Correlation between Chlorophyll-a Concentration and Carbohydrate Content of Gracilaria sp in Transition Season 2 and West Season in the Silvofishery Area of the North Coast of Central Java

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Abstract. The North Coast of Central Java has potential natural resources that can be developed, such as the cultivation of Gracilaria sp seaweed. in the silvofishery area either in monoculture or polyculture together with other biota cultivars. Different environmental characteristics in each silvofishery area with different seasons and research locations can influence the concentration of chlorophyll-a pigment Gracilaria sp. as well as the resulting carbohydrate content. The aim of this research is to determine the concentration of chlorophyll-a and carbohydrate content in Gracilaria sp., and to determine the relationship between these two parameters using a spatial approach (St 1: Brebes, St 2: Tegal, St 3: Pemalang, St 4: Pekalongan) and a temporal (transition season 2 and west season). Sampling Gracilaria sp. purposive sampling. Analysis of chlorophyll-a concentration using a spectrophotometer test and carbohydrate content using by difference. To determine the correlation between the two research parameters, the Pearson correlation test was used. The results showed that the chlorophyll-a concentration in transition season 2 ranged from 1,801-2,320 µg/mL with the highest concentration at St 1=2,320 µg/mL, while for the west season it ranged from 1,420-2,685 µg/mL with the highest concentration at St 1=2,685 µg /mL. The carbohydrate content of transition season 2 ranges from 37.088-57.135% with the highest content of St 3 (57.135%), while for the western season it ranges from 75.993-83.796% with the highest content of St 3 (83.796%). So it can be concluded that the relationship between chlorophyll-a concentration and carbohydrate content in transition season 2 is weak (r=-0.250; sig=0.750) and in the west season is strong (r=-0.846; sig=0.164). So it can be concluded that the relationship between chlorophyll-a and carbohydrate content is inversely proportional, that is, when the chlorophyll-a concentration is low, the carbohydrate content is low, and vice versa

Keywords: Gracilaria sp., chlorophyll-a, carbohydrates, silvofishery, transition season 2 and west

1. Introduction

The waters of the north coast of Java have a coastal area 92,000 meters long in the north of Java Island starting from Serang Regency to Gresik Regency. Geographically, it is located at latitude 060°44'14"S and longitude 108°34'53"E. The northern part of the coast of Central Java has potential natural resources that can be developed, namely seaweed. This is because the waters have mixed tides with a predominance of double types (diurnal) which supports optimal life for seaweed to grow and develop [1]. This type of diurnal tide also supports many anthropogenic activities in the surrounding area.

The waters north of Central Java have many anthropogenic activities such as fishing activities, ports, fishing market areas, and also factories. Waste from anthropogenic activities is thrown into the sea and affects the growth and quality of seaweed. One of the residents' efforts to preserve good quality seaweed is by creating seaweed cultivation ponds using the Silvofishery system. Silvo or forest cultivation as a conservation effort represents the ecological aspect, while fishery is a fishing activity as a utilization effort representing the economic aspect [2].

The benefits of this silvofishery include increasing harvest yields, reducing pond maintenance costs, reducing disease attacks on fisheries commodities, improving water quality, reducing coastal vulnerability and providing firewood for the population[3]. The silvofishery pattern currently being developed in Indonesia is according to Bengen [4] includes: (1) ditch pond, (2) improved ditch pond, and (3) komplangan. The results of this pond cultivation are to meet national seaweed production needs because its use continues to increase. It is proven that during the 2014-2019 period, national seaweed exports grew an average of 6.53% per year. In 2019, the export value of Indonesian seaweed reached 324.84 million USD or grew 11.31% compared to 2018 which reached 291.83 million USD [5]. One of the seaweed export products that is quite high is Gracilaria sp.

Gracilariasp. is a species of seaweed that has great tolerance to changes in environmental conditions, making it easier to cultivate [6]. Gracilaria sp has a fairly high tolerance for its environment, but it still needs to pay attention to several aspects such as the environment, seed quality, determining the method used and nutrient availability [7]. Environmental factors where Gracilaria sp. growth, namely the main nutrient content in the form of nitrates and phosphates, temperature, salinity DO (dissolved oxygen), pH (degree of acidity) can affect metabolism and synthesis and proximate [8]. Gracilaria sp. as an autotrophic biota, it is able to produce organic material through the process of photosynthesis.

The photosynthesis process in plants requires CO2, sunlight, chlorophyll and minerals [9]. Chlorophyll has three main functions in photosynthesis, including utilizing solar energy, triggering CO2 fixation to produce carbohydrates, and providing energy for the ecosystem as a whole. Sunlight contains all the colors of the visible spectrum from red to violet, but not all solid wavelengths are absorbed well by chlorophyll [10]. The effectiveness of absorbing light intensity will be related to the chlorophyll-a and b content which influences the rate of photosynthesis in producing carbohydrates. It is suspected that the higher the chlorophyll-a and b in seaweed, the more optimal the photosynthesis process will be seen from the higher carbohydrate content [11].

