Fisheries Journal, 15 (6), 3287-3301 (2025)
http://doi.org/10.29303/jp.v15i6.2007

RELATIONSHIP BETWEEN ECOLOGICAL FACTORS AND CAROTENOID CONTENT IN *Gracilaria* sp. IN THE SILVOFISHERY OF THE NORTH COAST OF CENTRAL JAVA

Keterkaitan Faktor Ekologis dengan Kandungan Karotenoid *Gracilaria* sp. Pada Silvofishery Pantai Utara Jawa Tengah

Swietenia Purnamasari*, Rose Dewi, Tjahjo Winanto

Marine Science Study Program, Jenderal Soedirman University

Jl. Dr. Soeparno, Komplek GOR Soesilo Soedarman, Purwokero, 531222 Indonesia

*Coresponding author: purnamasary.cms02@gmail.com

(Received December 8th 2025; Accepted December 28th 2025)

ABSTRACT

Gracilaria sp. is a seaweed with high economic value and is able to produce natural pigments in the form of carotenoids. The purpose of this study was to determine the value of the carotenoid content of *Gracilaria* sp., to determine the ecological conditions (physicochemical) of the water and to determine the relationship between these measurement variables. The method used was a survey method with a spatial approach (4 stations) and temporal (4 seasons). For the carotenoid variable, *Gracilaria* sp. was measured using a UV-Vis 1800V spectrophotometer. This study used Principal Component Analysis to determine the relationship between ecological factors and the carotenoid content of *Gracilaria* sp. The carotenoid content obtained spatially ranged from 14.03-31.10 mg/L and temporally 3.86-35.52 mg/L. The ecological conditions of the silvofishery area spatio-temporally have conditions that can be tolerated for the formation of carotenoids. The relationship between carotenoid content and ecological factors spatially produces an eigenvalue of 84.82% with F1 60.82% and F2 24.00%, with characteristics influenced by anthropogenic. Temporally produces an eigenvalue of 80.26% with F1 42.50% and F2 37.76%, with characteristics influenced by rainfall.

Keywords: Carotenoid, *Gracilaria* sp., Silvofishery

ABSTRAK

Gracilaria sp. merupakan rumput laut dengan nilai ekonomis tinggi dan mampu memproduksi pigmen alami berupa karotenoid. Tujuan dari penelitian ini yaitu mengetahui nilai kandungan karotenoid *Gracilaria* sp., mengetahui kondisi ekologis (fisika-kimia) air dan mengetahui keterkaitan antara variabel ukur tersebut. Metode yang digunakan yaitu metode survei dengan pendekatan spasial (4 stasiun) dan temporal (4 musim). Untuk variabel karotenoid, *Gracilaria* sp. diukur menggunakan spektrofotometer UV-Vis 1800V. Penelitian ini menggunakan *Principal Component Analysis* untuk mengetahui keterkaitan faktor ekologis dengan kandungan karotenoid *Gracilaria* sp. Kandungan karotenoid yang didapatkan secara spasial berkisar 14,03-31,10 mg/L dan temporal 3,86-35,52 mg/L. Kondisi ekologis area silvofishery secara spasio-temporal memiliki kondisi yang dapat ditoleransi untuk pembentukan karotenoid. Keterkaian antara kandungan karotenoid dengan faktor ekologis secara spasial menghasilkan *eigenvalue* sebesar 84,82% dengan F1 60,82% dan F2 24,00%, dengan karakteristik yang dipengaruhi oleh antropogenik. Secara temporal menghasilkan *eigenvalue* sebesar 80,26% dengan F1 42,50% dan F2 37,76%, dengan karakteristik yang dipengaruhi oleh curah hujan.

Kata Kunci: *Gracilaria* sp., Karotenoid, Silvofishery

INTRODUCTION

The North Coast of Java (Pantura) is a waterway located in the northern part of Java Island (Iskandar & Guntur, 2014). Central Java is the province on Java with the highest seaweed production. Seaweed-producing areas in Pantura include Tegal City, Brebes Regency, Pekalongan Regency, and Pemalang Regency. Brebes is the largest seaweed-producing region in Central Java. Seaweed cultivation in the Pantura region is often carried out using silvofishery pond methods. According to Tenriawaruwaty *et al*., (2021), silvofishery, or Wanamina, is an activity that combines mangrove planting and pond fisheries cultivation. Mangrove roots provide high nutritional value and act as water filters, maintaining water quality for fisheries cultivation (Wijaya *et al.,* 2019). *Gracilaria* sp. is a red seaweed that contains gel and has a high water-holding capacity. Through its secondary metabolism, *Gracilaria* sp. is able to produce natural pigments in the form of carotenoids.

