#### RESEARCH



# Sea grape (*Caulerpa lentillifera*) aquaculture in Van Phong Bay, Viet Nam: Evaluation of the post-harvest quality

Lara Elisabeth Stuthmann<sup>1</sup> · Hoang Trung Du<sup>2</sup> · Beatrice Brix da Costa<sup>1,3</sup> · Andreas Kunzmann<sup>1</sup> · Karin Springer<sup>3</sup>

Received: 29 March 2023 / Revised: 27 May 2023 / Accepted: 15 June 2023 © The Author(s) 2023

#### Abstract

*Caulerpa lentillifera*, known as `sea grapes' or `green caviar', is increasingly in demand as a sea vegetable for human consumption. The seaweed is cultivated in ponds in the Khánh Hòa province in Van Phong Bay, Viet Nam, during the dry season (March-October). The harvested sea grape fronds are graded into different qualities based on their physical characteristics for retail on the local market or for export. Based on systematic observations of sea grape fronds of two different qualities, the frond weight, frond length and rachis coloration were identified as physical characteristics important for grading. Fronds of the best quality had significantly longer  $(12.59 \pm 2.89 \text{ vs } 10.01 \pm 2.51 \text{ cm})$  and heavier  $(2.37 \pm 0.59 \text{ vs } 1.60 \pm 0.5 \text{ g})$  fronds with darker rachis than the other quality group. However, a logistic regression model revealed that frond weight was the best predictor of frond quality. The physiological parameter of  $F_v/F_m$  was slightly different between the qualities, but always with means > 0.7, whereas the antioxidant activity and the total phenolic content were similar (98.34 ± 19.22 vs 95.96 ± 24.98 mmol TE (100 g)<sup>-1</sup> DW and 163.8 ± 20.14 vs 149.85 ± 15.44 mg GAE (100 g)<sup>-1</sup> DW). To the best of our knowledge, this study took a first approach to identify quality characteristics of sea grape fronds from Van Phong Bay, Viet Nam, which can serve as a basis for adjusting cultivation parameters to improve the harvest quality by developing cultivation and post-harvest protocols. However, further research is needed to investigate the effect of certain cultivation parameters on the specific frond characteristics.

Keywords Chlorophyta · Macroalga · Phycoculture · Sustainable Food Production · Coloration · Post-Harvest Protocol

# Introduction

*Caulerpa lentillifera* J.Agardh is a green seaweed of the order Bryopsidales (Guiry and Guiry 2023) and known as a delicacy by the names `sea grapes' or `green caviar'. Sea grapes have traditionally been harvested, cultivated, and eaten in The Philippines and Okinawa in Japan (Trono and Toma 1993; Yap 1999). However, the interest in this sea vegetable has increased due to, among other things, its

- <sup>1</sup> Leibniz Centre for Tropical Marine Research, Fahrenheitstraße 6, 28359 Bremen, Germany
- <sup>2</sup> Institute of Oceanography, Vietnam Academy of Science and Technology, 01, Cau Da, Nha Trang, Khánh Hòa, Vietnam
- <sup>3</sup> Marine Botany, Faculty Biology/Chemistry, University of Bremen, BIOM, James-Watt-Straße 1, 28359 Bremen, Germany

nutritional benefits (reviewed by Syakilla et al. 2022) and the special texture of the fronds, which consist of small grape-like ramuli arranged around a central axis, called rachis (de Gaillande et al. 2017; Zubia et al. 2020). Aquaculture of C. lentillifera has also taken off in other countries in the Indo-Pacific region, including Viet Nam. Here, a long coastline with bays and lagoons provides various opportunities for aquaculture. It is therefore not surprising that Viet Nam ranks among the top five marine aquaculture producers for molluscs and crustaceans, and among the top seven for finfish (FAO 2022). The Khánh Hòa province is located in the Central South of the country and a recent hot spot for sea grape cultivation (So 2022). However, global production estimates of C. lentillifera are likely underestimated, and reports are limited to the Philippines (Cai et al. 2021a). Local news media from the Khánh Hòa province report production estimates of > 400 t year<sup>-1</sup> at an area of ~ 50 ha for 2020 (Son 2022). The sea grapes are cultivated in tidal ponds (Stuthmann et al. 2020) using the

Lara Elisabeth Stuthmann lara.stuthmann@leibniz-zmt.de

sowing or tray method (Rabia 2016), especially for export to countries such as Japan (Terada et al. 2018). However, sea grapes are also sold on local markets, supermarkets and served in restaurants. Biomass below the quality requirements for food use is discarded, which has been reported to be up to  $\sim 60-70\%$  of the total biomass in Thailand (Chaiklahan et al. 2020). The farmers grade the frond quality based on physical characteristics, including weight, length, number of branches, ramuli density, as well as coloration (Chaiklahan et al. 2020).

Caulerpa is a genus known for its morphological plasticity as response to environmental changes (Estrada et al. 2020). Changes of environmental parameters over seasons are common in the Indo-Pacific region and they are known to restrict or impact sea grape cultivation (Wichachucherd et al. 2019; Terada et al. 2021). The seaweed cultivation in the Khánh Hòa province is restricted to the dry season, since C. lentillifera is particularly sensitive to decreasing salinity (Guo et al. 2015a). Exposure of sea grapes to different stressors, including temperature, salinity, nutrient concentrations or PAR irradiances leads to changes in chlorophyll a, b and carotenoid composition, color, and stolon:frond composition of the thallus (Guo et al. 2015a, b; Cai et al. 2021b; Stuthmann et al. 2022). Biochemical parameters, including the antioxidant activity (AOA) or total phenolic content (TPC) and chlorophyll a fluorescence parameters are also expected to change in response to environmental parameters (Wichachucherd et al. 2019; Zhang et al. 2020; Cai et al. 2021b; Stuthmann et al. 2022), although this may not be visible.

