Optimizing cultivation and shipping environments for an edible green alga, Caulerpa chemnitzia var. laetevirens (Bryopsidales) from Japan: Effects of temperature, irradiance, desiccation, and salinity on photochemical efficiency

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Abstract

The effects of four stressors, temperature, irradiance, desiccation, and salinity, on the photochemical efficiency ($\Delta F/F_m'$) of a green alga, *Caulerpa chemnitzia* var. *laetevirens* from Kagoshima, Japan were determined for optimizing cultivation and shipping environments using a pulse amplitude modulation (PAM)-chlorophyll fluorometer. The $\Delta F/F_m'$ remained stable at 24–34°C during the 3-d temperature exposures ranging from 8–36°C; however, it dropped at higher and lower temperatures. During continuous 6-h exposure to irradiance levels of 400 (low), and 1000 (high) µmol photons m^{-2} s^{-1} at 16, 24, and 32°C, the decline of $\Delta F/F_m'$ was pronounced at high irradiance. Moreover, the $\Delta F/F_m'$ also dropped at 16°C even under low irradiance, suggesting the occurrence of low temperature–light stress. Desiccation experiments under 50% humidity and up to 5-h of aerial exposure at 24°C and dim-light (20 µmol photons m^{-2} s^{-1}) indicated that $\Delta F/F_m'$ was well tolerated within 1-h of desiccation; however, it dropped quickly as the desiccation period prolonged, suggesting that if more than 20% of the thallus interior water was lost, $\Delta F/F_m'$ dropped progressively. However, during a similar experiment up to 14 days of aerial exposure under saturated humidity (99%) and dim-light (12L12D photoperiod), as the interior water of the thallus was maintained due to the saturated humidity, $\Delta F/F_m'$ seemed to be well maintained for up to 5 days. Like desiccation, this alga exhibited stenohaline characteristics (30–40 psu) under the salinity gradient of 0–80 psu up to 7 days of culture at 24°C and dim light (12L12D). In conclusion, this alga can be cultivated by modifying the techniques used for cultivating Japanese *Caulerpa lentillifera* and adjusting its environment accordingly. To ensure appropriate shipping conditions, it is essential to maintain saturated humidity in a plastic container at room temperature.

Introduction

The species of *Caulerpa* (Bryopsidales) are mainly distributed in tropical and subtropical regions around the world, with some species found in warm temperate regions (Prud'homme van Reine et al. 1996; Yoshida 1998; Belton et al. 2019; Zubia et al. 2020). Some *Caulerpa* species are consumed as food, particularly in Japan (Ohno and Largo 1998; Pereira 2016; Zubia et al. 2020). *Caulerpa lentillifera* J. Agardh, is extensively cultivated in coastal facilities in the subtropical Ryukyu Islands, spanning the prefectures of Okinawa and Kagoshima (Ohno and Largo 1998; Shiroma 2012; Pereira 2016; Terada et al. 2018, 2021b). The cultivation techniques for this alga have been developed in Japan since the 1990s (Toma 1991, 2012; Shiroma 2012), due to an increase in consumer demand and to ensure a sustainable resource; the cultivation of this alga has also spread to southeast Asian countries (e.g., Philippines, Vietnam, Thailand, etc.; Trono 1998, 1999; Minh et al. 2019; Stuthmann et al. 2021). Hence, numerous comprehensive studies have contributed to the improvement of its cultivation (Kurashima et al. 2003; Matanjun et al. 2009; Paul et al. 2014; Liu et al. 2016; Guo et al. 2015a, b; Terada et al. 2018, 2021b; Stuthmann et al. 2021). For example, although this alga has a low tolerance to desiccation, harvested algae can be packed without seawater by using a simple moisture sheet in a plastic container and can be shipped and handled at room temperature for up to one week (Terada et al. 2018, 2021b). Additionally, the response of photosynthesis to temperature and irradiance has also been reported and has contributed

to optimizing the light and temperature environment for sustainable cultivation (Kurashima et al. 2003; Terada et al. 2018, 2021b).

In Japan, several other species of *Caulerpa*, such as *C. chemnitzia* var. *laetevirens* (Montagne) Fernández-García et Riosmena-Rodriguez (formerly known as *C. racemosa* var. *laetevirens*; Belton et al. 2014; Fernández-García et al. 2016; Norris et al. 2017), are also found, and these species are also distributed in the warm temperate regions of Japan (Yoshida 1998). In fact, while *C. chemnitzia* var. *laetevirens* is also important food resource in Japan, its utilization is limited to natural resources and harvest is limited to particular regions of Japan. Although culture and ecophysiological studies have been reported for this alga (Enomoto and Ohba 1987; Ohba and Enomoto 1987; Kurashima et al. 2003), knowledge of its adaptation and tolerance to regional environments remains insufficient.

