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Cultured and packed sea grapes (*Caulerpa lentillifera*): effect of different irradiances on photosynthesis

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Abstract

The green macroalga *Caulerpa lentillifera* (sea grapes, green caviar) is a promising source for future nutrition due to its beneficial composition for human consumption. It is cultured in tidal ponds, mainly in Vietnam and the Philippines, and stored for shipment and retail in plastic containers, like polystyrene (PS) and polyethylene terephthalate (PET), exhibiting different properties. This study investigates the influence of irradiances on the physiology of sea grapes under culture and packaging ambience in PET using pulse-amplitude modulated (PAM) fluorometry. F_v/F_m values of *C. lentillifera* significantly decreased < 0.54 ± 0.06 standard deviation (SD) after 7 days of culture under 100 μmol photons m^{-2} s^{-1}, but with the potential of recovery. In packaging ambience in the state of desiccation, sea grapes exposed to room irradiances (3 μmol photons m^{-2} s^{-1}) for 12 days were still physiologically in a good condition ($F_v/F_m = 0.70 \pm 0.06$). However, 12 days under irradiances of 70 μmol photons m^{-2} s^{-1} leads to decreased F_v/F_m (0.42 ± 0.11) and a moisture content of 88.2 ± 3.3% of initial. After re-immersion in sea water under room irradiances, F_v/F_m values recovered to a certain degree. In darkness, desiccation was followed by a decrease of F_v/F_m to 0.09 ± 0.19 and moisture content of 49.3 ± 20.2% of initial with no recovery after re-immersion under room irradiances. Results suggest shading of *C. lentillifera* in pond culture and PET containers as suitable packaging for sea grapes, but a dim light source should be provided during storage.

Keywords Aquaculture · Food · Green caviar · Packaging · Photosynthetic efficiency

Introduction

Seaweeds as a nutritious and abundant food product are one answer to an explosively growing and hungry world population (Pereira 2020). Many macroalgae naturally inhibit coastal zones, where they are exposed to fluctuations in physio-chemical environmental conditions which influence their physiology such as intensities of photosynthetically active radiation (PAR) and desiccation (Davison and Pearson 1996). Other than in the natural habitat, in aquaculture settings, environmental parameters can be partially adapted to the needs of the organism as long as these conditions are known.

Sea grapes (*Caulerpa lentillifera* J. Agardh; Caulerpaceae, Bryopsidales) are green, siphonous macroalgae with a special texture and thallus structure. The species is distributed in the Indo-Pacific region, where it is consumed as a food product eaten fresh in salads, as snack, as sushi, or in a salt preserved form (Long et al. 2020). The high nutritional composition consisting of polyunsaturated fatty acids, antioxidant activity, vitamins, minerals, and bioactive compounds makes sea grapes a nutritious food source and a good candidate to contribute to food security for the rising population, especially in coastal tropical areas (e.g., Saito et al. 2010; Nguyen et al. 2011; Paul et al. 2014; FAO, IFAD, WHO 2019). *Caulerpa lentillifera* are easily and sustainably culturable due to their propagation via fragmentation and the low need for expensive infrastructure or expertise (de Gaillande et al. 2017). Sea grapes are in particular cultured in open-tidal ponds as in the Philippines and Vietnam (de Gaillande et al. 2017; Zubia et al. 2020), and in the latter, pond culture is increasingly implemented at the coasts of the Central South in the Khánh Hòa province. In Japan and China, where the demand for sea grapes is especially high, land-based raceway culture is already practiced to some extent (Long et al. 2020; Zubia et al. 2020). A major factor during sea grape culture is solar radiation, which can be partially controlled through artificial shading

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of ponds. Although light is essential for seaweeds to maintain their metabolism, an excess of absorbed photosynthetically active radiation can oversaturate the electron transport chain capacity without driving the biochemical process of photosynthesis (Franklin and Forster 1997). This energy has to be emitted, e.g., through dynamic photoinhibition, a mosaic of photoprotective processes resulting in a declined transfer of excitation energy to the reaction centers in the antenna (non-photochemical quenching) (Osmond 1994; Häder et al. 1997; Hanelt et al. 1997). Otherwise, excess excitation energy can lead to irreversible photodamage or photooxidation with a loss of photosystem II (PSII) reaction centers (Demmig-Adams and Adams 1992, 1996; Aro et al. 1993). However, plants are able to respond to changing light regimes within hours to days by adjusting morphologically and physiologically (photoacclimation, e.g., Raniello et al. 2004; Marquardt et al. 2010; Aguilera and Rautenberger 2011). A common tool to quantify photosynthetic responses of seaweeds to different light conditions is the measurement of chlorophyll *a* fluorescence using pulse-amplitude modulated (PAM) fluorometry (Maxwell and Johnson 2000). Chlorophyll fluorescence is mostly produced by PSII, and the fluorescence pattern can be traced back to changes in the transfer of excitation energy to photochemistry (photochemical quenching) and energy dissipation (non-photochemical quenching). The chlorophyll fluorescence parameter maximum quantum yield of PSII (F_v/F_m) is widely used to assess the photosynthetic efficiency of PSII in dark-adapted leaves, and a decrease of which can be characterized as a result of photoinhibition (Demmig-Adams and Adams 1992; Maxwell and Johnson 2000).

Multiple studies investigated the effect of different irradiances on the photosynthesis of macroalgae and the potential of recovery after light stress exposure (García-Sánchez et al. 2012; Flores-Molina et al. 2014; Giovagnetti et al. 2018; Quintano et al. 2019). As benthic macroalgae, members of the genus *Caulerpa* are generally sensitive to high light radiation (Horstmann 1983; Ukabi et al. 2013; de Gaillande et al. 2017). Consistently, *C. lentillifera* has been found to thrive best under relatively low irradiances (10 to 100 μmol photons m^{-2} s^{-1}) of PAR and to show signs of photooxidation and photodamage under irradiances of 360 μmol photons m^{-2} s^{-1} (Guo et al. 2015a; Su et al. 2017; Kang et al. 2020). However, the physiological response of *C. lentillifera* to light irradiances over time spans > 1 week and the potential of recovery after light-induced stress exposure is still unknown, but crucial for farmers to adapt culture conditions accordingly.