Hence, the quality of the sea grapes might vary between harvests during the season. The average weight and length of preserved sea grape fronds from a Vietnamese company were reported to be  $0.73 \pm 0.18$  g and  $7.27 \pm 1.59$  cm, with a significant positive correlation between both parameters (Lapong et al. 2019). The sea grape fronds are usually graded directly on site based on experience of the workers. There has been an attempt to automatize the frond grading based on photographs and using a deep learning model. The authors of the study used a circular high transform method to detect the shape of the ramuli and the appearance was grouped into feature, shape, color, and compactness (Chinnasarn et al. 2022). The model estimations for each quality were considerably high with an accuracy of > 0.9 (relates to 90%). However, the study did not account for potential color differences between pictures taken with different cameras, nor did it determine the importance of each attribute in the grading. Knowledge of the priority of frond characteristics and interactive effect of cultivation stressors with C. lentillifera nutritional quality and the physical appearance of the species could enable farmers to estimate and manipulate their quality of harvest. Therefore, this study aimed to (1) report on the C. lentillifera cultivation cycle and environmental parameters at the VIJA farm in Van Phong Bay,

Khánh Hòa province and to (2) quantify the sea grape fronds of two different qualities over three sampling points in May and June 2022. (3) A binominal model was used to estimate the most important physical characteristics of sea grape fronds graded as different qualities. Based on observations at the sea grape farm, we hypothesize that the grading is influenced by the physical characteristics of frond lengths, weights, ramuli density, and color of the ramuli, as well as rachis.

# **Material and methods**

#### **Experimental location**

The Central South of Vietnam, including the Khánh Hòa province, is characterized by a monsoon weather regime with a wet (October-January) and a dry (January/February-October) season (Lam et al. 2002; Ilyash and Matorin 2007). The highest precipitation is usually recorded during the Northeast Monsoon (October - November,  $> 500 \text{ mm month}^{-1}$ ), with less rain from December onwards (Northwest Monsoon,  $< 200 \text{ mm month}^{-1}$ ) (Lam et al. 2002). Khánh Hòa province is comprised of of four bays, with Van Phong Bay being the northernmost and the largest bay of the province ( $\sim 510 \text{ km}^2$ ). The bay has an average depth of 15 m with a maximum of 34 m (Barthel et al. 2009; Phu et al. 2022). Several aquaculture activities are taking place in the bay, including lobster cage farming, longline cultivation of the red alga Kappaphycus alvarezii, pond cultivation of the shrimp Litopenaeus vannamei, the snail Babylonia areolata and the seaweed C. lentillifera (Phu et al. 2022, own observations).

Sea grape cultivation was introduced to Viet Nam about a decade ago (So 2022) and since then several companies have started commercial farming of this species, mainly for export to Japan (Terada et al. 2018; So 2022). The present study was conducted at one of these sea grape farms, called `VIJA´ (12°35'17.9"N 109°13'38.5"E), located in the southern part of Van Phong Bay.

### Sea grape production cycle

The cultivation season of *C. lentillifera* lasts about eight months, from March to October. The farm `VIJA' consists of several ponds of different sizes, which are connected through adjustable channels to the nearby coast. Water exchanges are conducted approximately every two days in accordance with the tidal range. The ponds are shaded with black gauze material to provide a light environment of ~ 50 µmol photons  $m^{-2} s^{-1}$  on average, but with high diurnal fluctuations (Stuthmann et al. 2022). The sea grapes are cultivated using trays (Fig. 1A) or the sowing method (Fig. 1B), depending



Fig. 1 Production cycle of *Caulerpa lentillifera* in Van Phong Bay, including the A) sowing cultivation and the B) trays from the tray cultivation. C) The process of sea grape frond harvest and D) place of collection and E) sorting. Sea grapes being sold fresh or F) dehydrated

on the nature of the material at the ground of the pond. In the case of tray cultivation, the farmers lift or float the trays at the surface for harvesting. The trays are being thinned by picking the healthy fronds (7–10 cm) in an interval of 7–15 days. In the case of the sowing method and sometimes also during tray cultivation, farmers use floating devices to swim on the surface of the ponds and pick the good-sized fronds (Fig. 1C). Fronds of the target size are harvested every ~15 days, yielding approximately 1000 kg wet weight in a 5000 m<sup>-2</sup> pond. However, the yield tends to decrease towards the end of the harvest season due to increasing rainfall and decreasing temperatures. After harvesting, the sea grape fronds are kept in clean sea water to ensure wound plug formation. The harvested fronds from the different ponds were collected in a central location (Fig. 1D) and sorted into two groups according to their appearance (Fig. 1E), which determines the fate of the product. Fronds of the highest quality are mainly used for the export to Japan, while fronds of the lesser quality are used for retail in local restaurants and markets. The two are referred to in the following as quality 1 (export grade) and quality 2 (local grade). Sea grape products are sold fresh or as dehydrated product (Fig. 1F). Sea grapes that do not reach the minimum requirements for sale (~30%, including stolons) are mostly discarded. A small amount is kept for other applications, such as cosmetics, especially if they do not meet the length requirements. The seedlings for the next season are kept in the cultivation ponds if the environmental parameters allow

it. When salinities are too low, the seaweeds are kept in cultivation tanks on land (personal and written communication with two farmers from different sea grape farms).

#### **Environmental parameters**

The environmental parameters salinity  $(S_A)$ , temperature (°C), pH and irradiance of photosynthetically active radiation (PAR,  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>) were monitored during several field trips to the VIJA farm in the years 2019, 2020, and 2022. The parameters were quantified using different methods. In the years 2019 (May - August) and 2020 (February - June) data loggers for light (MX2202, HOBO, USA) and salinity/temperature (U24-002-C, HOBO, USA) were installed in the ponds with a logging interval of 30 min. Data were recorded for the whole month or only for some days. An overview of the exact measurement days is presented in supplements I. The pH measurements and the measurements in June 2022 were carried out using a multiparameter probe (Manta2, Eureka, USA). Measurements were conducted for several minutes and at different locations in the pond (information on dates are presented in supplements I). All data are presented as mean  $\pm$  SD for each month. In June 2022, measurements were taken on two different days (09.06.22 and 23.06.22) and the data were averaged. Light irradiance data were quantified on the same farm and have already been published (Stuthmann et al. 2022).