Gracilariasp. has potential as a food supplement and can be used as a source of main ingredients in the industrial and pharmaceutical fields due to its high nutritional value [12]. As one of the main components of seaweed, carbohydrates are known to have many important roles for human health. Variations in carbohydrate content produced in Gracilaria sp. can be different because it is influenced by habitat, fiber content and water depth, especially water quality such as salinity, temperature and sunlight intensity [13]. Ahmed [14] conveying polysaccharides from sulfated carbohydrates in seaweed has potential in the blood clotting system, antiviral, antioxidant, anticancer, immunomodulatory and antilipedic activities.

Many things can have an impact on the bio-chemical conditions and physical factors of the waters in ponds, this will cause a direct decrease in water quality which will affect the survival of Gracilaria sp. One internal influence is season. The transition season causes harvest failure which is often experienced by seaweed cultivators due to extreme rainfall and continuous hot seasons, resulting in a decrease in the growth rate and quality of the seaweed itself [15]. This needs to be studied because spatially and temporally it can affect the nutrient content in waters and cause differences in chlorophyll-a levels and carbohydrate content in Gracilaria sp.

2. Methods

The materials used in this research are grouped into tools and materials. The tools used in this research are Water Quality Checker, hand refractrometer, bright sample bottles, plastic samples, syringes, labels, stationery, millimeter blocks, coolbox, then laboratory equipment such as mortars, porcelain dishes, analytical scales, spectrophotometers, cuvettes, electrical oven, Neycraft oven, hemp rope, electric stove, thermometer, distillation tube, condenser, Soxhlet, and F pipe. The materials used in this research were Gracilaria sp., sea water samples, ice cubes, distilled water, MnSO4, KOH-KI, Concentrated H2SO4, 1% starch indicator, Na2S2O3, 4% formalin, methanol, chloroform, 90% acetone, selenium powder, 30% NaOH, tashiro indicator, 0.1 N HCl, 4% boric acid, petroleum benzene, hexane solvent, potassium dihydrogen phosphate , ascorbic acid, sulfuric acid, and selenium.

In carrying out the research, the methods used were survey and descriptive. According to Adiyanta [16]. This survey method is a research method that uses systematic, logical and rational scientific steps and stages so that the entire process is dialectical between theory and data. This survey method was carried out in situ to take samples of Gracilaria sp. and sea water and measure physical and chemical parameters in the silvofishery area of the north coast of Central Java. Next, the data obtained was analyzed descriptively. The descriptive method is a research method carried out to describe a symptom, event, event that is occurring at this time [17]. The research flow diagram is presented in Figure 1.

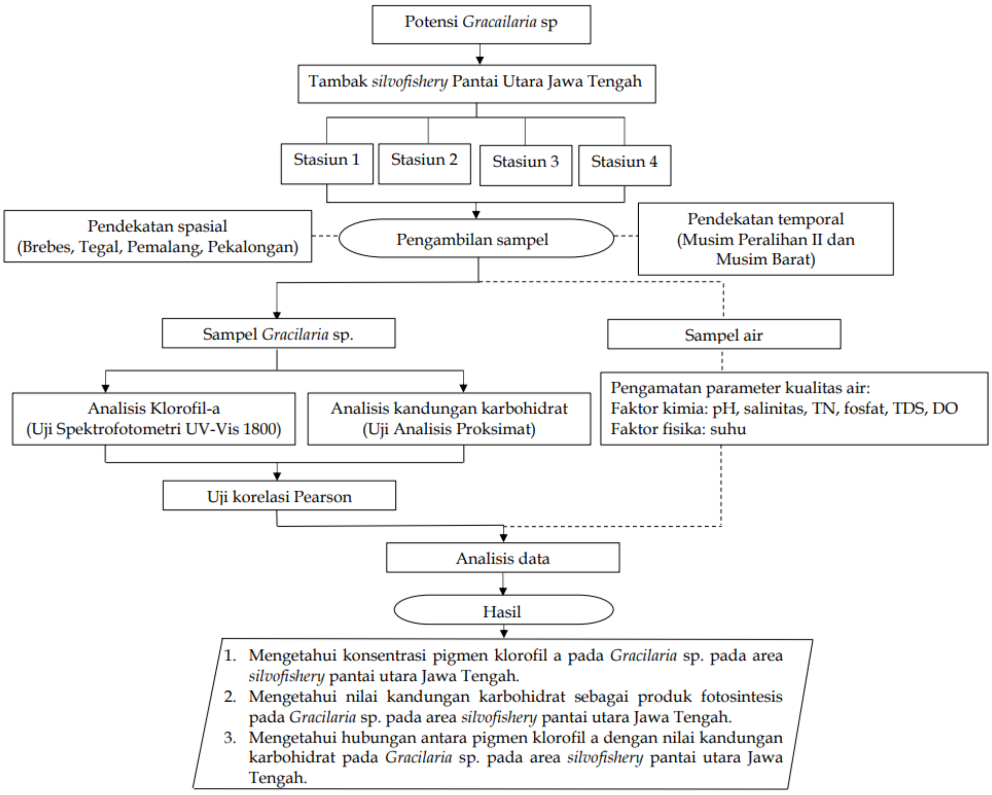

Figure 1. Research Flow Chart

The method for determining the research location was carried out using purposive sampling using a spatio-temporal approach. Determination of stations was carried out using purposive sampling at 4 points, then data collection was carried out in each season and assessed to be representative using a spatio-temporal approach. Each station has different characteristics so that it can be used as a research comparison parameter (Figure 2).