For sea grape trade, the place of production and retail often differs from each other such as most of the fresh harvested seaweeds in Vietnam are exported to Japan via air freight (de Gaillande et al. 2017; Terada et al. 2018). During transport and retail, *C. lentillifera* is stored in a variety of different plastic materials. Due to the thermo-isolating properties of polystyrene (PS) (Aditya et al. 2017), containers of this material, with moisture sheets to counteract desiccation, are commonly used to pack sea grapes for shipment (Terada et al. 2018). However, for retailing to the end consumer, packaging in different plastic materials is common and the plastic properties can strongly influence the physiology of packed sea grapes (Tuong et al. 2016). In Vietnam, sea grapes are frequently stored in polyethylene terephthalate (PET) containers, having the advantage that costumers can see the product through the transparent material. PS and PET do differ not only in their transparency and thermal isolation (Aditya et al. 2017), but also in their properties regarding oxygen permeability (Zeman and Kubík 2007). During storage, algae are in danger of desiccation, leading to dehydration and consequently a loss of weight (Holzinger and Karsten 2013). Desiccation stress is in this effect comparable to salinity stress, because both result in a decrease of the alga's water potential (Kirst 1990). However in contrast to salinity stress, during desiccation, cellular ion ratios remain constant, while ion concentrations increase (Kirst 1990; Holzinger and Karsten 2013). Therefore, desiccation can result in osmotic and ionic stress, which might ultimately lead to an inhibition of the electron flow at different sites at the photosynthetic apparatus (Wiltens et al. 1978; Satoh et al. 1983; Xia et al. 2004; Gao et al. 2011). Inhibitions may lead to accumulation of reactive oxygen species (oxidative stress, Kumar et al. 2014) and potentially photodamage (Kirst 1990). Multiple studies showed the loss of water is negatively correlated with maximum quantum yield of PSII, but partly, the potential for recovery of F_v/F_m after re-hydration can be observed (Gao et al. 2011; Flores-Molina et al. 2014; Holzinger et al. 2015; Xu et al. 2016). In nature, intertidal seaweeds are exposed to air, e.g., during low tide, where the common strategy is to reduce the metabolic activities and cope with the desiccation stress. However, packed sea grapes have desiccation times of ~ 1 week. In the airfreight packaging environment (PS), F_v/F_m values of *C. lentillifera* were found to decline from values of > 0.7 to 0.60 ± 0.22 and 0.47 ± 0.26 after 4 and 8 days of desiccation, respectively. After packaging over 12 days, algae were considered dead with F_v/F_m values of 0.10 ± 0.10 and a water loss of 72% (Terada et al. 2018). In Nha Trang, Vietnam, common practice is packaging in transparent PET containers, where algae are, additionally to desiccation stress, exposed to surrounding irradiances, in contrast to light impermeable PS packages. Therefore, light and desiccation are mutually influencing sea grape physiology.

In this study, we investigate the influence of irradiances on sea grapes in the culture and packing environment. We hypothesized that photosynthesis of *C. lentillifera* is best under pond irradiance conditions of 50 μmol photons m^{-2} s^{-1} and would be negatively influenced by irradiances above 100 μmol photons m^{-2} s^{-1} but could be maintained by irradiances around 25 μmol photons m^{-2} s^{-1}. Additionally, we indented to answer the question, whether sea grapes can recover from the potential stress after being transferred back to more suitable light conditions. For the packaging experiment, we hypothesized that sea grapes transported under dark conditions

would physiologically suffer, because the non-cyclic photophosphorylation process of photosynthesis requires light in addition to a constant supply of water molecules. Furthermore, we expect that higher irradiances will cause physiological stress reactions, because desiccation might lead to a lack of water essential for photosynthesis. We are making a first attempt in defining the optimal irradiances for sea grapes in the packaging environment.

Material and methods

Sample collection

The experiments presented in this study were carried out during July to August 2019 and February to March 2020 at the laboratory facilities of the Institute of Oceanography in Nha Trang (12° 14′ 25.2″ N; 109° 11′ 55.6″ E), located in the Central South coast of Vietnam (Fig. 1). The experiments are referred to as "culture" and "packaging" experiment, as the influences of different PARs on sea grapes during culture and under the packaging environment were investigated. For the culture experiment, sea grapes were collected at a sea grape farm ("VIJA") at Van Phong Bay (12° 35′ 11.8″ N; 109° 13′ 26.7″ E) in the Khánh Hòa province. *Caulerpa lentillifera* samples for the packaging experiment were purchased from a local market in northern Nha Trang.

Chlorophyll *a* variable fluorescence measurements

Photosynthetic performance was determined in vivo by measuring variable chlorophyll *a* fluorescence using a portable Diving-PAM chlorophyll fluorometer (Walz, Germany). F_v/F_m was measured in 7 min dark-adapted sea grape fronds (Schreiber et al. 1995; Maxwell and Johnson 2000). Sea grapes were considered unstressed when F_v/F_m values were ≥ 0.7.

Culture experiment: experimental setup, measurements, and data analysis

Based on the measured sea grape pond conditions of 50 μmol photons m^{-2} s^{-1}, two additional irradiance treatments were designed (25 and 100 μmol photons m^{-2} s^{-1}). Following common practice at sea grape farms, the algae were cultured in tray culture, where sea grapes are placed between plastic meshes. Trays (18.5 × 9.5 cm) were stocked with an initial of 35.0 ± 1.0 g fresh sea grapes and grown out in natural seawater in an outdoor tank under natural solar irradiances for approximately 1 month prior the start of the experiment. Three aquaria (59 × 25 × 25 cm; 37 L, fitting 9 algae trays) for the three treatments and two aquaria (30 × 20 × 20 cm, 12 L,