Over the past few years, we have elucidated how algae respond to various environmental factors, including temperature, irradiance, desiccation, and salinity gradients using a pulse amplitude modulation (PAM)-chlorophyll fluorometry (Borlongan et al 2020; Ito et al. 2021, 2023; Shindo et al. 2022a, b; Terada et al. 2021a, b). These studies aim to better understand the adaptation of algae to their natural habitats, with a particular focus, which is to optimize cultivation. Indeed, our previous studies on *C. lentillifera* have elucidated the effects of temperature, irradiance, and desiccation on photosynthesis (Terada et al. 2018, 2021b). These findings were effective in evaluating the optimal cultivation and shipping environments to maintain freshness and these studies are also essential for *C. chemnitzia* var. *laetevirens* to establish commercial-based cultivation.

In this study, we focused on elucidating the effect of four stressors, temperature, irradiance, desiccation, and salinity gradients on the photochemical efficiency of *C. chemnitzia* var. *laetevirens* to elucidate optimal environmental conditions for cultivation and shipping. We believe that these insights will contribute to the establishment of effective cultivation techniques for this alga.

Materials and methods

Sample collection and stock maintenance

The samples of *C. chemnitzia* var. *laetevirens* (ca. 600 g fresh weight) were collected using SCUBA from the natural population located 3 m deep near Kagoshima University Azumacho Marine Station at Nagashima Island (32.220780 N, 130.178520 E), Kagoshima Prefecture, Japan on 17 August 2022. The collected algae were immediately transported in a 20 L plastic bottle filled with seawater to the Laboratory of Marine Botany at Kagoshima University. The samples were kept in an aquarium tank (60 L), a salinity of 33 psu, pH 8.0, irradiance of 50 µmol photons m^{-2} s^{-1} (14L10D photoperiod) until examination. The algae were continuously maintained under these conditions until all experiments were completed, which took two months.

The effect of temperature on the photochemical efficiency of PSII

The chlorophyll fluorescence measurements (Mini Imaging-PAM, Heinz Walz GmbH, Effeltrich, Germany) followed the procedures outlined in our previous studies (Terada et al. 2021b, Shindo et al. 2022a). Portions of erect fronds (> 1 cm-long) were cut and acclimated for a few days to allow the wounded cell wall to recover (the same shall apply hereinafter). One day prior to the experiment, the samples were kept overnight in the dark at 24°C to ensure complete acclimation. Next, samples (n = 10 per temperature level) were placed in 300-mL flasks and cultured in the incubators (Eyela MTI-201B, Tokyo Rikakikai Co., LTD., Tokyo, Japan) at ten different temperatures (8, 12, 16, 20, 24, 28, 32, 34, 35, and 36°C) with an irradiance of 50 µmol photons m^{-2} s^{-1} (12L12D photoperiod) for 3 days (the date of cultivation start: 0 day).

The effective quantum yields of photosystem II (PSII), represented as $\Delta F/F_m' = (F_m' - F) / F_m' = \Phi_{PSII}$, were measured daily over a 3-day culture period at each temperature level. Samples were randomly selected for each measurement and placed in sterilized natural seawater in a petri dish (9 cm diam.) on an aluminum block incubator (BI-536T, Astec, Fukuoka, Japan). Temperature during the measurements was controlled using the same aluminum block incubator, which was monitored with a thermocouple (testo 925, testo AG, Lenzkirch, Germany). Initial values of $\Delta F/F_m'$ were also measured prior to starting the culture experiments to establish the initial state; however, these data were not included in the results.

Effect of continuous irradiance exposures on $\Delta F/F_m'$ at different temperatures, and their potential of recovery

