophyll-a* Content

Based on the research conducted, the *Chlorophyll-a* content value in *Ulva* sp. seaweed from measurements during 15 days of maintenance was highest in P0 at 8,06 mg/l, followed by P3 at 5,82 mg/l, then P1 at 5,31 mg/l and P4 at 3,14 mg/l, while the lowest value was in P2 at 2.45 mg/l. Based on normality results, chlorophyll was not normally distributed, so it could not be continued to One-Way ANOVA test at 0.05 level. Second-order polynomial regression test results obtained an R^2 of 0,5777 (57,77%), classified as moderate between the relationship of TAN concentration and *chlorophyll-a*.

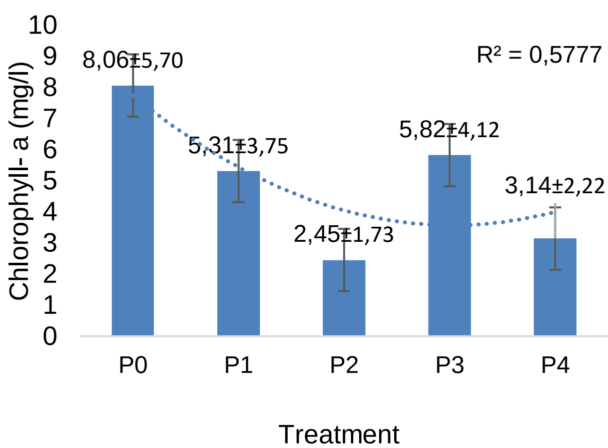
The highest *Chlorophyll-a* content was in P0 treatment, which indicates faster and higher *Chlorophyll-a* formation. This can be seen from the seaweed color that tends to be darker green compared to P2, which has a low *Chlorophyll-a* content. *Chlorophyll-a* formation in P0 is supported by inherent nutrients, and it is suspected that seaweed experiences stress levels so it regulates nutrient utilization in *Chlorophyll-a* formation. This is consistent with Hurd *et al*., (2014) who state that seaweed experiences nutrient stress, one of which is caused by low nutrient requirements; seaweed can utilize inherent nutrients stored in its tissues to maintain physiological functions, such as chlorophyll formation despite nutrient limitations. According to Afifah *et al*., (2021), nutrients in cell tissues are ammonium ions NH4+ from the reduction of nitrates and nitrites in cell tissues, enabling seaweed to uptake ammonia as a nutrient source.

Based on research results, the highest *Chlorophyll-a* amount was in P0 at 8,02 mg/l. According to regression tests categorized as moderate, the relationship between TAN concentration and pigment content during the *Ulva* sp. seaweed culture process can be observed through the morphological condition of thallus leaf color as a form of chlorophyll content presence. If seaweed has a dark green color, this indicates much more *Chlorophyll-a* formation, and this occurred in P0 which was high. This is consistent with Dewi (2018) that the difference in chlorophyll content causes variations in green pigment colors in both types

of chlorophyll where *Chlorophyll-a* forms blue-green while chlorophyll-b is yellow-green. The dark green color in P0 indicates abundant *Chlorophyll-a* pigment content but does not experience new tissue cell growth, while seaweed experiencing new tissue cell growth produces a light green pigment color. This is consistent with Mambai *et al*., (2014) that pigment color changes are related to cell division and energy use in regulating pigment levels, which is suspected due to *Chlorophyll-a* not functioning normally, as in P0.

The amount of *Chlorophyll-a* content in this research was highest in P0 at 8,02 mg/l, and when compared to Fitri *et al*., (2023) research that obtained a *Chlorophyll-a* value of 22,30 mg/l and according to them, this pigment is influenced by light supply needed for photosynthesis, which becomes a factor in the high or low chlorophyll content available. *Ulva* sp. has high chlorophyll content because it belongs to green algae, and chlorophyll in *Ulva* consists of *Chlorophyll-a* and chlorophyll-b but tends more towards *chlorophyll-a*. Low chlorophyll content is caused by lack of light intensity entering, resulting in less than maximum light absorption. Additionally, over time and age, the thallus's ability will decrease in light absorption, causing a decrease in *Chlorophyll-a* content.