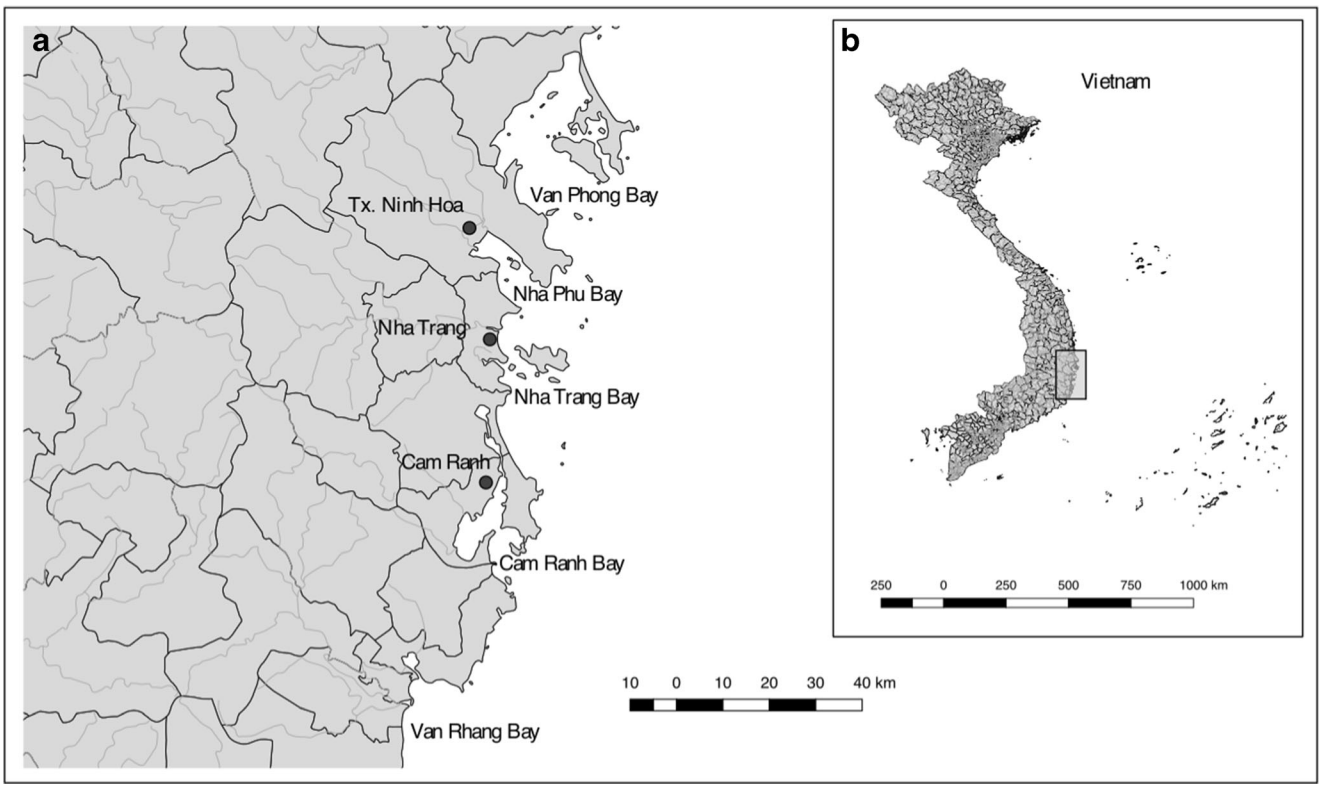

Fig. 1 **a** Coast around the city of Nha Trang and Van Phong Bay, where the VIJA sea grape farm is located. Each map has a scale bar at the bottom. **b** Map of Vietnam.

fitting 3 algae trays) for the recovery were set up with T5 High Output Fluorescence lights (2 × 39 W; 10,000 K) for illumination in a 12:12-h light:dark rhythm. The different irradiances were adjusted by adapting the height of lamps over the aquaria and monitored using a LI-1400 datalogger (LI-COR Biosciences, USA). Each treatment had a variation of ± 5 μmol photons m^{-2} s^{-1} within the aquaria. For the 25 μmol photons m^{-2} s^{-1} light treatment, gauze was additionally used for shading between the light source and the water surface. All aquaria were equipped with a constant air supply. Seawater from the adjacent coast was stored in a tank for water exchanges (every 2 days) in the experimental aquaria to ensure constant nutrient levels and water quality over the course of the experiment. Temperature, salinity, and pH were monitored to ensure constant conditions between and within aquaria. Prior to the start of the experiment, algae were acclimatized for 2 days (50 ± 5 μmol photons m^{-2} s^{-1}, 27.2 ± 0.4 °C, S_A 34.6 ± 0.5, pH 8.5 ± 0.3). During the experiment, changes in F_v/F_m were measured using a Diving-PAM fluorometer. F_v/F_m values were taken for each tray on the initial day of the experiment, as well as on days 1, 7, 14, and 21. In order to examine the potential of recovery after potential light-induced physiological stress, three replicates per treatment were transferred to the additional recovery aquaria (50 μmol photons m^{-2} s^{-1}) on days 7 and 14. F_v/F_m was monitored right before transfer and after 1 and 7 days under recovery irradiances (50 μmol photons m^{-2} s^{-1}). For statistical analysis, F_v/F_m of sea grape trays were averaged as mean and standard deviation (SD) per treatment (n = 3). Statistical differences between the treatments were analyzed using one-factor ANOVA (followed by Tukey's HSD test) with the fixed factor "treatment" (levels 50, 100, 25 μmol photons m^{-2} s^{-1}) which was conducted for each day of measurement over the experimental course between the alga groups without transfer to recovery, with recovery after 7 and 14 days, respectively. Analyses were conducted with a significance level of $P < 0.05$. All statistical tests were conducted in R Core Team (2019), and graphics were produced using ggplot2 (Wickham 2016).

Packaging experiment: experimental setup, measurements, and data analysis

The purchased sea grape fronds were already cut from the stolon, as common practice for consumption and retail of the fresh product. Sea grapes were acclimated in sea water (28.2 °C, S_A 34.2, pH 8.5) under room irradiances for 3 days prior start of the experiment. Four sea grape fronds were placed on the long side of PET containers (9 × 9 × 15 cm, capacity of 500 g) not attached to each other. A moisture sheet in each container kept the humidity constant at 100%. Initial F_v/F_m were measured for 50 randomly chosen fronds from the batch and initial biomass as wet-weight for sea grapes of each container was taken. Wet-weight and F_v/F_m values of the stored sea grapes were quantified after storage of 2, 4, 8, and 12 days under three different irradiances (darkness 0, room irradiance 3 ± 5, and high irradiance 70 ± 5 μmol photons m^{-2} s^{-1}). Five replicates per irradiance treatment for each time period were prepared. The containers for the dark treatment were wrapped in aluminum foil, and the caps were colored with black spray. A T5 High Output Fluorescence light (2 × 39 W; 10,000 K) was placed over the containers of the high and medium light treatment, and adjustments of the heights of the lamp ensured an irradiance of 70 ± 5 μmol photons m^{-2} s^{-1} of PAR in a 12:12-h light:dark rhythm. Temperature loggers (HOBO, USA) were placed in one container of each treatment to monitor the temperature over the course of the experiments in 30-min intervals. In order to determine the potential of recovery, the sea grapes were re-immersed in seawater under room irradiances of 3 ± 5 μmol photons m^{-2} s^{-1} after the desiccation period and F_v/F_m values were quantified 10 min, 3 h, 6 h, and 24 h after re-immersion. Percentage of difference in F_v/F_m over recovery period was calculated following the formula:

$$\text{Percent of initial after desiccation}(\%) = F_v/F_{mt} \times (100/F_v/F_{m\,i}) - 100,$$

with F_v/F_{mt} being measured after time t of desiccation and subsequent 24 h of re-immersion in seawater and $F_v/F_{m\,i}$ being the value measured directly after desiccation. Moisture content after each desiccation period (M_t %) was calculated following the formula:

$$M_t(\%) = ((W_i - W_t)/W_i) \times 100,$$

with W_i as the initial wet-weight of sea grapes after moisture removal at start of the experiment, and W_t as the wet-weight after desiccation period t in days (Seremet et al. 2016; Terada et al. 2018).