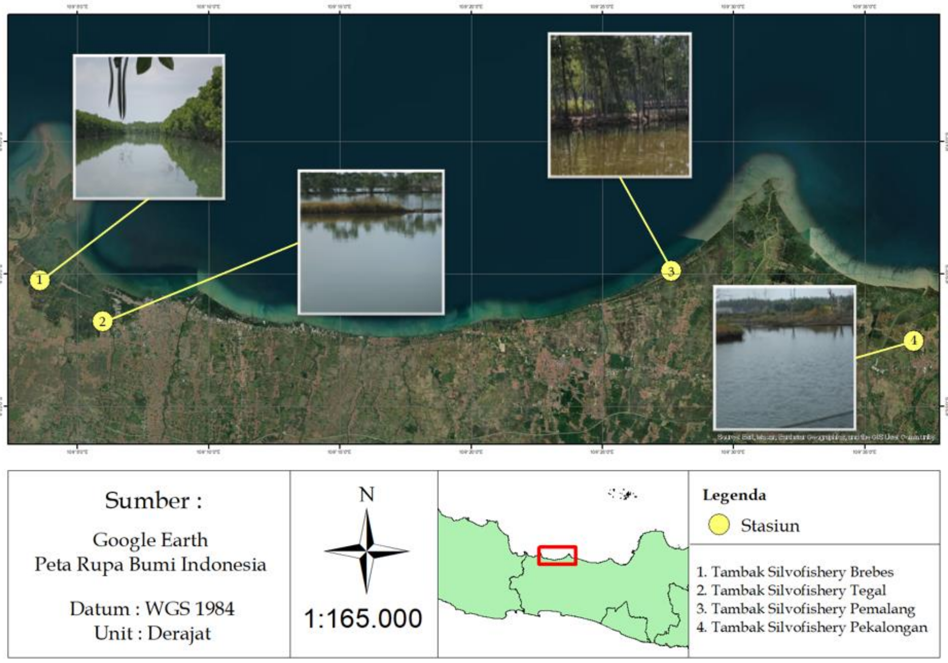

Figure 2. Research Location Map

This research was conducted during the Transition Season II (October 2022) and the West Season (February 2023) and in the North Java Waters with 4 research locations, namely stations in Brebes, Tegal, Pekalongan and Pemalang. Measurement of chlorophyll-a and carbohydrate content in Gracilaria sp. carried out at the Biochemistry Laboratory, Faculty of Mathematics and Natural Sciences, Jenderal Soedirman University. Physico-chemical water quality parameters at the Marine Biology Laboratory, Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University.

2.1.Sampling Gracilaria sp.

Sampling Gracillaria sp. This was carried out by in situ random purposive sampling at 4 different station points, namely at seaweed ponds in Brebes, Tegal, Pemalang and Pekalongan with repetition of each station 3 times. According to Insani et al. [18] sample of Gracilaria sp. after being taken directly from the pond, then washed with clean water to remove dirt and sand that sticks to it. The clean samples are then stored fresh by drying them first before putting them in a plastic and cool box. This is done to maintain the Gracilaria sp thallus. from damage during the collection process to research analysis in the laboratory [11].

2.2.Water Sampling

Water sampling was carried out at all station points with three repetitions. Observation of water samples in situ to determine the physical-chemical parameters of water in the form of temperature, salinity, DO, pH and TDS. Then observe the water samples ex situ to analyze the nitrate and phosphate content by putting the water samples in bottles and then taking them to the laboratory for analysis.

2.3. Chlorophyll-a content

Analysis of chlorophyll-a content using a Cole Pormer UV-Vis 1800 V spectrophotometer. Gracilaria sp samples were ground and then dissolved using 90% acetone for the maceration stage. This stage is to separate the loose color content from the tissue. Next, the extract was filtered using Whatman paper no. 42, then the chlorophyll sample was placed in a 4 mL cuvette. Measurement of chlorophyll-a content by measuring the light absorbance value using the UV-Vis spectrophotometer method. Absorbance was measured at wavelengths of 646 nm and 663 nm. Chlorophyll-a content was determined using the spectrophotometer method as used by Wellburn [19] with slight modifications:

Chlorophyll-a (µg/mL): 12.21 x A663 – 2.81 x A646

Information:
E 646: Absorbance 646 nm
E 646: Absorbance 663 nm

2.4. Carbohydrate Content

Analysis of carbohydrate content uses proximate analysis. The proximate analysis of this research includes: water content, ash content, fat content, protein and carbohydrate content. Testing water content uses the oven method, testing ash content using the furnace method, testing protein content using the Kjeldahl method, testing fat using the Soxhlet method, and testing carbohydrates by difference [20].