Carotenoids are one of the accessory pigments of *Gracilaria* sp. which has the ability to protect chlorophyll-a from damage (Febriani *et al.,* 2022). Carotenoids in seaweed are formed from carotenoid biosynthesis. Seaweed can experience stress due to low light intensity, which can cause a lack of chlorophyll-a to absorb light in the photosynthesis process (Santoso & Nugraha, 2008). When the ability of chlorophyll-a is reduced, carotenoids are responsible for providing energy for chlorophyll-a. High carotenoids can indicate a lack of light needed by chlorophyll-a during the photosynthesis process. The quality of *Gracilaria* sp. carotenoids can be influenced by ecological factors in the silvofishery area. Ecological factors (physicochemical) of water spatio-temporally have different characteristics, so they can affect the quality of *Gracilaria* sp. carotenoid content. This can certainly affect the utilization of *Gracilaria* sp. carotenoid content because the resulting carotenoid content value is not optimal. Therefore, this study is necessary to determine the relationship between ecological factors and the carotenoid content of *Gracilaria* sp. seaweed.

METHODS

This study used primary and secondary data. Secondary data consisted of rainfall data taken from the BMKG database, and depth data for the silvofishery area was taken from satellite imagery. Primary data consisted of *Gracilaria* sp. seaweed samples and ecological parameters. The research location was determined based on the presence of seaweed and silvofishery ponds. The research stations were determined using a purposive sampling technique. *Gracilaria* sp. samples were collected using simple random sampling.

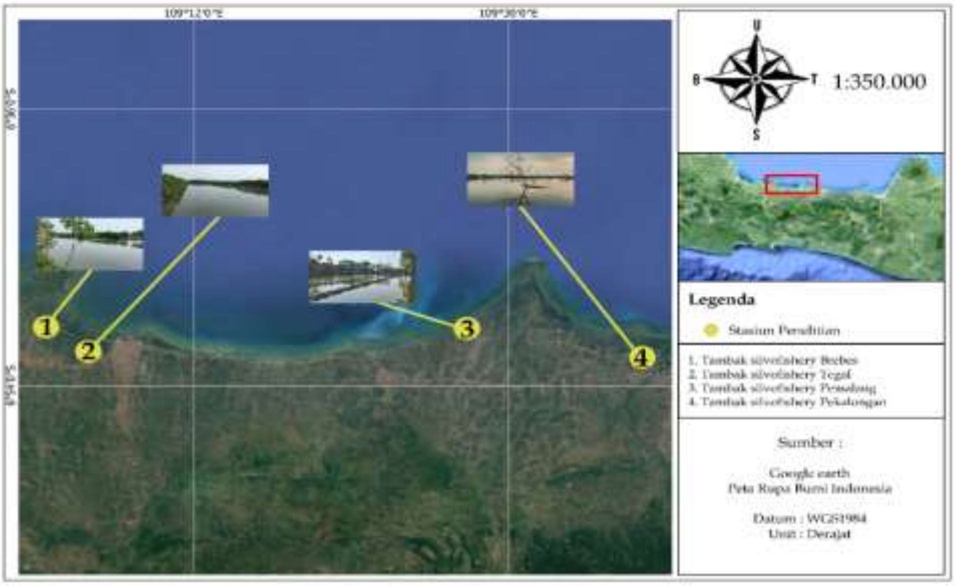

Figure 1. Research Location Determination

Gracilaria sp. sampling was conducted spatially at four stations: Station 1 at the Brebes silvofishery pond, Station 2 at the Tegal silvofishery pond, Station 3 at the Pemalang silvofishery pond, and Station 4 at the Pekalongan silvofishery pond. Temporarily, the sampling took place during the second transition season (October 2022), the west season (February 2023), the first transition season (May 2023), and the east season (August 2023). Sampling was conducted with three repetitions at each station.

The tools used for sampling included a water quality checker, 1-liter sample bottles, sample plastic, a cool box, a digital camera, and a GPS. Equipment for the carotenoid content test and total phosphate and nitrogen tests included a mortar, an 1800V UV-Vis spectrophotometer, an analytical balance, filter paper, a dropper, an Erlenmeyer flask, a boiling flask, an electric stove, a Kjeldahl flask, a dark bottle, a distillation apparatus, a digital camera, Microsoft Excel software, XLStat software, and QGIS software. The materials used included *Gracilaria* sp. samples, seawater samples, distilled water, 90% acetone, concentrated H2SO4 solution, 30% NaOH, 4% boric acid, Tashiro indicator, standard HCl, selenium powder, dye reagent, and standard phosphate solution.

Sampling

***Gracilaria* sp. Sampling**

5 g sample of *Gracilaria* sp. was taken and washed with fresh water to remove any dirt. The sample was then placed in a plastic bag and stored in a cool box. The *Gracilaria* sp. sample was taken to the laboratory and stored in a cooler at a temperature below -18°C (Farid *et al.,* 2013).

Ecological Parameter Collection

Ecological (physicochemical) water parameters were collected in situ and ex situ. In situ water parameter data collection was conducted to determine temperature, salinity, pH, DO, and TDS. Ex situ water parameter data observation was conducted to determine nitrogen and phosphate content, necessitating water sampling. Water sampling was carried out by taking a 1 liter sample of pond water and placing it in a sample bottle (Ahdiaty & Fitriana, 2020).