The additional irradiance treatment of 20 μmol photons m^{-2} s^{-1} was quantified following the same protocol described above. However, physiological response was only quantified by F_v/F_m values and recovery potential and moisture content were not conducted. The results are therefore presented separately as comparison between the three light treatments (3, 20, and 70 μmol photons m^{-2} s^{-1}). For statistical purposes, F_v/F_m of sea grapes were averaged per container and mean and SD were calculated (n = 3–5). Outliers were identified using Grubbs' test. Differences in F_v/F_m and moisture content of sea grapes measured after the desiccation period were compared between treatments on each day with a one-factor ANOVA (followed by Tukey's HSD test) and the fixed factor "treatment" (levels 0, 3, 70 μmol photons m^{-2} s^{-1}). In order to test for differences in F_v/F_m of sea grapes over the desiccation and recovery period, a one-factor ANOVA (followed by Tukey's honestly significant difference test) with the fixed factor "period" (levels "initial," "after desiccation period," "after 24 h recovery") was conducted and differences between the three light treatments were tested using a fixed term "treatment"

(levels 0, 3, 70 μmol photons m^{-2} s^{-1}). In all cases, Levene and Shapiro-Wilk tests were carried out, and if requirements for ANOVA were not met, a Kruskal-Wallis test (followed by pairwise Dunn test with Bonferroni correction) was conducted. Analyses were conducted with a significance level of $P < 0.05$. All statistical tests were conducted in R Core Team (2019), and graphics were produced using ggplot2 (Wickham 2016).

Results

Culture experiment

At the farm facility in Van Phong Bay, algae were maintained in shaded tidal ponds (~ 50 μmol photons m^{-2} s^{-1}), with F_v/F_m values indicating a good physiological state (≥ 0.7, unpublished data). Temperature in the experimental aquaria showed a mean of 28.4 ± 1.2 °C. Salinity and pH values ranged from 34.5 to 37.5 and 8.4 to 9.0, respectively. Initial F_v/F_m of all three treatments (25, 50, and 100 μmol photons m^{-2} s^{-1}) were similar, with values between 0.67 ± 0.02 and 0.7 ± 0.02 (Fig. 2). F_v/F_m of sea grapes cultured under 25 and 50 μmol photons m^{-2} s^{-1} did not change significantly from each over the 21 experimental days ($P > 0.05$). However, F_v/F_m of sea grapes exposed to 100 μmol photons m^{-2} s^{-1} was significantly lower after 7 (0.54 ± 0.06), 14 (0.54 ± 0.08), and 21 days (0.63 ± 0.03) than that of sea grapes under 25 and 50 μmol photons m^{-2} s^{-1} (≥ 0.70 ± 0.03), respectively (Fig. 2). However, algae cultured under 100 μmol photons m^{-2} s^{-1} showed a trend of increase in F_v/F_m values from day 14 to 21 of 0.09. After sea grapes were transferred from 100 μmol photons m^{-2} s^{-1} to recovery