2.4.1 Calculation of total carbohydrates

Carbohydrate levels are done by difference using the equation:

Carbohydrate content (%) = 100% - (%bb water + %bb ash + %bb fat + %bb protein)

2.4.2 Test water content(AOAC, 2005)

The water content test was carried out using the oven method. The cup to be used is dried in an oven at 105ºC for 10 minutes, then cooled in a desiccator and then weighed. The sample was weighed in a cup of 0.5 grams then dried again in an oven at 105ºC for 3 hours until a constant weight was obtained, then cooled and weighed until a constant weight was obtained. Water content is calculated using the formula:

$$\textbf{Water content (\%)} : \frac{a-b}{c} \times 100\%$$

Information:
a : Weight of cup and sample before drying
b : Weight of cup and sample after drying
c : Sample weight

2.4.3 Ash content test(AOAC, 2005)

The ash content test was carried out using the dry ashing method. The ashing cup was dried in an oven at 105ºC for 10 minutes, then placed in a desiccator and weighed. A sample of 1 gram was put into an ashing cup, then put into an ashing furnace at a temperature of 800ºC for 3 hours, then put into a desiccator and weighed. Ash content is calculated using the formula:

$$\textbf{Ash content (\%)} : \frac{a-b}{c} \times 100\%$$

Information:
a : Weight of the cup and sample after kilning
b : Weight of empty cup
c : Sample weight

2.4.4 Test fat content(AOAC, 2005)

Test fat content using the Soxhlet method. Before being used to wrap the samples, filter paper and thread were dried at 105ºC for 10 minutes, then placed in a desiccator and weighed. The sample is weighed at 2-3 grams then wrapped in filter paper and thread. Next, put it in a Soxhlet extraction tube and reflux the sample for at least 6 hours until the solvent drops back into the clear fat flask. The extracted samples were placed in an oven at 105ºC for drying, then cooled in a desiccator and weighed. Fat content is calculated using the formula:

$$\textbf{Fat level (\%)} : \frac{a-b}{c} \text{ x } 100\%$$

Information:
a : Weight of filter paper and sample before extraction
b : Weight of filter paper and sample after extraction
c : Sample weight

2.4.5 Test protein levels(AOAC, 2005)

The protein content test was carried out using the Kjeldahl method. Two grams of the sample was put into a Kjeldahl flask and digested using 10 mL of concentrated sulfuric acid by heating until the color was clear. The digestion results were diluted and distilled by adding 50 mL of 30% NaOH. The distillate was collected in 25 mL of 4% H3BO3 (Boric Acid) solution then titrated with 0.5 mL of standard HCl solution using methyl red (Tashiro) as an indicator. The titration results are used to determine the total nitrogen value. The protein content of the sample is calculated by multiplying the total nitrogen and the correction factor. Protein content is calculated by the formula:

$$\textbf{Total Nitrogen (\%)} : \frac{A-B}{g \, X \, 10} \text{ x N NaoH x 14.008}$$

$$\textbf{Protein content (\%) : \%N x f}$$

Information:
A : mL blank titration
B : mL sample titration
N : normality of NaOH
g : Sample weight
f : Conversion factor (6.25) for fishery products)[8]

2.5. Data analysis

The data analysis used in this research is comparative descriptive analysis. The data is displayed in table form which is then analyzed descriptively comparatively. Comparative descriptive analysis is research by descriptively comparing independent variables from more than one research sample [21].

Next, to determine the relationship between chlorophyll-a and carbohydrates in Gracilaria sp. in the silvofishery area of the north coast of Central Java using statistical analysis with the Pearson correlation test. This test is usually used to find relationships between variables for numerical data. The results obtained from this calculation will result in a correlation coefficient which functions to measure the strength of the linear relationship between two variables. If the relationship between two variables is not linear, then the Pearson correlation coefficient does not reflect the strength of the relationship between the two variables being studied even though the two variables have a strong relationship [22]. This data analysis was carried out using Microsoft Excel and SPSS version 23 applications.

Table 1. Pearson Correlation Test Interpretation Numbers

Interpretation Numbers	Correlation Relationship
0 – 0.2	Very weak correlation
0.2 – 0.4	Weak correlation
0.4 – 0.7	The correlation is quite close
0.7 – 0.9	Strong correlation
0.9 – 1	The correlation is very strong
1	Perfect correlation

3. Results And Discussion

3.1. Chlorophyll-a concentration of Gracilaria sp.
In this study, the results showed that the chlorophyll-a concentration value of Gracilaria sp. which varies at each research station. The chlorophyll-a concentration value of Gracilaria sp. in transition season 2 the highest average value was in St 1 of 2.320 ± 0.311 µg/mL and the lowest was in St 3 of 1.801 ± 0.006 µg/mL. This value is in line with the western season's chlorophyll-a concentration value for Gracilaria sp. with the highest average value at Station 1 of 2.695 ± 0.005 µg/mL and the lowest at Station 3 of 1.420 ± 0.006 µg/mL. The results obtained from this test are presented in Figure 3.

Figure 3. Chlorophyll-a Concentration ValueGracilaria sp.