### Study design and data collection

The sea grape fronds were graded at the collection facilities by workers and stored in tanks with natural sea water. The fronds at the collection facility were harvested from different ponds. Based on observations of the researchers and conversations with the farmers, the following physical characteristics of the fronds were identified as potentially important: Frond weight (g), frond length (cm), ramuli density (number of ramuli per cm frond), color of ramuli, and color of rachis (Fig. 2).

On each of the three sampling days (26 May 22, 09 June 22, 23 June 22), 100 fronds were randomly selected for each of the two qualities, respectively (n total = 600). The weight was quantified for each frond separately, before taking a photograph of ten fronds collectively (Fig. 2). The pictures were taken using a Canon EOS M50 camera (Canon Zoom Lens EF-M 14–45 mm), and for uniform illumination of the photographs a styrofoam box was equipped with two lamps. A grey reference scale, including a reference bar (B.I.G., photo equipment – Brenner Import and Handels GmbH, Germany) was placed next



**Fig. 2** Sea grape (*Caulerpa lentillifera*) fronds of **A**) grade 1 and **B**) grade 2. The pictures represent ten fronds of each grade from the sampling on the 09 June 2022 with ramuli and rachis indicated. Scale bar = 1 cm

to the fronds in each picture. The length was quantified using the software ImageJ (Schneider et al. 2012) and the respective reference bar in the picture. The ramuli of each frond were counted in a row along the right side of the respective frond and expressed as the number of ramuli per cm frond (ramuli density) according to the following formula:

Ramuli density = # of ramuli/frond length (cm),

with # ramuli denoting the count of ramuli along the right side and frond length (cm) being the length measurement of the frond, derived from Dobson et al. (2020).

The color of the rachis and ramuli of the sea grapes was analyzed following the description of Winters et al. (2009), adapted by Stuthmann et al. (2022) using the software Octave (Eaton et al. 2021). The color was expressed as value between 0 (black) and 255 (white) in the RGB (Red Green Blue) color space. On each frond, five measurement points (25 pixels) were randomly chosen along the rachis and from different ramuli, respectively. The mean value of these measurement points was used as respective measure for each individual frond. Following the procedure of Stuthmann et al. (2022), solely the Red color channel (R value) was used as a measure for the chlorophyll content and coloration. To quantify the physiological state of the fronds, the maximum quantum efficiency of photosystem (PS) II  $(F_v/F_m)$  was determined using a portable Diving-PAM chlorophyll fluorometer (Walz, Germany) after 7 min of dark adaptation. The parameters length,  $F_v/F_m$ , R values, and ramuli density were quantified for at least 50 of the 100 samples taken for each sampling day (n = 50), respectively. On each sampling day and for each quality, four replicates of frond biomass were collected for the antioxidant analysis (n = 4).

#### **Antioxidant analysis**

The biomass was stored under exclusion of light at -80 °C before freeze drying the samples. The biomass was pulverized using a FastPrep-24 (MP Biochemicals, Germany) for 20 s. For the extraction, approximately 40 mg of powder was weighed in and 1 mL ethanol (70%) was added. The samples were kept in a water bath (47 °C) for 4 h and vortexed hourly. After centrifuging the samples (2500 rpm; 20 °C, 10 min) the supernatant was transferred to an Eppendorf tube and frozen at -80 °C until analysis within the next two days.

For analysis of the AOA, an ABTS<sup>.+</sup> assay (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) was carried out following a modified method of Re et al. (1999). The ABTS<sup>.+</sup> stock solution (7 mM) was prepared at least 16 h before by oxidation with potassium disulfate (2.45 mM) in order to prepare the working solution. On the day of measurement, the stock was diluted with absolute ethanol until an absorption of  $0.7 \pm 0.02$  was reached. For the measurement, 10 µL of sample extract were mixed with 1 mL ABTS<sup>.+</sup> working solution and absorption was quantified after 6 min reaction time (734 nm). The AOA was expressed as Trolox Equivalents (TE).

For analysis of the TPC, the Folin-Ciocalteu (F–C) method was used with modifications (Ainsworth and Gillespie 2007). Sample extract (150  $\mu$ L) and 300  $\mu$ L 10% F–C reagent (v/v) were vortexed thoroughly before adding 1200  $\mu$ L sodium carbonate (700 mM). After 45 min incubation at room temperature and subsequent centrifugation (5000 rpm, 20 °C, 3 min), 1 mL was transferred to a cuvette and absorption was read at 765 nm. The TPC was expressed as Gallic Acid Equivalents (GAE).

#### **Data analysis**

Five different physical characteristics of the fronds, namely frond weight (g), frond length (cm), R value rachis (0–255), R value ramuli (0-255), and ramuli density (ramuli/ cm frond) were used to test the effect on the assigned quality.  $F_v/F_m$  values, AOA, and TPC were used to quantify the physiological state and the antioxidant content of the fronds. Levene's test (homogeneity of variance, p > 0.05) and the Shapiro–Wilk test (normal distribution, p > 0.05) were used to assess each data set. A one-way analysis of variance (ANOVA) with a Tukey's honesty significant difference (HSD) post-hoc test was conducted to explain the effect of quality on the respective quantified variable. In case the requirements for an ANOVA were not met, a Kruskal-Wallis test followed by a Dunn-Bonferroni post-hoc test was applied. Correlations between variables weight (g) and length (cm) and AOA and TPC were conducted using Spearman's rank correlations, because data were non-parametric (Shapiro–Wilk test, p > 0.05) and Pearson correlation test, respectively.

A logistic regression model was used to estimate the influence of the different physical characteristics as explanatory variables on the binary outcome (quality 1 or 2). A univariant analysis (generalized linear model, glm with family "binomial") was run for each explanatory variable separately. Subsequently, a multivariate model was designed including all explanatory variables with a significant effect on the response. However, since weight (g) and length (cm) were highly correlated and their effect in the univariant models was highly significant as well, two models with either weight (g, glm (quality ~ (weight (g), R value rachis (0–255))) or length (cm, glm (quality ~ (length (cm), R value rachis (0-255))) were constructed. The uni- and multivariate models were evaluated based on the Akaike Information Criterion (AIC), the Accuracy and the area under the curve (AUC) received from conducting a receiver operating characteristic curve (ROC curve). The Accuracy was calculated from a confusion matrix comparing the predicted vs observed values. The univariate logistic model of weight (g) reached a comparable high accuracy, AUC and a low AIC, compared to the multivariate models and simultaneously the quantification of frond weight (g) required comparable little effort for the farmers, compared e.g. to length to color measurements. Therefore, this model was tested to predict the quality based on the frond weights (g) of the 300 samples which were not included in the analysis. All analyses were conducted using R Studio (R Core Team 2019) with the meta package tidyverse (Wickham et al. 2019) and pROC (Robin et al. 2011). The level of significance was set to  $\alpha = 0.05$ . The results of all statistical tests are included in the supplements (TSII-IV).