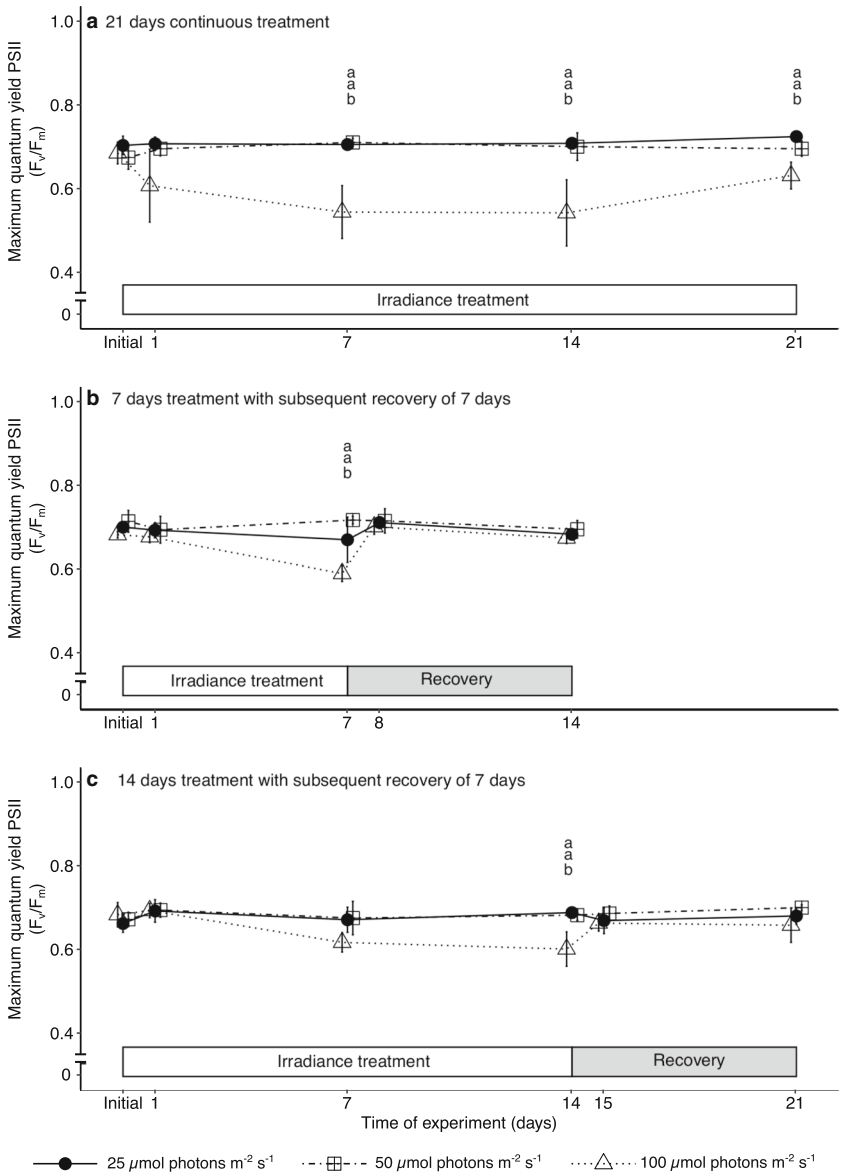

Fig. 2 Chronological change of maximum quantum yield of PSII (F_v/F_m) of *Caulerpa lentillifera* exposed to three different irradiance treatments (25, 50, 100 μmol photons m^{-2} s^{-1}). The potential of recovery under control conditions (50 μmol photons m^{-2} s^{-1}) after exposure to different treatments has been investigated at different time steps: **a** 21 days continuous treatment without transfer to recovery conditions, **b** 7 days treatment with subsequent recovery of 7 days, and **c** 14 days treatment with subsequent recovery of 7 days. White and gray bars indicate the time of exposure to the different treatment irradiances or recovery, respectively. Data are mean values ± SD ($n = 3$). Letters indicate significant differences between treatments (one-factor ANOVA followed by Tukey's HSD, $P < 0.05$) and are assigned to treatments top down according to order in graph

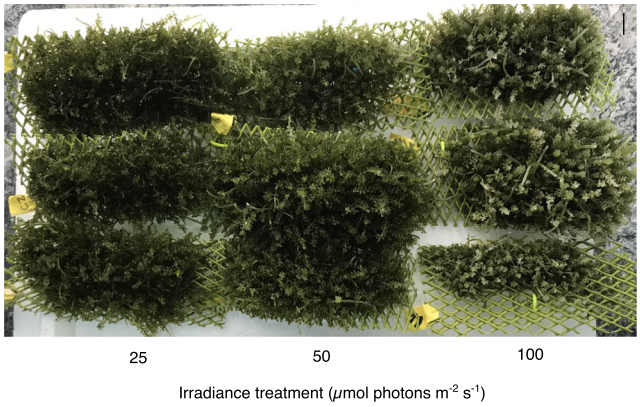

Fig. 3 Photograph of sea grape (*Caulerpa lentillifera*) trays ($n = 3$) after exposure to irradiances of 25, 50, and 100 μmol photons m^{-2} s^{-1} for 21 days. Black scale bar in the right top corner represents 2 cm

conditions (50 μmol photons m^{-2} s^{-1}) after 7 and 14 days of exposure, F_v/F_m increased instantaneously by 0.11 and 0.06 over 1 day and no significant difference between all three treatments was observed. Sea grapes under high irradiances (100 μmol photons m^{-2} s^{-1}) showed a fading of color after 21 days of culture (Fig. 3).

Packaging experiment

The temperature measured by HOBO loggers in the packaging containers did not vary between the three treatments (25.8 ± 0.5 °C, 25.7 ± 0.4 °C, and 26.8 ± 0.8 °C for 0, 3, and 70 μmol photons m^{-2} s^{-1}, respectively). Sea grapes were in a good physiological state at the start of the experiment (0.74 ± 0.03, $n = 50$). F_v/F_m developed differently between

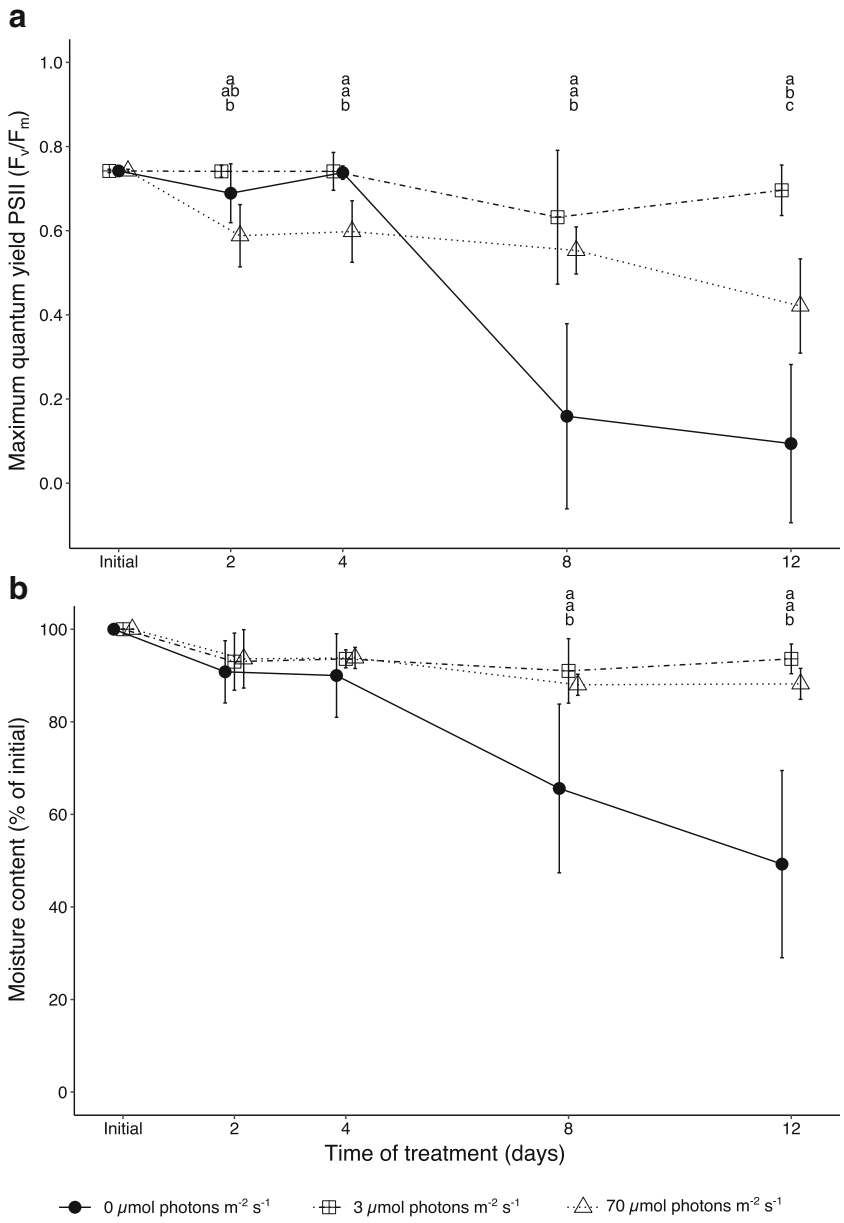

Fig. 4 **a** Maximum quantum yield of PSII (F_v/F_m) and **b** moisture content (percentage of initial) of *Caulerpa lentillifera* packed in transparent polyethylene terephthalate (PET) containers exposed to three different irradiances (0, 3, 70 μmol photons m^{-2} s^{-1}) over a period of 12 days, respectively. Data represent mean values ± SD ($n = 4$–5). Letters indicate significant differences between treatments (one-factor ANOVA followed by Tukey's HSD or Kruskal-Wallis test followed by pairwise Dunn test with Bonferroni correction, $P < 0.05$) and are assigned to treatments top down according to order in graph