Testing Method

Carotenoid Content Test

1 g sample of *Gracilaria* sp. was weighed and then ground using a mortar. The ground sample was added to 50 ml of 90% acetone solution. Then, it was filtered using Whatman No. 42 filter paper into a dark bottle. The mortar was rinsed with 50 ml of 90% acetone solution and returned to the same bottle. The sample was then tested using a 1800 V UV-Vis spectrophotometer. To determine the sample's absorbance, the wavelength used was 470 nm. Total carotenoids were determined by measuring the sample at a wavelength of 470 nm (Thaipong *et al.,* 2006).

Total Nitrogen Test

The total nitrogen test for water used the Kjeldahl method (Legowo & Nurwanoro, 2004). The first stage is the digestion stage. The seawater sample was heated in concentrated sulfuric acid to break down into its constituents. 10 ml of pond water was placed in a Kjeldahl flask, then 5 ml of concentrated sulfuric acid and 0.25 g of selenium powder were added. The next process is distillation, this process aims to separate the desired substance, namely breaking down ammonium sulfate $(NH_4)_2SO_4$ into ammonia (NH_3) by adding NaOH and then heating. The function of adding NaOH is to provide a basic atmosphere because the reaction cannot take place in acid. The released ammonia will then be captured by a standard acid solution. The standard acid that can be used is a 4% boric acid solution (H_3BO_3) which has previously been mixed with a tashiro indicator. The distillation ends when the distillate is blue. Boric acid that reacts with ammonia can be identified by titration. The sample is then titrated using 0.1 N hydrochloric acid, the end of the titration is marked by a change in the color of the solution from blue to pink (Legowo & Nurwanoro, 2004).

Total Phosphate Test

The total phosphate test for water was performed using the digestion method. First, 100 ml of seawater and 10 ml of 25% HCl were placed in a 250 ml Erlenmeyer flask. The mixture was then homogenized by gently shaking the flask for 30 minutes. The solution was then allowed to stand until clear. A standard phosphorus solution was prepared by diluting a 1000 ppm standard phosphorus solution to 500 ppm. Pipette 1 ml of each sample extract into a beaker tube. Add 9 ml of distilled water and shake 20 times. Pipette 2 ml of each diluted sample extract and add 10 ml of dye reagent P, shake 20 times, and let stand for 1 hour. The next step was to measure the absorbance using a UV-Vis spectrophotometer at a wavelength of 693 nm (Umaternate *et al.,* 2014).

Data Analysis

The data analysis used in this study included comparative descriptive analysis and quantitative analysis. The data are presented in tabular form and discussed descriptively. To determine the relationship between ecological (physicochemical) factors in water and the carotenoid content of *Gracilaria* sp., the researchers used Principal Component Analysis (PCA). This study used calculations for carotenoid content, total water nitrogen, and total water phosphate.

1. Calculation of Carotenoid Content Values according to Hendriyani *et al.,* (2018):

$$\text{Carotenoid (mg/L)} = \frac{(A470 + (0{,}114 \text{ x } A663) - (0{,}638 \text{ x } A645)) \text{x V x } 1000}{112{,}5 \text{ x } 0{,}1 \text{ x } 10} \text{ x } 27.25 \text{ mg/L}$$

Where:
A_{663} = Absorbance of wave length 663 nm
A_{646} = Absorbance of wave length 646 nm
A_{470} = Absorbance of wave length 470 nm
V = Extract volume (ml)
1 µmol/L = 27.25 mg/L

2. Calculation of total nitrogen in water according to APHA (2017):

$$N \text{ value (mg/L)} = \frac{y-a}{b}$$

Where:
y = sample absorbance
a = intercept in the linear equation
b = slope in the linear equation

3. Calculation of total phosphate in water according to Angraini & Yanti (2021):

$$P \text{ value (mg/L)} = C \text{ x } fp$$

Where:
C = phosphate content from instrument measurement results (mg/L)
Fp = dilution factor

Solution dilution formula according to Supriyantini *et al*., (2018):

$$V_1 \text{ x } N_1 = V_2 \text{ x } N_2$$

Where:
V_1 = Stock solution volume (ml)
N_1 = Stock solution concentration (ppm)
V_2 = Treatment test solution volume (ml)
N_2 = Treatment concentration (ppm)

RESULTS

Spatial Carotenoid Content

The results of this study, using a spatial approach, show fluctuations at each station. The highest average content was at Brebes station at 31.10 mg/L, and the lowest at Pemalang station at 14.03 mg/L. Tegal station had the second-highest carotenoid content after Brebes station at 20.84 mg/L.