**Table 1** Environmental data derived from data loggers (HOBO, USA) and multiparameter (Manta2, Eureka, USA) measurements from a sea grape farm (VIJA) in Van Phong Bay, derived over the years 2019—2022. Letters give details about measurements; A: monthly mean $\pm$  standard deviation (SD) values calculated from logger measurements in 30 min intervals; B: mean $\pm$ SD of point measurements over shorter periods (<10 min) on one or two (June 2022, 09.06.22 and 23.06.22) single days within the month; C: only a few days of the month considered; Pond 1, 2, 3 are different ponds oper-

ated by VIJA. Pond 1 and 2 were demolished/not used anymore in the second and third year of fieldwork due to constructions in the area, therefore, field measurements were moved accordingly. Ponds were located in the immediate vicinity of each other. Data on irradiances of photosynthetically active radiation (PAR) were sourced from Stuthmann et al. (2022). For specific information on dates and sampling frequencies refer to supplements I. The raw data of the salinity, temperature and MANTA data can be accessed online

Month, Year	Temperature °C	Absolute Salinity $S_A$	рН	Irradiance $\mu$ mol photons m <sup>-2</sup> s <sup>-1</sup>	Pond
February 2020	$26.8 \pm 0.5^{A,C}$	$32.4 \pm 1.4^{A,C}$	$9.0\pm0.05^{\rm B}$	-	2
March 2020	$28.9 \pm 0.8^{A}$	$32.5 \pm 0.3^{A}$	-	-	2
April 2020	$29.4 \pm 0.9^{\text{A}}$	$32.5 \pm 0.5^{A}$	-	-	2
May 2019	$30.7 \pm 0.7^{A}$	$30.1 \pm 0.6^{A}$	-		1
May 2020	$30.9 \pm 0.8^{\text{A}}$	$31.9 \pm 0.4^{A}$	-	-	2
June 2019	$29.8 \pm 0.9^{\text{A}}$	$31.5 \pm 1.1^{A}$	$8.4\pm0.02^{\rm B}$	$71.0 \pm 62.9^{A}$	1
June 2020	$30.9 \pm 0.6^{\text{A}}$	$31.2 \pm 0.2^{A}$	-	-	2
June 2022	$30.5 \pm 0.1^{B}$	$33.8 \pm 0.4^{B}$	$8.6\pm0.05^{\rm B}$		3
July 2019	$29.0 \pm 1.1^{A}$	$34.2 \pm 0.6^{A}$	$8.4 \pm 0.1^{B}$	$56.9 \pm 52.8^{A}$	1
August 2019	$27.9 \pm 1.0^{\rm A}$	$33.5 \pm 0.3^{A}$	$8.3\pm0.1^{\rm B}$	$69.4 \pm 74.2^{\text{A}}$	1

**Table 2** Results (p-values) of three different logistic regression models and model evaluation criteria Akaike Information Criterion (AIC), the Accuracy score and the area under the curve (AUC) received from conducting a receiver operating characteristic curve (ROC curve). The asterisks \*\*\*, \*\*, \* represent different significance levels 0.001, 0.01, 0.05, respectively

Variable	Univariate Frond weight (g)	Multivariate Frond weight (g)	Multivariate frond length (cm)
Frond weight (g)	5.83e <sup>-16</sup> ***	4.91e <sup>-15</sup> ***	-
Frond length (cm)	-	-	3.85e <sup>-11</sup> ***
R value rachis (0—255)	-	2.16e <sup>-09</sup> ***	$2.47e^{-10***}$
ACI	290.1	242.12	293.45
Accuracy score	0.79	0.79	0.76
AUC	0.845	0.892	0.837
Accuracy score based on test data-set	0.773		

# Results

# **Environmental parameters**

Data on salinity, temperature and pH are presented for the months of February to August (Table 1). Overall, the temperature measured in the sea grape ponds ranged between mean values of  $26.8 \pm 0.5$  and  $30.9 \pm 0.6$  °C. The temperature increased from February to April, with highest values of ~30—31 °C in May and June (Table 2), and a trend of

decrease can be seen in July and August. The salinity values ranged between  $S_A$  30.1±0.6 and 34.2±0.6, with lowest values in May (Table 2). The pH ( $8.3\pm0.1-9.0\pm0.05$ ) was rather similar between the measurements, with the highest mean quantified in February 2020 (Table 2).

# Sea grape quality parameters

The fronds assigned to quality 1 (very good) were significantly heavier  $(2.37 \pm 0.59 \text{ vs } 1.60 \pm 0.5 \text{ g}$ , Chi-Square (1) = 103.71, p < 0.001, Fig. 3A) and longer  $(12.59 \pm 2.89 \text{ vs } 10.01 \pm 2.51 \text{ cm}$ , Chi-square (1) = 61.37, p < 0.001, Fig. 3B), compared to quality 2. Both parameters were significantly positively correlated ( $r_s = 0.818$ , p < 0.001). The R value (0-255) of the ramuli was similar between the different qualities (p = 0.232, Fig. 3D), whereas the rachis of fronds of quality 1 had significantly lower R values (hence darker), compared to quality 2 ( $43.48 \pm 10.98$  vs  $54.13 \pm 9.32$ , Chi-square (1) = 46.94, p < 0.001, Fig. 3C). The ramuli density of the fronds was similar between the qualities (p = 0.303, Fig. 3E).