The chlorophyll-a concentration value in the west season appears to be higher than in the transition season 2 from all research stations. In the west season and transition season 2, St 1 and 2 have quite high values compared to other stations. This is thought to be because the nutrients in both seasons are optimal enough to produce chlorophyll-a (Figure 4) and the characteristics of the silvofishery area (Table 4) of the station are classified as good for Gracilaria sp. in absorbing nutrients resulting in high chlorophyll production. The characteristics of the silvofishery area at St 1 and 2 are monoculture silvofishery (only Gracilaria sp.) so that there is no competition in nutrient consumption in the water column. According to [23]. The presence of biota in seaweed can disrupt the growth of seaweed which can later cause growth to decrease.

Furthermore, the low chlorophyll-a concentration values in the transition season 2 and the west season in St 3 and 4 are also thought to be due to insufficient nutrients for Gracilaria sp. in producing chlorophyll-a. The characteristic of the silvofishery area at St 3 and 4 is that it is a polyculture silvofishery (together with milkfish) thus allowing for competition in nutrient consumption in the water column. Apart from that, the movement of milkfish can affect turbidity in the silvofishery area, this is indicated by high TDS values while DO values are low. This turbidity can affect the photosynthesis process of Gracilaria sp. So it is suspected that chlorophyll-a is not optimal in absorbing light. According to [23] cultivating seaweed requires waters with a low level of turbidity, so that sunlight energy can enter the waters optimally to support the photosynthesis process for seaweed growth.

Differences in chlorophyll-a concentrations are caused by differences in light intensity and nutrient concentrations in the water [24]. There are differences in light intensity for Gracilaria sp. It is thought to influence the formation of chlorophyll-a. Sufficient intensity of sunlight received by Gracilaria sp. really determines the speed of Gracilaria sp. to meet its nutritional needs [25]. In this research, to measure the intensity of light in waters using the TDS (total dissolved solid) value. According to [26]. The higher the TDS value will increase the turbidity value in the water, if the turbidity value is high the oxygen content will decrease. Silvofishery characteristics in each study can influence TDS values such as the presence of anthropogenic activities, polyculture between Gracilaria sp. with milkfish, tourism areas, and near tidal areas. The TDS value in the west season is higher compared to the transition season 2.

The highest TDS value in transition season 2 is located in St 1 at 3.73 mg/L and the lowest is in St 2 and St 3 at 2.41 mg/L, while in the west season the highest TDS value is in St 4 at 11.03 mg /L and the lowest was at St 3 at 7.04 mg/L. This is thought to be because St 1 and 4 have the characteristics of polyculture silvofishery Gracilaria sp. with milkfish cultivation and the presence of anthropogenic activities can affect the TDS value in it. Other factors that influence high turbidity are the presence of inorganic particles, organic colloids (such as microorganisms), and the increasing levels of feed in the water, the more the water turbidity will increase [27]. Another thing that is thought to influence the TDS value is the intensity of rainfall.

Figure 4. Area Rainfall FluctuationsSilvofishery North Coast Waters of Central Java

Fluctuations in rainfall can be seen in the western season (December 2022-February 2023) showing higher rainfall intensity than in transition season 2 (September 2022-November 2022) (Figure 4). In transition season 2 with low fluctuations in rainfall and high nutrient concentrations (Figure 5), it is thought that the high temperature causes an increase in the decomposition of organic matter by microbes [28]. The increase in decomposition is in line with an increase in nitrogen followed by an increase in phosphate.

Furthermore, nutrients in the west season with high fluctuations in rainfall have nutrient concentration values that are not much different from one station to another (Figure 5). This is thought to be because rainwater carries nutrients into the water column. When it rains, surface flow will carry nutrients from land or sediment from land will also flow into the waters [29]. This allows the concentration of this nutrient to be the same, but not the chlorophyll-a content which has differences between one station and another. This is thought to be because there are other factors that influence it, such as temperature, TDS value, salinity and others.

Another thing is that the rainy season allows lower nutrient levels in the waters compared to the dry season. This is in accordance with Nirmalasari et al [30] that when the rainy season occurs, the nutrient concentration will be lower compared to the dry season, this condition is caused by light penetration, salinity, low temperature, while turbidity is high compared to the dry season. The high and low levels of nutrients (nitrate and phosphate) are parallel to the pigment concentration values [31].

The research results show that if the TN and TP values are high, they are followed by high chlorophyll-a concentration values (Figure 5). High nutrients will increase the production of chlorophyll-a in plants, so it is possible that the chlorophyll-a content in Gracilaria sp. also high. According to [8]. Nitrogen (in the water in the form of nitrate) is one of the elements that forms chlorophyll-a so that increasing the amount of nitrogen in the water will increase the production of chlorophyll-a.