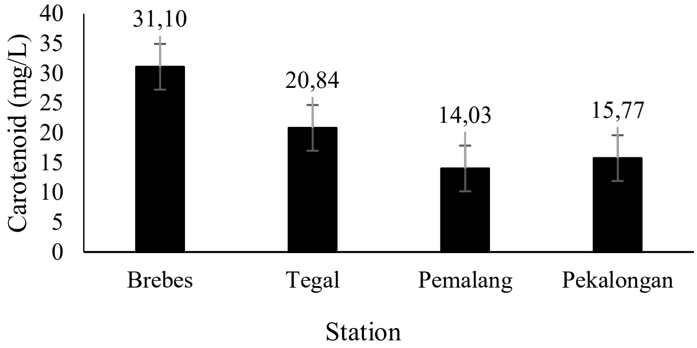

Figure 2. Average Carotenoid Content of *Gracilaria* sp. Spatially

Temporal Carotenoid Content

The average content was highest during the west monsoon at 33.52 mg/L, and lowest during the east monsoon at 3.86 mg/L. The high carotenoid content during the west monsoon is thought to be due to high rainfall. High rainfall can carry nutrients into the water, resulting in nutrient enrichment (Yuniarti *et al.,* 2022).

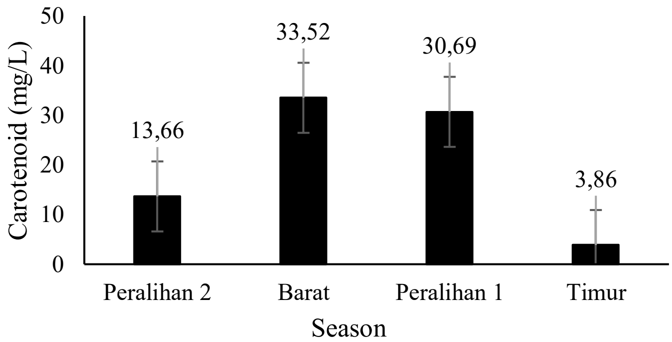

Figure 3. Average Carotenoid Content of *Gracilaria* sp. Temporally

Ecological (Physico-Chemical) Water Factors Spatial

The highest pH range was at Tegal Station, ranging from 7.84 to 8.91, and the lowest was at Brebes Station, ranging from 7.03 to 7.74. Based on SNI (2011), the optimum pH range for seaweed growth is 7.0 to 8.6. The highest salinity range was at Brebes Station, at 24.5 to 34 ppt, and the lowest was at Pemalang Station, at 17 to 28 ppt. Based on SNI (2011), the optimum salinity range for seaweed cultivation is 28 to 34 ppt.

Table 1. Average Spatial Ecological (Physico-Chemical) Water Factors in the Silvofishery Area on the North Coast of Central Java

Parameter	Brebes	Tegal	Pemalang	Pekalongan
pH	7.03-7.74	7.84-8.91	7.02-8.38	6.98-8.02
Salinity (ppt)	24.5-34	18-32	17-28	20-32
Temperature (°C)	25-32	27-33	27.6-34.5	31.3-33.9
TDS (mg/L)	3.62-10.00	2.4-7.94	2.36-7.48	3.2-11
DO (mg /L)	2.6-12.56	5.8-15.4	4.55-13.36	5.85-15.11
TN (mg /L)	0.42-5.37	0.17-4.48	0.17-4.59	0.70-3.29
TP (mg /L)	1.78-4.34	2.26-7.18	1.75-4.45	2.11-7.52
Rainfall (mm)	1.09-27.07	0.15-26.19	0.15-26.19	0.15-26.19
Depth (m)	0.84-1.23	0.77-1.15	0.50-0.75	0.59-0.86

Temporal Ecological (Physico-Chemical) Water Factors

The highest pH range is during the second transitional season, ranging from 7.3 to 8.4, and the lowest is during the east season, ranging from 7.0 to 7.9. According to SNI (2011), the optimum pH range for seaweed growth is 7.0 to 8.6. The highest salinity range is during the east season, ranging from 28 to 34 ppt, and the lowest is during the first transitional season, ranging from 17 to 25 ppt. According to SNI (2011), the optimum salinity range for seaweed growth is 28 to 34 ppt.

Table 2. Average Temporal Ecological (Physico-Chemical) Water Factors in the Silvofishery Area on the North Coast of Central Java

Parameter	Transition 2	West	Transition 1	East
pH	7.6-8.9	7.0-7.9	7.3-8.4	7.0-7.9
Salinity (ppt)	18-34	18-32	17-25	28-34
Temperature (°C)	31.5-34	28.2-31.5	30-34	25-33.5
TDS (mg/L)	2.36-3.84	6.74-11.10	5.91-5.95	7.04-11.03
DO (mg /L)	11.91-15.36	2.82-6.72	7.17-12.33	3.67-6.60
TN (mg /L)	2.78-5.77	3.29-4.62	0.18-1.05	0.28-0.77
TP (mg /L)	1.76-2.92	1.78-2.50	4.34-7.52	4.34-7.52
Rainfall (mm)	6.33-12.51	16.88-27.07	2.92-8.92	0.15-5.71
Depth (m)	0.51-0.85	0.75-1.23	0.63-1.00	0.50-0.73

Spatial Relationship of Ecological Factors with Carotenoid Content of *Gracilaria* sp.