# Logistic model estimation

The multivariate logistic regression model including R values of rachis (0–255) and frond length (cm) or frond weight (g), respectively, as well as the univariate model of frond weight (g) performed best among all tested models (Table 2, Supplements TSIII). The multivariate model incorporating weight, as well as the univariate frond weight model

Fig. 3 Explanatory variables A) Frond weight (g), B) Frond length (cm), C) Red (R) value from the Red Green Blue color space of the C) rachis and the **D**) ramuli, as well as the **E**) ramuli density (Ramuli per cm frond) of Caulerpa lentillifera fronds from two different quality standards (1 = very good,2 = ok). Data are presented as median with the box drawn from the first to the third quantile and the whiskers presenting the 1.5 interquartile range (n = 45 for A-F and n = 11 - 12for G-H). Different letters represent significant differences of the variables between the different qualities, analyzed using a one-way analysis of variance (ANOVA) or a Kruskal-Wallis test (significance level  $\alpha = 0.05$ )



outperformed the multivariate length model. Using the test data of 150 frond weights for each quality, the univariate frond weight model reached an accuracy score of 0.77 (meaning 77% of fronds were correctly assigned to a quality 1 or 2 group), comparable to the score based on the data used for the model construction (Table 2).

### Physiological and biochemical parameters

The  $F_v/F_m$  values were lower for sea grape fronds of quality 2 (0.71 ± 0.03), compared to those of quality 1

(0.71 ± 0.02, Chi-Squared (1) = 9.60, p < 0.01, Fig. 4A). The AOA and TPC on the other hand were not significantly different between fronds of both qualities with means and SDs of 98.34 ± 19.22 mmol TE (100 g)<sup>-1</sup> DW and 163.8 ± 20.14 mg GAE (100 g)<sup>-1</sup>DW for quality 1 and 95.96 ± 24.98 mmol TE (100 g)<sup>-1</sup> DW and 149.85 ± 15.44 mg GAE (100 g)<sup>-1</sup> DW for quality 2, respectively (Fig. 4B, C). However, there was a trend towards lower values for fronds of quality 2, compared to quality 1 (Fig. 4B, C). AOA and TPC were moderately positively correlated ( $r_p = 0.66$ , p < 0.001). Fig. 4 Physiological and biochemical parameters A)  $F_v/F_m$  value, **B**) antioxidant activity (AOA, mmol Trolox equivalents, TE (100 g)<sup>-1</sup> dry weight, DW) and C) total phenolic content (TPC, mg gallic acid equivalents, GAE  $(100 \text{ g})^{-1} \text{ DW})$  of *Caulerpa* lentillifera fronds from two different quality standards (1 = very good, 2 = ok). Data are presented as median with the box drawn from the first to the third quantile and the whiskers presenting the 1.5 interquartile range (n = 45 for A-F and n = 11 - 12 for G-H). Different letters represent significant differences of the variables between the different qualities, analyzed using a one-way analysis of variance (ANOVA) or a Kruskal-Wallis test (significance level  $\alpha = 0.05$ )



# Discussion

The environmental parameters were almost exclusively quantified during the cultivation period of the sea grapes from ~ March to October, and they reflected the reported trend of lower temperatures towards the beginning and end of the cultivation season. Growth rates, as well as photosynthetic performance of C. lentillifera, are temperature dependent (Guo et al. 2015b; Cai et al. 2021b; Terada et al. 2021), with higher growth rates at 27.5 °C, compared to 30 °C (Guo et al. 2015b) and a maximum gross photosynthetic rate at 30.7 °C (Terada et al. 2021). However, the salinity was rather stable without decreases towards the off-season months and still within the reported window of growth (Guo et al. 2015a; Tanaka et al. 2020). Nonetheless, salinities could spontaneously decrease due to heavy rainfall, which might be indicated by the trend of higher SDs of logger values quantified in February and March 2019. Regenerated sea grape stolons showed lower chlorophyll a and b contents within one week at salinities of  $S_A \ge 30$  compared to 35, as well as lower growth rates (Guo et al. 2015a; Tanaka et al. 2020). However, as the highest rainfall is expected during the Northeast monsoon from October - November, the values might decrease towards the end of the respective year (Lam et al. 2002).

The frond length of *C. lentillifera* is highly variable, potentially due to the high phenotypic plasticity of the

species (Estrada et al. 2020), as well as their growth cycle. Frond lengths between ~3 cm and ~13 cm have been reported in the literature (Paul et al. 2014; Lapong et al. 2019; Estrada et al. 2020; Thi et al. 2020) and the quantified sea grape fronds at the VIJA farm were in the upper end of this range (quality 1:  $12.59 \pm 2.89$ ; quality 2:  $10.01 \pm 2.51$  cm). Additionally, they met or exceeded the length guidelines reported by farmers (7-10 cm). The biochemical composition of sea grape fronds might change during their growth cycle, as a reported negative correlation of frond length with nutritionally interesting compounds beta-carotene and eicosatetraenoic acid (EPA) suggests (Paul et al. 2014). Hence, even though shorter fronds seem to be perceived as less valuable by farmers, their nutritional composition might be an argument to enhance their market value. The strong correlation between frond length and weight was not surprising and similarly reported by Lapong et al. (2019). Based on literature reports (Chaiklahan et al. 2020) and observations at the sea grape farm, we hypothesized that the ramuli density would also be a quality characteristic. Thi et al. (2020) reported similar frond morphometrics between treatments of water levels and exchange rates. But the mean values of frond length, ramuli density and diameter over time suggested a higher ramuli density (~13–14 vs~11 ramuli cm<sup>-1</sup>) and diameter (~2.2 vs~2.0 mm) of shorter fronds (~8 cm), compared to longer fronds (~10-11 cm). Hence, the frond morphometrics could change with the growth cycle of the algae. However, in contrast to the ramuli density, the ramuli diameter might have differed between the grades, creating the visual impression of a higher ramuli density.