The $\Delta F/F_m'$ of the samples was monitored using a Mini Imaging-PAM for a 6-hour exposure to different temperature and irradiance levels, followed by an additional 12-hour acclimation period under dim-light conditions (20 µmol photons m^{-2} s^{-1}; Terada et al. 2021b; Shindo et al. 2022a). One day prior to the experiment, the samples were pre-incubated overnight (12 hours) in the dark at each temperature treatment (16, 24, and 32°C). Before the start of experiment, the $\Delta F/F_m'$ values were measured to obtain initial state for each irradiance (400 and 1000 µmol photons m^{-2} s^{-1}) and temperature treatment (16, 24, and 32°C). Thereafter, samples were placed in separate beakers (200 mL) containing sterile natural seawater maintained at a specific temperature in a water bath (14L) with a circuit chiller (Coolnit CL-150R, Taitec, Inc., Tokyo, Japan). The exposure to 400 and 1000 µmol photons m^{-2} s^{-1} (metal-halide lamp) lasted for 6 hours, during which the samples were continuously stirred using a magnetic stirrer to uniformly expose each replicate to the light. The $\Delta F/F_m'$ (n = 10 per temperature/irradiance treatment) was measured every 2 hours of continuous exposure to each irradiance treatment. After the 6-hour exposure, the samples were acclimated to dim-light conditions (20 µmol photons m^{-2} s^{-1}) for 12 hours (similar to the duration of night in their natural state) at their experimental temperatures. After the 2-hour and 12-hour dim-light acclimation (i.e., 8 and 18 hours from the initial state), the $\Delta F/F_m'$ was measured again to confirm the potential recovery of PSII.

The chronic effect of desiccation on the photochemical efficiency under 50% humidity and the potential of recovery after rehydration in seawater

This experiment was conducted based on the procedures outlined in our previous studies (Terada et al. 2021a, b; Sato et al. 2022; Shindo et al. 2022b). The $\Delta F/F_m'$ of the samples was monitored using a Mini Imaging-PAM during each desiccation period and its subsequent rehydration period in seawater.

Forty explants of the erect frond were used for the experiment. One day prior to the experiment, the explants were pre-incubated in the dark at 24°C for 12 hours. After measuring the initial state of $\Delta F/F_m'$ *in situ* for all explants, five explants were randomly assigned to each eight different desiccation levels: 0 (always immersed), 0.17 (10-min), 0.5 (30-min), 1, 2, 3, 4, and 5-hour periods of air exposure. The surface moisture on the samples was wiped off using Kimwipes (Nippon Paper Crecia Co., Ltd, Tokyo, Japan) at the start of the experiment, and each explant was placed on a separate petri dish without seawater for the respective desiccation treatment. Throughout the experiment, the temperature, irradiance, and humidity were maintained at approximately 24°C, 20 µmol photons m^{-2} s^{-1}, and 50%, respectively. Humidity was measured using a hygrometer (Testo 610, Testo AG, Lenzkirch, Germany). Subsequently, $\Delta F/F_m'$ was measured for each desiccation treatment (n = 10 per explant; 50 measurements in total for each desiccation treatment, with n = 50 per period, using five explants per treatment). Since the desiccation process in the thallus was not uniform, ten different portions were measured for each individual.

To examine the $\Delta F/F_m'$ status and potential recovery of desiccated thalli following rehydration in seawater after each aerial exposure period, the $\Delta F/F_m'$ was determined (n = 50 per desiccation period) under four different conditions: *in situ* initial state, after each desiccation treatment, 30 minutes after rehydration in seawater, and 24 hours after rehydration in seawater.

The effect of chronic desiccation on water loss from the thalli was examined in the dehydrated state, as well as after 30-minute and 24-hour rehydration periods. The relative water content (RWC, %) after each desiccation treatment is as follows:

$$RWC\,(\%) = \frac{(W_t - W_d)}{(W_0 - W_d)} \times 100$$

1

where W_t is the weight of the alga at time t after desiccation, W_0 is the initial weight of the alga (i.e., the wet weight after the gentle removal of surface moisture), and W_d is the dry weight of the alga after drying at 60°C in a sterilizer (MOV-202S, Sanyo Electric Co., Ltd., Osaka, Japan) for 48 hours (Terada et al. 2021 b; Shindo et al. 2022 b). The weight of the samples was measured using an analytical balance (AP 125WD, Shimadzu Corporation, Kyoto, Japan).

The chronic effect of desiccation on the photochemical efficiency under saturated humidity (99%) and the potential of recovery after rehydration in seawater

The experimental design followed the previous experiment, which maintained a humidity level of 50%. However, in this experiment, the saturated humidity (99%) was maintained throughout the desiccation

treatment, which lasted 14 days (336 hours).

Prior to treatment, the initial state of $\Delta F/F_m'$ was measured *in situ* for all explants. Then, five explants were randomly assigned to each of nine different desiccation levels, which included periods of 0 (always immersed), 0.21 (5-hour), 1, 2, 3, 5, 7, 10, and 14 days of air exposure (the date of cultivation start: 0 day). Surface moisture on the samples was removed using Kimwipes. At the beginning of the experiment, each explant was placed on a separate petri dish without seawater and covered with a transparent plastic container (12 cm × 8 cm × 5 cm) for the respective desiccation treatment. The temperature and irradiance were maintained at approximately 24°C and 20 µmol photons m^{-2} s^{-1} (12L12D photoperiod), respectively, throughout the experiment. Humidity was maintained at 99% by placing water moistened Kimtowels (Nippon Paper Crecia Co., Ltd, Tokyo, Japan) at the bottom of each container, but not in contact with the samples, and measured using a hygrometer. The state of $\Delta F/F_m'$ after desiccation and subsequent rehydration in seawater was examined using a similar protocol to the first experiment.