treatments over the desiccation period (Fig. 4a). Sea grapes under room irradiance (3 μmol photons m^{-2} s^{-1}) showed only a slight decrease of F_v/F_m to 0.70 ± 0.06 after 12 days of desiccation with moisture content not dropping below 91.0 ± 7.0% (Fig. 4b). However, desiccation over 2 days under an irradiance of 70 μmol photons m^{-2} s^{-1} leads to significantly decreased F_v/F_m of 0.59 ± 0.07 compared to room irradiances. The decrease continued to a value of 0.42 ± 0.11 after 12 days. However, F_v/F_m values showed a trend of recovery after re-hydration under room irradiances. The moisture content after 12 days under 70 μmol photons m^{-2} s^{-1} was with 88.2 ± 3.3%, only slightly lower than in the treatment of irradiance of 3 μmol photons m^{-2} s^{-1}. Under exclusion of light, F_v/F_m values remained stable over the first 4 days (0.74 ± 0.02) but decreased rapidly after 8 and 12 days of packaging to significantly lower values compared to other two treatments (0.16 ± 0.22 and 0.10 ± 0.19, respectively). Exemplary pictures of sea grape fronds depict strong differences in thallus structure when packed under darkness; therefore, two pictures were provided for desiccation period of 8 and 12 days (Fig. 5). No recovery of F_v/F_m was observed, but rather a further decrease of the values (Fig. 6, absolute values see Online Resource 1). Moisture content decreased strongly from 90 ± 9.0% (4 days) to 49.25 ± 20% (12 days) (Fig. 4b). Sea grapes under 20 μmol photons m^{-2} s^{-1} had constantly slightly lower F_v/F_m values than algae under room irradiances (Fig. 7). This difference was significantly lower 4 days under packaging ambience with 0.61 ± 0.04. However, F_v/F_m values were consistently higher than of sea grapes under 70 μmol photons m^{-2} s^{-1}.

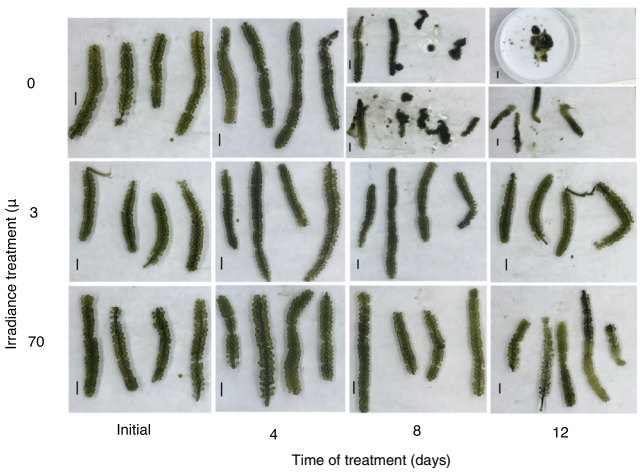

Fig. 5 Pictures of *Caulerpa lentillifera* packed in polyethylene terephthalate (PET) containers from initial state, and after 4, 8 and 12 days under irradiance treatments 0, 3, and 70 μmol photons m^{-2} s^{-1}. After days 8 and 12 under packaging ambience in darkness, sea grapes have very different thallus structures; therefore, two pictures are presented in order to demonstrate the pigmentation ranges of different desiccation stages of algae. Black scale bar in the left corner of each picture represents 1 cm

Discussion

In this study, we found that light irradiances have a considerable impact on sea grapes' physiological constitution, both in the culture as well as in the packaging environment. Inappropriate irradiances seem to adversely affect the alga's physiology. However, in some cases, the sea grapes have the potential to recover. We used PAM fluorometry with F_v/F_m and can confirm that this tool is suitable to quantify the physiological state of *C. lentillifera* (Guo et al. 2015a, b; Terada et al. 2018).

Culture experiment

Based on the results of the culture experiment, we can confirm our hypothesis that sea grapes thrive best under irradiances of 25 and 50 μmol photons m^{-2} s^{-1} by maintaining their F_v/F_m values over the course of 21 days, indicating that they were in a good physiological state and not negatively impacted by the irradiances they were exposed to. These results are in line with studies identifying *C. lentillifera* and other representatives of the genus *Caulerpa* (e.g., *C. racemosa*) as shade-adapted low light plants, which is evident for some benthic seaweeds (Horstmann 1983; Ukabi et al. 2013; de Gaillande et al. 2017). Furthermore, the decline in F_v/F_m under 100 μmol photons m^{-2} s^{-1} accompanied by the observed bleaching of the fronds is in line with observations by Guo et al. (2015b). The authors observed a decline in F_v/F_m of 0.16 in sea grape fronds over 7 days exposure to 100 μmol photons m^{-2} s^{-1} along with a significant decrease in chlorophyll *a* content. The abrupt decrease in F_v/F_m as a consequence of high irradiances is a characteristic sign of photoinhibition (Goh et al. 2012) and has been observed widely in different temperate species of the genus *Caulerpa* (Ukabi et al. 2013) and also in *C. lentillifera* (Guo et al. 2015b). However, the immediate and full recovery of F_v/F_m values of *C. lentillifera* within 24 h after transfer to recovery conditions demonstrates the ability of the sea grapes to rapidly restore previous photosynthetic efficiency after certain stress exposure (Osmond 1994; Häder et al. 1997; Hanelt et al. 1997). This process of recovery from high irradiances was also observed in other green macroalgae (e.g., *Ulva rotunda*; Franklin et al. 1992). Han et al. (2007) found *Ulva pertusa* and *Umbraulva japonica* showing a decline of F_v/F_m values with exposure to increasing doses of PAR. Subsequent recovery under dim light increased F_v/F_m within 24 h completely and partially in connection with the habitat-related sensitivity, respectively. *Ulva pertusa* thrives in the intertidal, comparable with *C. lentillifera* (Norashikin et al. 2013). However, intertidal algae are exposed to highly fluctuating environmental conditions (Davison and Pearson 1996) and an elasticity of light requirements for photosynthesis might therefore be a coping mechanism of the seaweed survival, potentially related to their xanthophyll cycle or antioxidant activity (Han et al. 2003). The