The color of the sea grapes' rachis had an essential effect on the grading, in contrast to the color of the ramuli. The R value is likely to be highly correlated with the chlorophyll a content of the biomass (Stuthmann et al. 2022). Color plays a crucial role in costumers decision making (Pathare et al. 2013) and a color change of green vegetables has been found to be unacceptable by consumers (Shewfelt 2002). Hence, a dark rachis color induced by high chlorophyll content could be valued by costumers. The chlorophyll distribution in the single-celled Caulerpa (Zubia et al. 2020) arguably changes through chloroplast migration and degradation as a result of excess irradiances (Stuthmann et al. 2022) or according to surrounding nitrate composition, temperature or salinity (Guo et al. 2015a; Cai et al. 2021b). The logistic regression models confirmed that frond length, weight, and rachis R value were crucial characteristics for quality grading of sea grape fronds. However, both univariate and multivariate models including frond weight outperformed models based on frond length (univariate length model evaluation shown in supplements). The deep learning model constructed by Chinnasarn et al. (2022) successfully extracted the roundshaped ramuli from photographs and graded them based on their features, which enforces the potential role of ramuli in the grading of the fronds. The frond weight variable might contain information about the arguably important trait of ramuli diameter, compared to frond length, potentially resulting in a better prediction of quality. Additionally, frond weight can be easily and quickly quantified with a balance, whereas measuring color, ramuli morphometrics or length is more time-consuming and costly for farmers. The univariate frond weight model grouped > 77% of the test fronds in the right quality grade and hence frond weight seemed like the major predictor of the sea grape quality.

 $F_v/F_m$  indicated a good physiological state of all fronds with values ≥ 0.7 (Stuthmann et al. 2020). However, quality 2 sea grape fronds seemed to contain more individuals showing signs of photoinhibition compared to quality 1, as revealed by lower  $F_v/F_m$  values (Goh et al. 2012). Different environmental stressors such as salinity, temperature or light can enhance photoinhibition in plants (Takahashi and Murata 2008). Oxidative stress, induced by exposure to the respective stressors could have resulted in decreased chlorophyll contents and hence color, in addition to reduced  $F_v/F_m$ values (Guo et al. 2015a, b; Stuthmann et al. 2020; Cai et al. 2021b), causing the lower grading of fronds into quality 2. The AOA and TPC values were within the range of values reported for *C. lentillifera* (Stuthmann et al. 2022).

*Caulerpa* species contain a variety of secondary metabolites acting as antioxidants, including carotenoids, ascorbic acid (vitamin C), tocopherols (vitamin E), as well as

polyphenols like flavonoids (Matanjun et al. 2008; Tanna et al. 2018, 2019). Hence, the positive correlation between AOA and TPC was in line with previous reports, suggesting that phenolic compounds contributed essentially to the overall AOA of C. lentillifera (Nguyen et al. 2011). Antioxidants are involved in the scavenging of reactive oxygen species (ROS) on one hand as part of the physiological oxidative stress reaction of seaweeds and on the other hand as essential feature in the human diet (Young and Woodside 2001; Dring 2005). AOA and TPC were not significantly different between qualities, but fronds of quality 2 tended to have lower values. The chemo-diversity of the algae could be caused by natural variability of abiotic conditions between pond microhabitats, as well as by differences between developmental stages (Stengel et al. 2011). Additionally, the pre-harvest conditions of the algae are likely to influence the physiology during post-harvest storage similar to other plant products (Sams 1999), and especially when sea grapes are deprived of water (Stuthmann et al. 2020). Therefore, the physiological development of different quality fronds should also be observed from harvest until retail.

Sea grape farmers harvest sea grape fronds in tray or sowing cultivation directly in the pond environment, based on judgement and experience, when they expect them to have reached the harvestable size. Grading of frond qualities is performed on land, and weight, as well as rachis coloration seemed to be import. Frond weight was the best predictor of quality grading of sea grapes. The nutritional value of sea grapes, including the antioxidative capacities, could be an interesting additional marketing instrument for the species, which was already denoted as `functional food candidate' (Nurkolis et al. 2023). Identification of potential biochemical fluctuation patterns of the sea grapes' developmental phase could increase the value of smaller fronds (Paul et al. 2014).

The fronds grading and the associated differences in value enforce the importance of such intraspecific physiological, morphometric and biochemical differences of the seaweed cultivar for aquaculture (Demes and Pruitt 2019). Modern technologies, including the high computational power and various algorithms enable on one hand the quick quantification of information, e.g. through pictures and on the other hand the rapid analysis of large data sets. In agriculture, different methods are used for crop phenotyping, namely the *in-situ* estimation of plant traits, which can be set in relation to their environment and to determine genotypes for plant breeding (Yang et al. 2017; Araus et al. 2022). Seaweeds, like sea grapes, often reproduce through fragmentation, but the interplay of different traits with their environment could be a valuable tool for aquaculture to increase the harvests quality and quantity (Demes and Pruitt 2019). Tadmor Shalev et al. (2022) use

field spectroscopic data and a machine learning algorithm to assess the protein content of *Gracilaria* sp. providing the opportunity for farmers to make in-site decisions for the seaweeds use. This study has only taken a first step to quantify farmers frond grading criteria of importance and further research should target the interlink of those frond properties with different environmental parameters over the production chain. By coupling phenotyping techniques with the farmers' experience plus time-efficient and accurate cultivation and post-harvest protocols could be developed for seaweed cultivation.

Conclusions

This study demonstrated that frond weight, length, and rachis color are important quality criteria for grading of sea grapes. However, from an economic perspective, quantification of frond weight is expected to be less time consuming and costly for farmers compared to rachis color or length. Sea grapes of the better quality are longer, heavier and have darker rachis, compared to the other quality group. However, the antioxidant activity and the total phenolic content were similar.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10811-023-03030-y.

**Acknowledgements** The authors would like to thank the staff of the Institute of Oceanography in Nha Trang and farm VIJA for their support, as well as Aaron Cordes and Mia Pribbernow for the help in the field.

Authors contribution Lara E. Stuthmann: Conceptualization, Investigation, Formal Analysis, Visualization, Methodology, Writing – Original Draft Preparation; Hoang Trung Du: Writing – Review & Editing, Resources; Beatrice Brix da Costa: Investigation, Writing – Review & Editing; Andreas Kunzmann: Writing – Review & Editing, Supervision, Funding Acquisition; Karin Springer: Writing – Review & Editing, Supervision, Funding Acquisition.