The parameters in the factors generated by the PCA analysis were determined based on the largest factor loadings (marked in bold). The variable characteristics formed in F1 contributed 60.82% of the total variance (Table 3). The F1 factor was constructed by carotenoids, pH, salinity, temperature, DO, TN, rainfall, and depth. The F2 factor contributed 24.00% of the total variance (Table 3), constructed by pH, salinity, and TDS. The F2 factor contributed 100% of the total variance (Table 3).

Table 3. Factor Loading, Variability, Cumulative Variance, and Factors Formed from Ecological Parameters in the North Coast of Central Java Silvofishery Area Spatially Based on Research Stations

Parameter	Factor		
	F1	F2	F3
Carotenoid	**-0.978**	-0.006	0.210
pH	0.642	**-0.708**	0.294
Salinity	**-0.704**	0.700	0.123
Temperature	**0.913**	0.403	-0.062
TDS	0.208	**0.866**	0.454
DO	**0.989**	-0.137	0.056
TN	**-0.809**	-0.574	0.128
TP	0.545	0.081	**0.834**
Rainfall	**-0.973**	0.202	-0.112
Depth	**-0.682**	-0.317	0.659
Eigenvalue	6.082	2.400	1.518
Variability (%)	**60.821**	**24.003**	15.176
Cumulative %	60.821	**84.824**	100.000

Description:
- Processed from primary and secondary research data
- Bold numbers indicate that the variable is the main factor in F1 and F2

The biplot from the PCA analysis using a spatial approach shows the relationship between ecological factors and the carotenoid content of *Gracilaria* sp., divided into four quadrants (Figure 4). Based on the proximity in the biplot, Brebes station is closely related to the carotenoid content of *Gracilaria* sp., along with the parameters of salinity, rainfall, depth, and TN. The carotenoid content at Brebes station is high. This indicates that the high carotenoid content at Brebes station is influenced by the parameters of salinity, rainfall, depth, and TN (Figure 4).

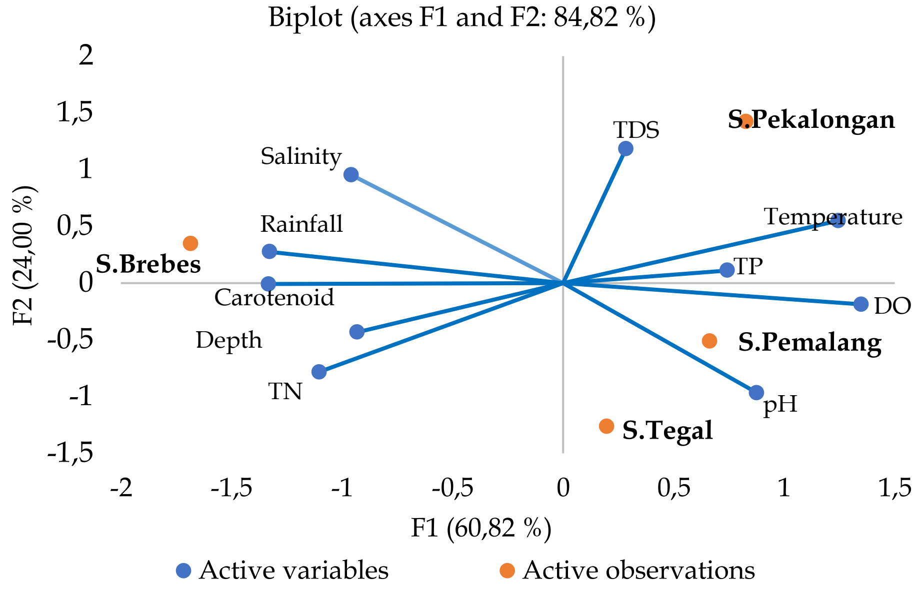

Figure 4. Biplot of Principal Component Analysis Results Showing the Relationship Between Ecological Factors and Carotenoid Content Using a Spatial Approach in the Silvofishery Area of the North Coast of Central Java

Description:

- Processed from primary and secondary data
- Ecological parameters (blue dots) and research stations (orange dots)
- S for station
- Parameters in the red circle are related to carotenoid content

Temporal Relationship of Ecological Factors with Carotenoid Content of *Gracilaria* sp.

The parameters in the factors generated by the PCA analysis were determined based on the largest factor loadings (marked in bold black). The variable characteristics formed in F1 contributed 42.50% of the total variance (Table 4), which was constructed by pH, temperature, TDS, and DO. Factor F2 contributed 37.76% of the total variance (Table 4), which was constructed by carotenoids, TN, TP, rainfall, and depth.