The effect of salinity on the photochemical efficiency

The $\Delta F/F_m'$ was measured for erect fronds cultured in 11 different salinities (0, 5, 10, 20, 30, 34, 40, 50, 60, 70, and 80 psu) for 1, 3, 5, and 7 days (the date of cultivation start: 0 day) at a temperature of 24°C and an irradiance of 20 µmol photons m^{-2} s^{-1} (12L12D photoperiod).

One day prior to the experiment, the explants were pre-incubated in the dark at 24°C for 12 hours. The explants (n = 10 per salinity treatment) were placed in a 300 mL flask assigned to one of the 11 different salinities and then cultured under the aforementioned conditions. Salinities were prepared by adding distilled water or sodium chloride to natural seawater, and Provasoli's enriched seawater (PES; Starr and Zeikus 1993) was added to all culture media to minimize nutrient heterogeneity and deficiency. The experimental details were followed with past studies (Shindo et al. 2022b; Yonemori et al. 2023; Ito et al. 2023).

Evaluation of the appropriate shipping and preservation environment

To evaluate the appropriate shipping and preservation environment in relation the temperature and desiccation experiments, we measured $\Delta F/F_m'$ under a commercial-based package environment. The experiment conducted under a desiccation environment with saturated humidity (99%) under 24°C and 4°C temperature treatments (n = 50 per temperature treatment), which were chosen to represent room temperature and refrigerator conditions, respectively.

Before the experiment, we measured the initial state of $\Delta F/F_m'$ *in situ* (I). Each explant was then placed on a separate petri dish without seawater and covered with a transparent plastic container for overnight preservation. Humidity was maintained at 99% by placing water moistened Kimtowels at the bottom of each container, but without contact with the samples. After one day of preservation (P) and subsequent rehydration (R) in seawater, we examined the state of $\Delta F/F_m'$ using a similar protocol to the above-mentioned experiment.

Modeling the photosynthetic response to temperature

We used a Bayesian approach to analyze the response of photochemical efficiency to temperature. To model this response, we applied a thermodynamic non-linear model (Eq. 2) in which physiological rates enter a less active state beyond the optimal temperature (Thornley and Johnson 2000; Alexandrov and Yamagata 2007).

$$y = \frac{y_{max} H_d \exp\left(\frac{H_a}{R(K_{opt}^{-1} - K^{-1})}\right)}{H_d - H_a\left(1 - \exp\left(\frac{H_d}{R(K_{opt}^{-1} - K^{-1})}\right)\right)}$$

2

In this equation, *y* is the response variable, which is the photochemical efficiency of PSII ($\Delta F/F_m'$). The temperature scale is Kelvin (*K*). The model has four parameters: y_{max} scales the model to the range of *y*. K_{opt} is the absolute temperature where *y* is maximized, H_a is the activation energy in kJ mol^{-1} and H_d is the deactivation energy in kJ mol^{-1}. *R* in this model is the ideal gas constant, and has a value of 8.314 J K^{-1} mol^{-1}. The optimal value of y_{opt} at K_{opt} can be determined by substituting K_{opt} into the equation.

Due to zeros occurring at the higher temperatures, we used a zero-inflated beta distribution modified after Borlongan et al. (2020), and modeled the zero-inflation rate as a linear function of day, temperature, and their interaction. The shape parameter (Φ) for the beta distribution was modeled as a linear function of cultivation day, and model parameters were determined for each cultivation day. The link function for the data was the identity function, while a log function was used as the link function for the shape parameter (Φ) of the beta distribution, and a logit function was used as the link function for the zero-inflation rate.

Statistical analyses

Statistical analyses of all models were performed using R version 4.3.0 (R Development Core Team, 2023), and model fitting was carried out using the package brms version 2.19.0 (Bürkner 2023). The parameters were estimated by fitting the relevant models using Bayesian methods. The brms package in RStan (Stan Development Team 2023) was used as the backend to sample from the posterior distributions of the parameters, and four chains of at least 8000 samples per chain were generated and assessed for convergence. Typically, at least 2000 samples of the parameters of interest were generated. Informative normal priors were placed on all parameters of the model using values from a previous study (Kokubu et al. 2015), and a Student's prior distribution was placed on the scale parameter of the models (Gelman 2004, 2006).