**Funding** Open Access funding enabled and organized by Projekt DEAL. Funding has been received from the Leibniz Centre for Tropical Marine Research inhouse-funds.

**Data availability** The datasets generated and analysed during the current study are available from the corresponding author on reasonable request. Environmental data are available at fig share at https://doi.org/10.6084/m9.figshare.23214956 and https://doi.org/10.6084/ m9.figshare.23214950.

#### Declarations

Competing interests The authors declare no competing interests.

**Conflict of interest disclosure** The authors declare no competing conflict of interest.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

## References

- Ainsworth EA, Gillespie KM (2007) Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc 2:875–877
- Araus JL, Kefauver SC, Díaz OV, Gracia-Romero A, Rezzouk FZ, Segarra J, Buchaillot ML, Chnag-Espino M, Vatter T, Sanchez-Bragado R, Fernandez-Gallego J, Serret MD, Bort J (2022) Crop phenotyping in a context of global change: what to measure and how to do it. J Integr Plant Biol 64:592–618
- Barthel K, Rosland R, Thai NC (2009) Modelling the circulation on the continental shelf of the province Khanh Hoa in Vietnam. J Mar Syst 77:89–113
- Cai J, Lovatelli A, Aguilar-Manjarrez J, Cornish L, Dabbadie L, Desrochers A, Diffey S, Garrido Gamarro E, Geehan J, Hurtado A, Lucente D, Mair G, Miao W, Potin P, Przybyla C, Reantaso M, Roubach R, Tauati M, Yuan X (2021a) Seaweeds and microalgae: an overview for unlocking their potential in global aquaculture development. FAO Fisheries and Aquaculture Circular No. 1229, Rome. NFIA/C1229 pp 1–36
- Cai Y, Li G, Zou D, Hu S, Shi X (2021b) Rising nutrient nitrogen reverses the impact of temperature on photosynthesis and respiration of a macroalga *Caulerpa lentillifera* (Ulvophyceae, Caulerpaceae). J Appl Phycol 33:1115–1123
- Chaiklahan R, Srinorasing T, Chirasuwan N, Tamtin M, Bunnag B (2020) The potential of polysaccharide extracts from *Caulerpa lentillifera* waste. Int J Biol Macromol 161:1021–1028
- Chinnasarn K, Yookwan W, Chinnasarn S, Yuenyongputtakal W (2022) Green caviar quality image classification based on enhanced deeply learn model. In: 2022 13th International Conference on Information and Communication Technology Convergence (ICTC). IEEE, pp 458–462. https://doi.org/10.1109/ ICTC55196.2022.9952839
- de Gaillande C, Payri C, Remoissenet G, Zubia M (2017) *Caulerpa* consumption, nutritional value and farming in the Indo-Pacific region. J Appl Phycol 29:2249–2266
- Demes KW, Pruitt JN (2019) Individuality in seaweeds and why we need to care. J Phycol 55:247–256
- Dobson GT, Duy NDQ, Paul NA, Southgate PC (2020) Assessing potential for integrating sea grape (*Caulerpa lentillifera*) culture with sandfish (*Holothuria scabra*) and Babylon snail (*Babylonia areolata*) co-culture. Aquaculture 522:735153
- Dring MJ (2005) Stress resistance and disease resistance in seaweeds: The role of reactive oxygen metabolism. Adv Bot Res 43:175–207
- Eaton JW, Bateman D, Hauberg S, Wehring R (2021) GNU Octave version 6.3.0 manual: a high-level interactive language for numerical computations. https://www.gnu.org/software/octave/doc/v6.4.0/

- Estrada JL, Bautista NS, Dionisio-Sese ML (2020) Morphological variation of two common sea grapes (*Caulerpa lentillifera* and *Caulerpa racemosa*) from selected regions in the Philippines. Biodiversitas 21:1823–1832
- FAO (2022) The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. FAO, Rome
- Goh CH, Ko SM, Koh S, Kim Y-J (2012) Photosynthesis and environments: photoinhibition and repair mechanisms in plants. J Plant Biol 55:93–101
- Guiry MD, Guiry GM (2023) AlgaeBase. World-wide electronic publication. National University of Ireland, Galway. https://www.algaebase.org
- Guo H, Yao J, Sun Z, Duan D (2015a) Effects of salinity and nutrients on the growth and chlorophyll fluorescence of *Caulerpa lentillifera*. Chin J Oceanol Limnol 33:410–418
- Guo H, Yao J, Sun Z, Duan D (2015b) Effect of temperature, irradiance on the growth of the green alga *Caulerpa lentillifera* (Bryopsidophyceae, Chlorophyta). J Appl Phycol 27:879–885
- Ilyash LV, Matorin DN (2007) Features of the spatial distribution of phytoplankton in Nhatrang Bay of the South China Sea during the rainy season. Oceanology 47:788–796
- Lam NN, Anh NTM, Hai DN, Van TH (2002) Seasonal variations in the abundance of phytoplankton in the shallow waters of Cua Be River Estuary, Nha Trang Bay, Central Vietnam. Collect Mar Res Work 12:129–148
- Lapong I, Paul N, Reza A (2019) Characterization of sea grapes (*Caulerpa lentillifera*) from Vietnamese company's products. Mar Chim Acta 20:51–57
- Matanjun P, Mohamed S, Mustapha N, Muhammad K, Ming C (2008) Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. J Appl Phycol 20:367–373
- Nguyen VT, Ueng J-PP, Tsai G-JJ (2011) Proximate Composition, total phenolic content, and antioxidant activity of seagrape (*Caulerpa lentillifera*). J Food Sci 76:C950–C958
- Nurkolis F, Taslim NA, Qhabibi FR, Kang S, Moon M, Choi J, Choi M, Park MN, Mayulu N, Kim B (2023) Ulvophyte green algae *Caulerpa lentillifera*: metabolites profile and antioxidant, anticancer, anti-obesity, and in vitro cytotoxicity properties. Molecules 28:1365
- Pathare PB, Opara UL, Al-Said FA-J (2013) Colour measurement and analysis in fresh and processed foods: A review. Food Bioproc Technol 6:36–60
- Paul NA, Neveux N, Magnusson M, de Nys R (2014) Comparative production and nutritional value of "sea grapes" - the tropical green seaweeds *Caulerpa lentillifera* and *C. racemosa.* J Appl Phycol 26:1833–1844
- Phu LH, Kim-Hong PT, Van CT et al (2022) Environmental concerns for sustainable mariculture in coastal waters of south-central Vietnam. Sustainability 14:8126
- R Core Team (2019) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
- Rabia MDS (2016) Cultivation of *Caulerpa lentillifera* using tray and sowing methods in brackishwater pond. Environ Sci 4:23–29
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26:1231–1237
- Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanches J-C, Müller M (2011) pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 12:77
- Sams CE (1999) Preharvest factors affecting postharvest texture. Postharvest Biol Technol 15:249–254
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Meth 9:671–675
- Shewfelt R (2002) Color. In: Bartz JA, Brecht JK (eds) Postharvest physiology and pathology of vegetables. Marcel Dekker, New York, pp 287–296