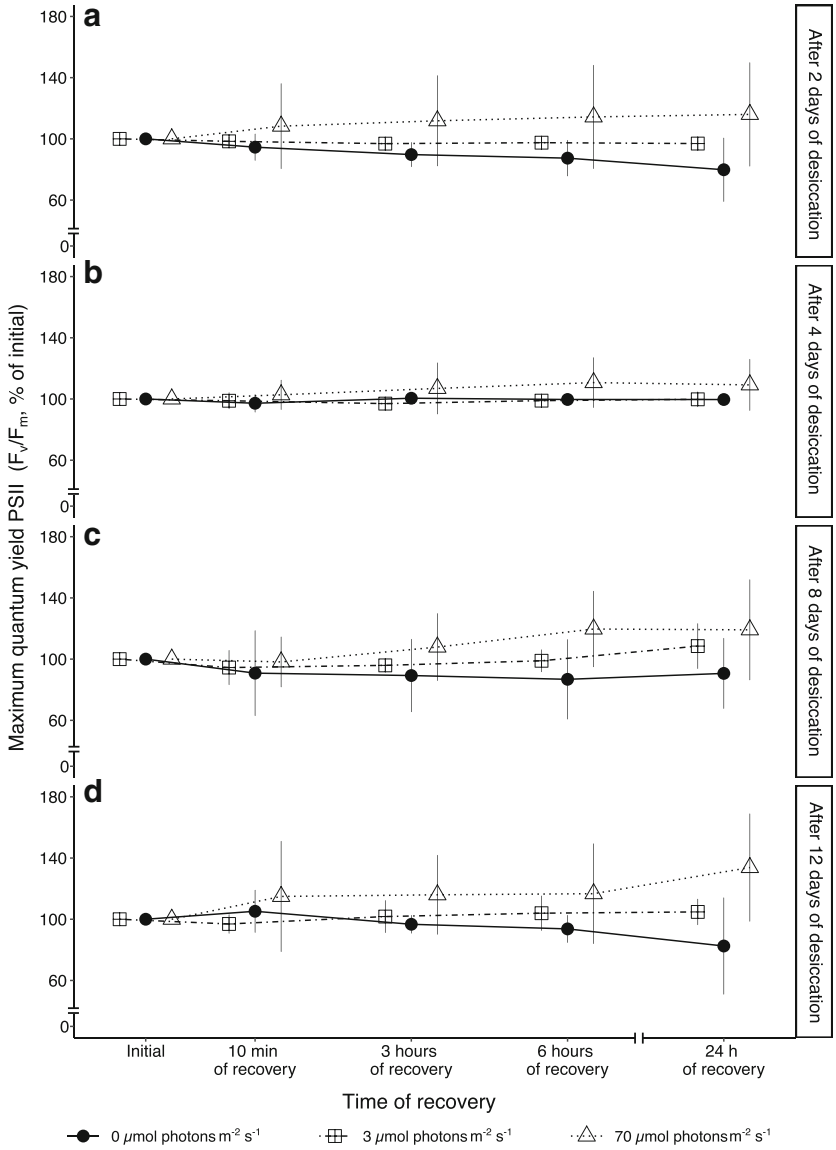

Fig. 6 Chronological development of maximum quantum yield of PSII (F_v/F_m as percentage of initial) of *Caulerpa lentillifera* under re-hydration conditions (10 min–24 h) at an irradiance of 3 μmol photons m^{-2} s^{-1} after desiccation period in transparent polyethylene terephthalate (PET) containers of **a** 2, **b** 4, **c** 8, and **d** 12 days under three different irradiances (0, 3, 70 μmol photons m^{-2} s^{-1}) is depicted. Calculations of percentage of initial relate to absolute F_v/F_m values measured at the end of the desiccation and the start of the recovery period (Online Resource 1). Data represent mean values ± SD (n = 5). No significant differences were found (one-factor ANOVA followed by Tukey's HSD or Kruskal-Wallis test followed by pairwise Dunn test with Bonferroni correction, P < 0.05)

increase of F_v/F_m within the third week under 100 μmol photons m^{-2} s^{-1} might potentially be due to a long-term acclimation of *C. lentillifera* to the changed irradiance environment. Long-term photoacclimation as an answer to changes in photo-regime, e.g., through morphological and physiological alternations, has been observed in several *Caulerpa* species (e.g., Horstmann 1983; Riechert and Dawes 1986; Raniello et al. 2004, 2006; Malta et al. 2005; Marquardt et al. 2010). Raniello et al. (2004) describe the capacity of *C. racemosa* to reorganize the photosynthetic apparatus, change pigment composition, and eventually display different photosynthetic traits in relation to light availability over seasons and in the canopy. The observed trends are particularly interesting taking into account the economic value of sea grapes. Photoinhibition can decrease productivity and growth and therefore critically impact the harvest of *C. lentillifera* (Goh et al. 2012). However, if sea grapes have the capacity to acclimate to higher irradiances, farmers could use the opportunity to their benefits. Therefore, this potential capacity should be explored further.