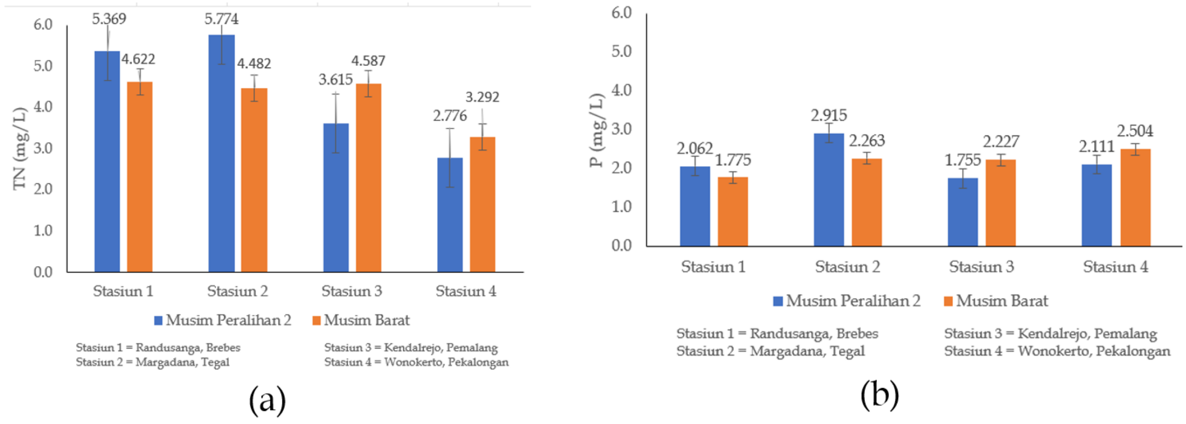

Figure 5. Concentration Values of (a) Total Nitrogen and (b) Phosphate Area Silvofishery

Nitrates and phosphates function in compiling protein compounds in cells. If these two elements are deficient, it will cause the protein content to decrease and this will be followed by the degradation of several cell components including chlorophyll-a [8]. According to Zainuddin and [32].The source of nitrogen comes from the decomposition of organic materials by bacteria and scientific water metabolism and industrial waste. The source of phosphate is through river flows consisting of various industrial wastes containing organic compounds.

The role of phosphate is in the transfer of energy in the form of ATP (adenosine triphosphate) and other high energy compounds present in the processes of photosynthesis and respiration. Chlorophyll synthesis requires ATP and NADPH (Nicotinamide adenine dinucleotide phosphate), so sufficient phosphate is needed for the formation of ATP [33]. The ability of seaweed to utilize nutrients in the waters is influenced by physical factors (temperature, light and water movement), chemical (nutrient concentration and nutrient form) and biology (type of seaweed, age, nutritional history and thalus size).

The results of this TN value research data are different from research conducted by Patahiruddin [34] ranged from 2,179-2,895 mg/L. This concentration is suitable for carrying out cultivation activities if seen from the statement, nitrate is a limiting factor if the concentration is <0.1 mg/L and >4.5 mg/L. The lowest limit of phosphate concentration for optimal growth of algae ranges between 0.018-0.09 mg/L and the highest limit ranges between 8.90-17.8 mg/L. Seaweed has a Nitrogen absorption efficiency of 32% and Phosphate 19%.

Stations 1 and 2 have high chlorophyll-a concentration values and high nutrient values, presumably because the silvofishery has the characteristics of being close to residential areas, thus allowing organic material to enter the silvofishery area thereby increasing the nutrients therein, as well as the condition of the few mangroves in the silvofishery area. allows light to be absorbed by Gracilaria sp. better. Stations 3 and 4 have lower chlorophyll concentration values compared to stations 1 and 2, even though the condition of the silvofishery area is because it is an estuary area and at the same time as milkfish cultivation, this allows little light penetration into the waters so that chlorophyll-a levels are lower than with station 1 and station 2. Zunnuraini et al. [25] states that light penetration is one of the limiting factors for seaweed growth, if the light received is below the required level, then the energy produced through the photosynthesis process is unbalanced or not fulfilled, if the light received continuously can cause disease.

The chlorophyll-a content can also be influenced by the levels of other pigments that are more dominant in Gracilaria sp. what's in it. The area of the blade and thallus can also influence the absorption of light entering Gracilaria sp. to carry out the process of photosynthesis [35]. The results of this research are different from research conducted by Yudiati et al. [8] amounted to 4.88 µg/mL and 2.31 µg/mL in Litopenaeus vannamei shrimp ponds. The chlorophyll-a concentration value is different for each individual due to different species, sunlight, water environmental conditions and different sample handling [36].

3.2. Carbohydrate content of Gracilaria sp.

Carbohydrate content of Gracilaria sp. In this study, the highest results were shown in transition season 2, namely at St 3 at 57.135% and the lowest at St 4 at 37.088%. Meanwhile, for the west season, the highest is on St 3, namely 83.796% and the lowest is on St 1, namely 75.993%. The results obtained from this test are presented in Figure 6.

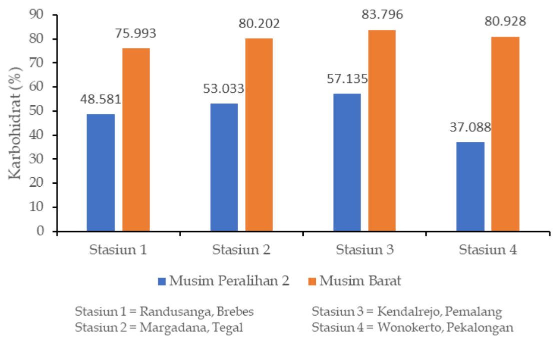

Figure 6. Carbohydrate Content ValueGracilaria sp.