Table 4. Factor Loading, Variability, Cumulative Variance and Factors Formed from the Ecological Parameters of the North Coast of Central Java Silvofishery Area Temporally Based on the Research Season

Parameter	Factor		
	F1	**F2**	**F3**
Carotenoid	0.171	**0.825**	-0.538
pH	**0.957**	-0.285	-0.046
Salinity	-0.525	-0.426	**0.737**
Temperature	**0.866**	-0.188	-0.464
TDS	**-0.926**	0.279	-0.256
DO	**0.942**	-0.327	0.069
TN	0.527	0.589	**0.612**
TP	-0.505	-0.609	**-0.611**
Rainfall	-0.011	**0.971**	0.239
Depth	0.030	**0.975**	-0.219
Eigenvalue	4.250	3.776	1.974
Variability (%)	**42.496**	**37.764**	19.740
Cumulative %	42.496	**80.260**	100.000

Description:

- Processed from primary and secondary research data
- Bold numbers indicate that the variable is the main factor comprising F1 and F2

The ecological factor correlations from the PCA analysis were dominated by two main factors, F1 and F2, which yielded an eigenvalue of 80.26% (Table 4). This value indicates that the information obtained was 80.26% of the total information. This means that F1 contributed 42.50% of the information, and F2 contributed 37.76%. The correlation between ecological factors and carotenoid content in *Gracilaria* sp. using a temporal approach (Figure 5). The biplot presented is composed of two axes, F1 and F2.

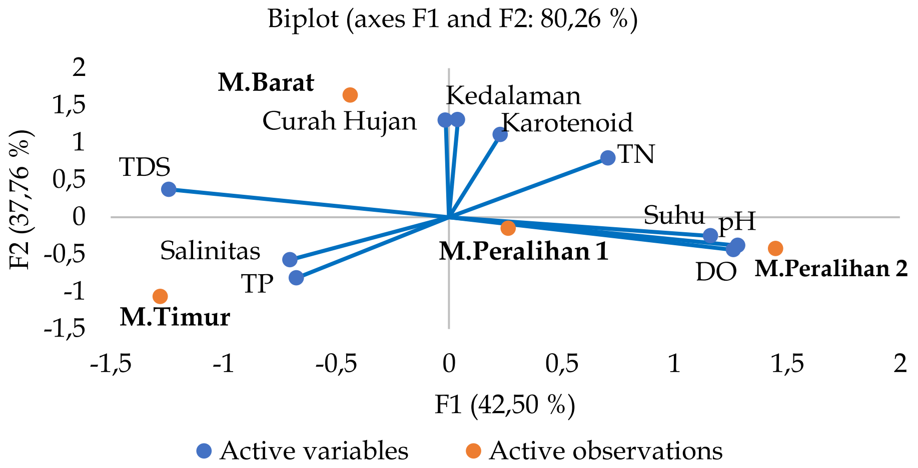

Figure 5. Biplot of Principal Component Analysis Results Showing the Relationship Between Ecological Factors and Carotenoid Content Using a Temporal Approach in the Silvofishery Area on the North Coast of Central Java

Description:

- Processed from primary and secondary data
- Ecological parameters (blue dots) and research stations (orange dots)
- M for season
- Parameters in the red circle are related to carotenoid content

DISCUSSION

Carotenoid Content

High carotenoid content is influenced by several factors, one of which is nutrients. Nutrients play a crucial role in photosynthesis, contributing to the formation of chlorophyll in seaweed metabolism. Seaweed requires nutrients to support photosynthesis. The lowest average carotenoid content occurs during the east monsoon. This is thought to be due to low rainfall, which tends to reduce the availability of nutrients in the water. The low carotenoid content during the east monsoon is thought to be due to photoinhibition caused by low rainfall. Photoinhibition is the inhibition of photosynthesis caused by excessively high light intensity (Booy *et al.,* 2019). In photosynthesis, carotenoids act as accessory pigments and triplet quenchers, protecting photosynthetic components from photooxidative damage (Merdekawati *et al.,* 2017).

Spatial Ecological (Physico-Chemical) Water Factors

Low salinity at Pemalang Station results in low carotenoid content in *Gracilaria* sp. The highest temperature was at Pekalongan Station, ranging from 31.33-33.9°C, and the lowest was at Brebes Station, ranging from 25-32°C. According to SNI (2011), the optimum temperature range for seaweed growth is 26-31°C. The highest Total Dissolved Solids (TDS) was at Tegal Station, at 2.4-7.94 mg/L, and the lowest was at Pemalang Station, at 2.36-7.48 mg/L. The optimum TDS value for seaweed physiological processes ranges from 10.22-45.15 mg/L (Nasmia & Raihani, 2012). Dissolved Oxygen (DO) was highest at Tegal station, ranging from 5.8 to 15.4 mg/L, and lowest at Brebes station, ranging from 2.6 to 12.56 mg/L. According to Mudeng *et al*., (2015), the optimum DO value for seaweed growth is more than 6 mg/L. According to Dewa (2016), DO can be used as an indicator of water freshness and plays a crucial role in water quality. This is because DO plays a role in the oxidation and reduction of organic matter. Oxygen oxidizes organic and inorganic matter under aerobic conditions, producing nutrients that contribute to water fertility (Rahman, 2019).