- So K (2022) Vietnam's largest sea grape exporter: Learning lessons from failure. https://vietnamagriculture.nongnghiep.vn/vietnamslargest-sea-grape-exporter-learning-lessons-from-failure-d312552. html. Accessed 22.03.2023
- Son T (2022) Khanh Hoa: Stable income from Seaweed grapes farming. https://ocopvietnam.com.vn/news/khanh-hoa-stable-incomefrom-seaweed-grapes-farming.html29851. Accessed 19.05.2023
- Stengel DB, Connan S, Popper ZA (2011) Algal chemodiversity and bioactivity: Sources of natural variability and implications for commercial application. Biotechnol Adv 29:483–501
- Stuthmann LE, Achuthan R, Pribbernow M, Du HT, Springer K, Kunzmann A (2022) Improving the nutritional value of edible *Caulerpa lentillifera* (Chlorophyta ) using high light intensities. A realistic tool for sea grape farmers. Algal Res 66:102785
- Stuthmann LE, Springer K, Kunzmann A (2020) Cultured and packed sea grapes (*Caulerpa lentillifera*): effect of different irradiances on photosynthesis. J Appl Phycol 33:1125–1136
- Syakilla N, George R, Chye FY et al (2022) A review on nutrients, phytochemicals, and health benefits of green seaweed, *Caulerpa lentillifera*. Foods 11:2832
- Tadmor Shalev N, Ghermandi A, Tchernov D et al (2022) NIR spectroscopy and artificial neural network for seaweed protein content assessment in-situ. Comput Electron Agric 201:107304
- Takahashi S, Murata N (2008) How do environmental stresses accelerate photoinhibition? Trends Plant Sci 13:178–182
- Tanaka Y, Ashaari A, Mohamad FS, Lamit N (2020) Bioremediation potential of tropical seaweeds in aquaculture: low-salinity tolerance, phosphorus content, and production of UV-absorbing compounds. Aquaculture 518:734853
- Tanna B, Brahmbhatt HR, Mishra A (2019) Phenolic, flavonoid, and amino acid compositions reveal that selected tropical seaweeds have the potential to be functional food ingredients. J Food Process Preserv 43:1–10
- Tanna B, Choudhary B, Mishra A (2018) Metabolite profiling, antioxidant, scavenging and anti-proliferative activities of selected tropical green seaweeds reveal the nutraceutical potential of *Caulerpa*. Algal Res 36:96–105
- Terada R, Nakazaki Y, Borlongan IA, Endo H, Nishihara GN (2018) Desiccation effect on the PSII photochemical efficiency of cultivated Japanese *Caulerpa lentillifera* under the shipping package environment. J Appl Phycol 30:2533–2538
- Terada R, Takaesu M, Borlongan IA, Nishihara GN (2021) The photosynthetic performance of a cultivated Japanese green alga *Caulerpa lentillifera* in response to three different stressors, temperature, irradiance, and desiccation. J Appl Phycol 33:2547–2559
- Thi N, Anh N, Thong LV, Lam NP, Thi T, Lien K, Hoa NV (2020) Effects of water levels and water exchange rates on growth and production of sea grape Caulerpa lentillifera J. Agardh 1837. Int J Fish Aquat Stud 8:211–216
- Trono CG, Toma T (1993) Cultivation of the green alga Caulerpa lentillifera. In: Ohno M, Critchley AT (eds) Seaweed cultivation and Marine Ranching. Kanagawa International Fisheries Training Center; JICA, Yokosuka pp 75–88
- Wichachucherd B, Pannak S, Saengthong C, Koodkaew I, Rodcharoen E (2019) Correlation between growth, phenolic content and antioxidant activity in the edible seaweed, *Caulerpa lentillifera* in open pond culture system. J Fish Environ 43:66–75
- Wickham H, Averick M, Bryan J et al (2019) Welcome to the Tidyverse. J Open Source Softw 4:1686
- Winters G, Holzman R, Blekhman A, Beer S, Loya Y (2009) Photographic assessment of coral chlorophyll contents: Implications for ecophysiological studies and coral monitoring. J Exp Mar Bio Ecol 380:25–35
- Yang G, Liu J, Zhao C, Li C, Huang Y, Yu H, Xu B, Yang X, Zhu D, Zhang X, Zhang R, Feng H, Zhao X, Li Z, Li H, Yang H (2017)

Unmanned aerial vehicle remote sensing for field-based crop phenotyping: Current status and perspectives. Front Plant Sci 8:1111

- Yap WG (1999) Rural aquaculture in the Philippines. Regional office for Asia and the Pacific Food and Agriculture Organization of the United Nations (FAO), Bangkok, Thailand, Publication, 20, 82
- Young IS, Woodside J (2001) Antioxidants in health and disease. J Clin Pathol 54:176–186
- Zhang M, Ma Y, Che X, Huang Z, Chen P, Xia G, Zhao M (2020) Comparative analysis of nutrient composition of *Caulerpa lentillifera* from different egions. J Ocean Univ China 19:439–445
- Zubia M, Draisma SGA, Morrissey KL, Varela-Álvarez E, De Clerck O (2020) Concise review of the genus *Caulerpa* J.V. Lamouroux. J Appl Phycol 32:23–39

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.