The research data in Figure 6 shows that the carbohydrate content in Gracilaria sp. in both seasons, the value appears to be higher in the west season compared to transition season 2. Nutrient availability (Figure 5) in transition season 2 has a fairly high value but this is not followed by the carbohydrate content. This is thought to be because the photosynthesis phase to produce carbohydrates has not yet reached its optimum point so the carbohydrate content is still low. Another thing that causes low carbohydrate content is due to the characteristics of polyculture silvofishery areas (along with milkfish) so that carbohydrates are low. This is in accordance with the research results [23] which stated that attacks by seaweed predatory fish were marked by cutting off the thallus of Gracilaria sp. is still a factor inhibiting the growth of seaweed.

The carbohydrate content in the west season is higher than in transition season 2. This is thought to be because the nutrients in the west season are more evenly distributed compared to transition season 2 as presented in (Figure 5). These high nutrients can produce high levels of carbohydrates because the photosynthesis process that is taking place has reached optimum. This statement is supported by Dewi et al. [11] that the photosynthesis process will be more optimal, which is characterized by the high carbohydrate content produced. Another reason that could be the reason why these carbohydrates are higher in the west season compared to transition season 2 is because of the rainy season (Figure 4).

High rainfall fluctuations in the west season (Figure 4) result in high carbohydrate content. According to [37]. High rainfall can affect salinity levels in waters. Salinity is an important factor for seaweed. Low salinity ranges can cause abnormal growth. Tolerant of a high salinity range, but optimal growth is only at a salinity of 30 ppt. The seaweed osmoregulation mechanism can occur by using amino acids or types of carbohydrates [38].

The increase in carbohydrates when osmoregulation occurs is supported by the statement [39] that when plants experience osmoregulation they will try to make physiological changes as a form of adaptation in order to survive. One form of adaptation is the ability of Gracilaria sp. to maintain turgor pressure or osmotic adjustments and accumulate dissolved compounds including sugar, amino acids, proline and glycine betaine. Carbohydrate solubility can help plants to survive and maintain cell osmotic pressure caused by suboptimal salinity.

According to Winarno (1990) in Ma'aruf et al. [40]. The chemical composition of algae varies depending on the habitat. Apart from that, differences in nutrition are also possible due to differences in age and environmental parameters of the Gracilaria sp habitat. Other research such as that carried out by [41] that the chemical composition of seaweed can vary because it is influenced by several environmental factors such as temperature, water, salinity, light, nutrition, species, geographical location, and season. This research also measured several physico-chemical parameters from the silvofishery area (Table 2).

Table 2. Area Physico-chemical ParametersSilvofishery Transition Season 2 and West Season

Parameter	Musim	Stasiun				**Referensi**
		St 1	St 2	St 3	St 4	
Suhu (°C)	Peralihan 2	31.8	32.8	32	33.9	27 -31
	Barat	28.2	28.6	30.3	31.4	(Harahap *et al.*, 2022)
Salinitas (ppt)	Peralihan 2	33.3	19	20.3	28.3	15-35
	Barat	29.6	18	19	31.3	(Widyorini, 2010)
pH	Peralihan 2	7.6	8.9	8.1	7.9	6-8
	Barat	7.1	7.9	7	7.7	(Syam *et al.*, 2020)
DO (mg/L)	Peralihan 2	11.9	15.3	12.8	14.6	4.5-9.8
	Barat	2.8	6.5	5.4	6.7	(Rukisah *et al.*, 2020)
TDS (mg/L)	Peralihan 2	3.7	2.4	2.4	3.2	10.22-45.15
	Barat	10	7.8	7	11	(Nasmia dan Zakirah, 2012)

The characteristics of the silvofishery area on St 1 and St 2 which are close to residential areas are the disposal of household waste and sewage into one irrigation stream to the pond which allows for high levels of nutrients which also influence the high chlorophyll-a concentration values which also allows for increased carbohydrate content of Gracilaria sp. Apart from that, several silvofisheries in Brebes and Tegal Gracilaria sp. polyculture is carried out simultaneously with shrimp commodities [42]. It can be seen in (Figure 6) that the carbohydrate content in the west season is higher than in the transition season 2, this is thought to be due to the high chlorophyll-a content followed by high carbohydrates. This result is in line with [11] that the more chlorophyll is formed, the more optimal the photosynthesis process is, indicated by the high levels of carbohydrates produced.

St 3 and 4 are characterized by being close to residential areas, tourist areas and polyculture silvofishery areas with milkfish and soft shell crabs so the turbidity is higher. High turbidity is indicated by high TDS values while DO is low in both seasons which results in Gracilaria sp. difficult to absorb light to carry out photosynthesis. Overall, it can be seen that the chlorophyll-a concentration values for St 3 and 4 are lower than those for St 1 and 2 (Figure 4). However, the value of carbohydrate content at St 3 is the highest among the three other stations presented in (Figure 6).