Packaging experiment

We attempted to contribute in defining suitable storage irradiances for sea grapes. The stable F_v/F_m values with only minimal loss of moisture content of *C. lentillifera* stored under room irradiances (3 μmol photons m^{-2} s^{-1}) in PET containers suggest a good physiological state of the alga and thus a sufficient quality of the product for the end consumer even after 12 days of storage. However, Terada et al. (2018) found F_v/F_m of *C. lentillifera* packed in PS containers (irradiances of 3 μmol photons m^{-2} s^{-1}) declining to 0.10 ± 0.10 along with 72% critical water loss and absence of recovery after re-

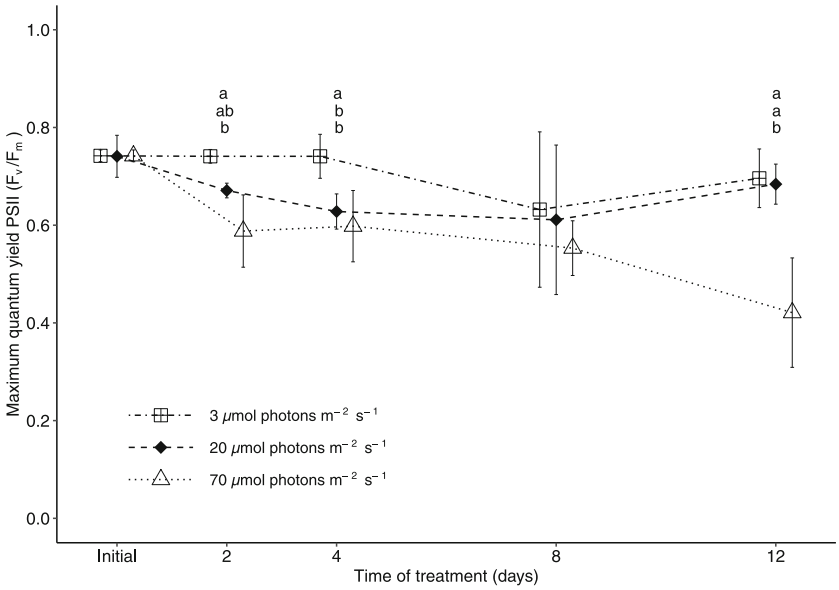

Fig. 7 Maximum quantum yield of PSII (F_v/F_m) of *Caulerpa lentillifera* during desiccation period packed in transparent polyethylene terephthalate (PET) containers under three different irradiances (3, 20, 70 μmol photons m^{-2} s^{-1}) for a period of 2, 4, 8, and 12 days. Data are mean values ± SD (n = 3–5). Letters indicate significant differences between treatments (one-factor ANOVA followed by Tukey's HSD or Kruskal-Wallis test followed by pairwise Dunn test with Bonferroni correction, $P < 0.05$) and are assigned to treatments top down according to order in graph

immersion in sea water. The authors suggest this might be caused by cellular alterations resulting in dysfunctional algae. These results imply potentially more favorable conditions of storage in PET than PS containers. However, potential explanations for the strong deviation between the results are the properties of packaging materials (PET vs PS) and different storage temperatures (~ 26 °C vs 20 °C). Polymer type of containers has been found to influence the amount of total aerobic bacteria on packed sea grapes (Tuong et al. 2016), possibly due to differences in gas and especially oxygen permeability (Zeman and Kubík 2007; Siracusa 2012). Accordingly, the microbial community was also found to influence the postharvest physiology of seaweeds (Liot et al. 1993).

Our hypothesis that packaging of sea grapes under dark (0 μmol photons m^{-2} s^{-1}) as well as high light (70 μmol photons m^{-2} s^{-1}) environments negatively influences the physiological status of *C. lentillifera* was supported by the results. Absence of light clearly constitutes a source of limitation stress for the seaweeds. Insufficient irradiance leads to a lack of carbon assimilation by plants, and under carbohydrate starvation, plants have to substitute sugar with protein and lipids before running out of energy to sustain metabolism (Brouquisse et al. 1998; Lavaud et al. 2020). However, some polar seaweeds have been found to be adapted to survival under extended periods of darkness, e.g., through substantial starch storages (Gómez et al. 1997; Weykam et al. 1997; Wiencke et al. 2007). Other plants are physiologically not that well equipped for extended dark or even light limiting periods, as studies on, for example, *Laminaria*, sea grasses, and microalgae show (Smayda and Mitchell-Innes 1974; Dieck 1993; Silva et al. 2013). *Caulerpa paspaloides* was found to have significant lower starch concentrations following overwintering, along with stolons forming a higher percentage of the whole thalli biomass compared to the alga's fronds (O'Neal and Prince 1988). Thus, cutting of the sea grape's stolons before packaging might even decrease carbon storage of the algae and therefore adversely affect survival. Over 4 days of packaging in darkness, sea grapes were still active with minor loss of water, indicating sufficient storage of essential nutrients. But the rapid decrease of moisture content over 8 and 12 days of packaging with simultaneously declining F_v/F_m values and without potential of recovery indicates an irreversible damage of the photosystem. However, a high variability in moisture contents, F_v/F_m values, and thallus structure (soft vs intact) after 8 and 12 days of desiccation under dark conditions might be traced back to unequal nutrient storages of the organisms. Interestingly, the decreased photosynthetic performance provoked by high light stress was reversible under recovery conditions, whereas induced by darkness, a further decrease of F_v/F_m was observed. This indicates that desiccation under darkness distinctively affected the ultrastructure of the sea grape's membrane (Davison and Pearson 1996; Holzinger and Karsten 2013; Flores-Molina et al. 2014), whereas the thalli under light stress were still intact but showed a faster decrease of F_v/F_m. Desiccation and the resulting hypersalinity in the cells seem to affect the process of photosynthesis at different steps. It might have restricted the inflow of water molecules as essential electron donor at the water splitting side of PSII, as well as interrupting the electron transport from PSII to PSI and energy transfer between pigments (Satoh et al. 1983; Gao et al. 2011). The reduced ability to use absorbed light energy requires a corresponding increase in processes that dissipate excess solar energy to avoid damage (Davison and Pearson 1996). Consequently, desiccation stress seems to lower the threshold of increased non-photochemical quenching occurrence caused by high irradiances. This observation could explain the successive decrease of F_v/F_m with increasing irradiances (3, 20,

70 μmol photons m^{-2} s^{-1}), which was observed under desiccation conditions. The decreased F_v/F_m under 20 μmol photons m^{-2} s^{-1} compared to room irradiances in the packaging ambience on one hand and stable photosynthesis activity under similar irradiances under immersed conditions suggests that energy absorption exceeded the limit to be used in photochemical quenching under the desiccation packaging conditions. The potential of recovery and the apparently intact thallus structure, however, imply that no lasting photodamage appeared, but that protective mechanisms were still intact.

Conclusion

Our objective to investigate suitable irradiances for sea grapes in culture and packaging conditions resulted in certain recommendations for sea grape farmers and retailers. For outdoor sea grape culture, our results suggest that shading of sea grapes is beneficial. Additionally, PET containers equipped with moisture sheets seem to be a suitable opportunity for the product's storage over at last 12 days, but the additional provision of a dim light environment is essential to maintain a good physiological state of *C. lentillifera* and therefore offer a fresh product of high quality to the end consumer.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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