Controlled cultivation methods using lamps can increase chlorophyll content because light supply is continuously available. Light from lamp rays with 12-hour illumination from morning to evening is an energy source for photosynthesis; with increased photosynthesis, the seaweed's ability to absorb nutrients also increases. This makes light a limiting factor if the light received is less than its growth requirements. This can result in non-optimal growth and can cause death (Zunnuraini *et al*., 2023).



Treatment	Concentration	Chlorophyll-a (mg/l)	SD
P0	0 (no treatment)	8,06	5,70
P1	0,010	5,31	3,75
P2	0,025	2,45	1,73
P3	0,050	5,82	4,12
P4	0,075	3,14	2,22

(P0) No Treatment; (P1) 0,010 mg/l; (P2) 0,025 mg/l; (P3) 0,050 mg/l; (P4) 0,075 mg/l

Figure 6. *Chlorophyll-a* Content of *Ulva* sp. Seaweed at Different TAN Concentrations.

3.6 Water Quality

Water quality parameters observed during the research activities were pH, temperature, salinity, and light intensity. Measurements were taken daily for 15 days of cultivation. The results obtained during the research from all parameters were optimal. Water quality data results can be seen in Table 6.

Table 6. Water Quality During Research

No	Parameter	Unit	Result	Optimal	Reference
1	pH	-	7,1 – 8,48	7-8,5	(Fitri *et al*., 2023)
2	Temperature	°C	26,18 - 29	24-35	(Fitri *et al*., 2023)
3	Salinity	Ppt	30 – 30,80	30-31	(Fitri *et al*., 2023)
4	Light	Lux	1,321-1,413	1,300,6- 4,1605	(Zunnuraini, 2023)

The pH quality in the culture water was already optimal, reaching 7.1-8.48. This is consistent with what was stated by Fitri *et al*., (2023) that *Ulva* sp. seaweed has optimal growth if within that pH range. This aligns with Ikbal *et al*., (2023) who stated that seaweed cultivation tends to have a basic pH above 7,0 and if too acidic or basic, it will cause metabolic disorders. Low pH in water can be caused by photosynthesis processes, temperature, and salinity (Hamuna *et al*., 2018). Normal water temperature is 24-35°C (Fitri *et al*., 2023) while the temperature during *Ulva* sp. seaweed culture activities was 26,18-29°C. If water temperature is not good, it can result in less than maximum seaweed growth, thus inhibiting the growth of *Ulva* sp. seaweed, which can cause the seaweed thallus to become pale with a yellowish color, and temperature greatly affects chlorophyll content, where the photosynthesis process will be faster when the temperature increases (Ikbal *et al*., 2023). Salinity is the concentration of salt solution in the sea that affects water osmotic pressure; differences in salinity are caused by differences in evaporation (Hamuna *et al*., 2018). Salinity during *Ulva* sp. seaweed culture activities reached the optimal limit of 30-30,80 ppt and in this case, obtained values that align with salinity for cultivation at 30-31 ppt (Fitri *et al*., 2023). Light intensity is very important during cultivation activities, which obtained a value of 1.321-1.413 lux, and this value is still within optimal limits; this is consistent with the fact that the light intensity value for *Ulva* sp. seaweed culture activities is 1.300,6-4.160,5 Lux (Zunnuraini *et al*., 2023).

4. CONCLUSION

Based on the ANOVA results that have been conducted, different TAN concentrations tested did not have a significant effect on each treatment, including absolute weight growth, specific weight growth rate, thallus sheet width, and thallus sheet length. This is because the TAN concentration in the treatments <0,1 mg/l can only support survival. Second-order polynomial regression test results showed a strong relationship between *Ulva* sp. seaweed growth and TAN concentration, and a moderate relationship between *Chlorophyll-a* content and TAN concentration. Therefore, research with different TAN concentrations is only used for survival purposes for seaweed.

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