It is suspected that the concentration of chlorophyll-a has decreased due to the photosynthesis phase when the light reaction occurs, while the high carbohydrate content produced in the dark reaction phase has reached its optimum. This is in line with research [43]. The carbohydrate content produced is a process resulting from the dark reaction, where in this dark reaction phase what actually works directly is chlorophyll-b.

3.3. Relationship between Chlorophyll-a Concentration and Carbohydrate Content of Gracilaria sp.

Results of data analysis carried out to test chlorophyll-a and carbohydrate content in Gracilaria sp. then compared using the Pearson correlation test. The results obtained from these tests are presented in Figure 7 and Figure 8.

Correlations

		Klorofil-a	Karbohidrat
Klorofil-a	Pearson Correlation	1	-.250
	Sig. (2-tailed)		.750
	N	4	4
Karbohidrat	Pearson Correlation	-.250	1
	Sig. (2-tailed)	.750	
	N	4	4

Picture 7. Pearson correlation test for chlorophyll-a with carbohydrate contentGracilaria sp. in transition season 2

Correlations

		Klorofil a	Karbohidrat
Klorofil a	Pearson Correlation	1	-.851
	Sig. (2-tailed)		.149
	N	4	4
Karbohidrat	Pearson Correlation	-.851	1
	Sig. (2-tailed)	.149	
	N	4	4

Picture 8.Pearson correlation test for chlorophyll-a with carbohydrate contentGracilaria sp. in the west season

The results obtained in (Figures 12 and 13) show that the correlation test results for the relationship between chlorophyll-a concentration and carbohydrate content in the transition season have a correlation of r=-0.250 and the significance value is 0.750. This value is according to [44] fall into the weak category. So it can be interpreted that the carbohydrate content can be influenced by the concentration of chlorophyll-a by 25% and by other factors by 75%. Furthermore, the results of the Pearson correlation test can be interpreted to mean that the values are inversely proportional to each other, that if the chlorophyll-a concentration is low then the carbohydrate content is high, and vice versa.
Likewise, the results obtained in the western season in the Pearson correlation test show a value of r=0.846 and a significance value of 0.154. This value is according to Nasution et al. [44] falls

into the strong category. So it can be interpreted that the carbohydrate content can be influenced by the concentration of chlorophyll-a by 84.6% and by other factors by 15.4%. Furthermore, the results of the Pearson correlation test can be interpreted to mean that the values are inversely proportional to each other, that if the chlorophyll-a concentration is low then the carbohydrate content is high, and vice versa.

Likewise, the results obtained in the western season in the Pearson correlation test show a value of r=0.846 and a significance value of 0.154. This value is according to Nasution et al. [44] falls into the strong category. So it can be interpreted that the carbohydrate content can be influenced by the concentration of chlorophyll-a by 84.6% and by other factors by 15.4%. Furthermore, the results of the Pearson correlation test can be interpreted to mean that the values are inversely proportional to each other, that if the chlorophyll-a concentration is low then the carbohydrate content is high, and vice versa.

It is suspected that the thallus of Gracilaria sp. has experienced a decreased ability to respond to photosynthetic activity due to the initial performance of chlorophyll-a being too high so it cannot produce optimal content [11]. Dewi et al. [11] states that what actually plays a direct role in the results of carbohydrates is chlorophyll-b because it will work when the dark reaction occurs by utilizing ATP and NADPH produced from the light reaction (chlorophyll-a).

In the light reaction, many chemical reactions take place which will ultimately produce O2 and also energy (ATP and NADPH) for the formation of C6H12O6 (carbohydrates) in the dark reaction. Gracilaria sp. undergo a growth process through the processes of respiration and photosynthesis as well as from the quality of the water and nutrients contained therein [45]. At certain concentrations, nutrients are not the main elements in the formation of carbohydrates so that carbohydrates as primary macromolecules will no longer be produced and continue with the biosynthesis of secondary metabolites including the formation of pigments [8].

4. Conclusions And Recommendations

4.1. Conclusion

The chlorophyll-a concentration value in Gracilaria sp in the silvofishery area of the north coast of Central Java, namely in the transition season 2, ranges from 1,801-2,320 µg/mL with the highest concentration in St 1 (2,320 µg/mL), while for the west season it ranges from 1,420-2,685 µg/mL. mL with the highest concentration in St 1 (2,685 µg/mL).

The value of carbohydrate content in Gracilaria sp in the silvofishery area of the north coast of Central Java, namely in the transition season 2, ranges from 37.088-57.135% with the highest content in St 3 (57.135%), while for the west season it ranges from 75.993-83.796% with the highest content in St 3 (83.796%).

The relationship between chlorophyll-a concentration and carbohydrate content in Gracilaria sp in the silvofishery area of the north coast of Central Java, namely in transition season 2, there is a weak relationship of r=-0.250 with a significance of 0.750. Meanwhile, in the west season there is a strong relationship of r=-0.846 with a significance of 0.154.

4.2. Suggestion

Further research can carry out specific analysis on the seaweed species Gracilaria sp, then also analyze the concentration of chlorophyll-b in Gracilaria sp and also add a more complete spatial and temporal approach, such as for research spanning 4 seasons.

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