Total Nitrogen (TN) was highest at Brebes station, ranging from 0.42 to 5.37 mg/L, and lowest at Pekalongan station, ranging from 0.70 to 3.29 mg/L. This range is still tolerable and supports the growth of *Gracilaria* sp. This is in accordance with the opinion of Patahiruddin (2010), the optimum range of TN which is good for seaweed growth is around 2.2-2.9 mg/L. The highest average Total Phosphate (TP) value is at Pekalongan station ranging from 2.11-7.52 mg/L and the lowest is at Brebes station ranging from 1.78-4.34 mg/L. According to Patahiruddin (2010), the optimum range of TP which is good for seaweed growth is around 0.09-1.80 mg/L. High phosphate values can supply the phosphate needs for the growth of *Gracilaria* sp. (Patahiruddin, 2010). The highest rainfall is at Brebes station ranging from 1.09-27.07 mm. Sufficiently high rainfall can bring nutrient content into the water, resulting in nutrient enrichment (Yuniarti *et al.,* 2022). Based on the research results of Afandi & Syam (2020), nitrate levels are quite good during the rainy season, this is due to nutrient input through rain. The highest depth is at Brebes station, ranging from 0.84-1.23 m, and the lowest is at Pemalang station, ranging from 0.50-0.75 m. According to Priono (2013), the optimum depth range for seaweed cultivation is 0.6-2.1 m. The depth of the pond can affect the intensity of light entering the water; the closer it is to the water surface, the higher the light intensity.

Temporal Ecological (Physico-Chemical) Water Factors

The highest temperature range is during transition season 1, at 30-34°C, and the lowest is during the east season, at 25-33.5°C. The highest Total Dissolved Solids (TDS) range is during the east season, ranging from 7.04-11.03 mg/L, and the lowest is during transition season 2, at 2.36-3.84 mg/L. Based on research by Dewa (2016), the lower the TDS value, the better it is for seaweed cultivation. The highest Dissolved Oxygen (DO) range is during transition season 2, at 11.91-15.36 mg/L, and the lowest is during the west season, at 2.82-6.72 mg/L. The highest Total Nitrogen (TN) range is during transition season 2, at 2.78-5.77 mg/L, and the lowest is during the east season, at 0.28-0.77 mg/L. The low total nitrogen content in the east monsoon is thought to be due to low rainfall. Supported by research by Yuniarti *et al.*, (2022), nutrient content in the rainy season is higher than in the dry season. The highest Total Phosphate (TP) values are in Transition Season 1 and the east monsoon, ranging from 4.34 to 7.52 mg/L, while the lowest are in the west monsoon, ranging from 1.78 to 2.50 mg/L. High phosphate values can supply the phosphate needs for the growth of *Gracilaria* sp. (Patahiruddin, 2010). The highest rainfall values are in the west monsoon, ranging from 16.88 to 27.07 mm, and the lowest are in the east monsoon, ranging from 0.15 to 5.71 mm. High rainfall can carry nutrient content into the water, resulting in nutrient enrichment (Yuniarti *et al.,* 2022). The highest depth is in the west season, ranging from 0.75-1.23 m and the lowest is in the east season, ranging from 0.50-0.73 m.

The Relationship between Ecological Factors and the Carotenoid Content of *Gracilaria* sp. Spatially

The relationship between ecological factors from the PCA analysis results was dominated by two main factors, F1 and F2, which produced an eigenvalue of 84.82% (Table 3). This value indicates that the information obtained was 84.82% of the total information. This means that F1 contributed 60.82% of the information, and F2 contributed 24.00%. The relationship between ecological factors and the carotenoid content of *Gracilaria* sp. in the silvofishery area is illustrated by a biplot (Figure 5) composed of two axes, F1 and F2. Salinity at the Brebes station ranged from 24.5 to 34 ppt, which is optimal for the physiological processes of *Gracilaria* sp. According to Yuliana *et al.,* (2015), salinity is a chemical factor that can affect the physical properties of water, including the osmotic pressure present in seaweed. This balance will create favorable conditions for nutrient absorption as a nutrient for photosynthesis. Excessively high salinity can cause osmotic stress, which can inhibit the absorption of water and nutrients through osmosis.

Brebes Station has relatively high rainfall compared to other stations, ranging from 1.09 to 27.07 mm. High rainfall can affect salinity, nutrient uptake, and light penetration into the water column. Research by Rema *et al.,* (2019) found that high rainfall can reduce water salinity, while low rainfall can increase salinity. Brebes Station has a high TN water content compared to other stations, ranging from 0.42 to 5.37 mg/L. The high TN value at Brebes Station is thought to be due to high rainfall. This is supported by research by Yuniarti *et al.,* (2022) that high rainfall can carry nutrients such as TN into the water, resulting in nutrient enrichment. Nutrients are needed by seaweed to support photosynthesis. Nutrients are a factor that can increase chlorophyll-a content (Cokrowati & Diniarti, 2019). Research by Minsas *et al.,* (2023) supports this finding, stating that carotenoid content in seaweed increases directly with chlorophyll-a content.

Brebes Station has a depth ranging from 0.84 to 1.23 m, which is optimal for seaweed physiological processes. Pond depth can affect light penetration. According to Astriana *et al.*, (2019), pond depth can affect the intensity of light entering the water; the closer the water surface is, the higher the light intensity. The intensity of light entering the water affects photosynthesis, where chlorophyll-a absorbs light for seaweed metabolic processes. Carotenoid content is also influenced by temperature and DO. Temperature plays a crucial role in seaweed physiological processes. According to research by Rachmawati and Abdillah (2019), high temperatures cause the seaweed thallus to turn pale yellowish, which hinders proper seaweed growth. The highest temperature range in this study was at Pekalongan station, ranging from 31.33 to 33.9°C. This range is still tolerable for seaweed. Supported by research by Amiluddin (2007), seaweed thallus will turn yellow and die at a temperature of 35°C. DO can be used as an indicator of freshness in water. In this study, the highest DO range was found at Tegal station, ranging from 5.8 to 15.4 mg/L. DO is closely related to the processes of respiration and photosynthesis, where respiration produces CO_2 and photosynthesis requires CO_2 (Effendi, 2003).

The Relationship of Ecological Factors with the Carotenoid Content of *Gracilaria* sp. Temporally

The relationship between ecological factors from the PCA analysis was dominated by two main factors, F1 and F2, which produced an eigenvalue of 80.26% (Table 4). This value indicates that the information obtained was 80.26% of the total information. This means that F1 contributed 42.50% of the information, and F2 contributed 37.76%. The relationship between ecological factors and the carotenoid content of *Gracilaria* sp. was demonstrated through a temporal approach. The biplot presented was composed of two axes, F1 and F2. The biplot from the PCA analysis using a temporal approach showed the relationship between ecological factors and the carotenoid content of *Gracilaria* sp., divided into four quadrants. Based on the proximity in the biplot, the west monsoon season was closely related to the carotenoid content of *Gracilaria* sp., along with the parameters of rainfall, depth, and TN. The carotenoid content of the west monsoon season was higher compared to other seasons. This indicates that the high carotenoid content in the west monsoon is influenced by rainfall, depth, and TN.

The highest rainfall ranges from 16.88 to 27.07 mm during the west monsoon. According to Yuniarti *et al.,* (2022), February (the west monsoon) is characterized by high rainfall. High rainfall during the west monsoon affects nutrient input into the water. Nutrients are essential for seaweed to support photosynthesis. TN values during the west monsoon range from 3.29 to 4.62 mg/L. Compared to the east monsoon, the west monsoon has higher TN values. Research by Yuniarti *et al.,* (2022) supports this finding, stating that nitrate levels are highest during the rainy season and lowest during the dry season.

Nitrate (NO_3-) is the primary form of nitrogen in natural waters. Nitrate is a nutrient compound essential for animal and plant protein synthesis (Ernawati & Restu, 2021). According to Minsas *et al.,* (2023) Nitrate is one of the nutrient contents that can increase chlorophyll-a content. Carotenoid content in seaweed can develop directly with the development of chlorophyll-a content. TN values can be affected by pond depth. According to Raymont (1967), nitrate will increase at a certain depth. Carotenoid content is also affected by pond depth. The west season has a pond depth of 0.75-1.23 m. Pond depth can affect the intensity of light entering the water, the closer to the water surface, the higher the light intensity obtained. According to Astriana *et al.,* (2019) the intensity of light entering the water will affect the photosynthesis process, where chlorophyll-a will absorb light for the seaweed metabolic process.

CONCLUSION

The results of this study are generally in accordance with the expected objectives and it can be concluded that the average value of carotenoid content obtained through a spatial approach is at Brebes station of 31.10 mg/L, Tegal station of 20.84 mg/L, Pekalongan station of 15.77 mg/L, and Pemalang station of 14.03 mg/L. The average value of carotenoid content obtained through a temporal approach is in the west season of 33.52 mg/L, transition season 1 of 30.69 mg/L, transition season 2 of 13.66 mg/L, and east season of 3.86 Mg/L. The ecological (physico-chemical) conditions of water in the silvofishery area of the north coast of Central Java through a spatio-temporal approach have ecological conditions that can be tolerated for the formation of *Gracilaria* sp. carotenoid content. Ecological factors of the silvofishery area with *Gracilaria* sp. carotenoid content. Spatially, it produces an eigenvalue of 84.82% with F1 of 60.82% and F2 of 24.00%. It produces the proximity of Brebes station to the carotenoid content of *Gracilaria* sp. accompanied by the parameters of salinity, rainfall, depth, and TN. Ecological factors of the silvofishery area with the carotenoid content of *Gracilaria* sp. Temporally, it produces an eigenvalue of 80.26% with F1 of 42.50% and F2 of 37.76%. It produces the proximity of the west season to the carotenoid content of *Gracilaria* sp. accompanied by the parameters of rainfall, depth, and TN.

ACKNOWLEDGEMENT

The author would like to express his gratitude to all parties who played a vital role in the preparation of this research. He hopes that this research will contribute both academically and practically to the utilization of *Gracilaria* sp. seaweed.

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