The response of photosynthesis to salinity was analyzed using a Generalized Additive Model (GAM), where the basis was a thin-plate spline with 10 knots. Zeros occurred at both low and high salinity; therefore, a zero-inflation beta distribution was used in the model. A GAM was also used to model the

shape and zero-inflation rate parameters, where the basis was a thin-plate spline and with 10 knots. Student's prior distribution was used for all parameters in the model. The link function for the data and the zero-inflation rate was the logit function and the link function for the shape parameter was the log function.

To examine if continuous irradiance exposures affected $\Delta F/F_m'$ for each irradiance-temperature treatment, paiwise Tukey-HSD t-tests were used. Time was considered a factor with three levels: 0, 6, and 18 hours after the start of the experiment (i.e., initial $\Delta F/F_m'$ after 6-h irradiance exposure and the final $\Delta F/F_m'$ after 12 hours of dim-light acclimation).

RESULTS

The effect of temperature on the photochemical efficiency of PSII

The measured $\Delta F/F_m'$ of the algae cultured for 1 to 3 days at temperatures of 20–34°C under 50 μmol photons m^{-2} s^{-1} (12L12D photoperiod) were consistently above 0.500. However, at lower temperatures of 8, 12, and 16°C, the measured $\Delta F/F_m'$ values for 3-day cultures were 0.097 ± 0.058 (mean ± standard deviation, SD), 0.143 ± 0.076, and 0.401 ± 0.052, respectively (Fig. 1C). The values for 1- and 2-day cultures were also similar (Fig. 1A, B). The highest measured $\Delta F/F_m'$ values during 1- to 3-day cultures were observed at 32°C (0.679 ± 0.017 in 1-day, 0.672 ± 0.018 in 2-day, and 0.670 ± 0.012 in 3-day). However, at 35°C, the $\Delta F/F_m'$ values dropped significantly from 1-day (0.557 ± 0.032) to 3-day cultures (0.134 ± 0.149). Given the model and data, optimum temperatures where $\Delta F/F_m'$ showed the maximum was estimated at 29.3°C (27.8–30.7°C, 95% highest density credible intervals, HDCI), 28.8°C (26.6–32.8°C), and 32.3°C (29.4–33.2°C) for 1-, 2-, and 3-day measurements, respectively ($T_{opt}^{\Delta F/Fm'}$; Table 1). Other model parameter estimates are presented in Table 1.

Table 1
The parameter's mean and 95% highest density credible intervals (95% HDCI) estimated for the effective quantum yield ($\Delta F/F_m'$) – temperature model in *Caulerpa chemnitzia* var. *laetevirens* from Kagoshima, Japan exposed for 1-, 2-, and 3-day culture. $\Delta F/F_m'$ = effective quantum yield; H_a = activation energy for photosynthesis (kJ mol^{-1}); H_d = deactivation energy (kJ mol^{-1}); $T_{opt}^{\Phi F/Fm'}$ = optimum temperature (°C).

	1-d		2-d		3-d	
Parameter	**Mean**	**95% HDCI**	**Mean**	**95% HDCI**	**Mean**	**95% HDCI**
$\Delta F/F_m'_{(max)}$	0.69	0.64–0.73	0.71	0.65–0.77	0.77	0.72–0.82
H_a	56	36–79	59	32–85	43	35–58
H_d	157	99–242	345	130–1186	1476	246–2138
$T_{opt}^{\Delta F/Fm'}$	29.3	27.8–30.8	28.8	26.6–32.8	32.3	29.4–33.2

Effect of continuous irradiance exposures on $\Delta F/F_m'$ at different temperatures, and their potential of recovery

The $\Delta F/F_m'$ of the algae varied across the different irradiance-temperature treatments during the 6-hour continuous exposures to 400 (low) and 1000 (high) µmol photons m^{-2} s^{-1} at 16, 24, and 32°C, as well as during their subsequent recovery period following 12 hours of acclimation to dim light (Fig. 2). For both irradiances, a significant decline in the $\Delta F/F_m'$ was observed at 16°C (low irradiance: from the initial 0.604 ± 0.024 SD to 0.207 ± 0.037 after 6 hours of exposure; $P < 0.0001$) during the irradiance exposure, and the decline was more pronounced under high irradiance (high irradiance: from the initial 0.669 ± 0.030 SD to 0.120 ± 0.013 after 6 hours of exposure; $P < 0.0001$; Fig. 2A, B). Moreover, the $\Delta F/F_m'$ for both irradiance treatments did not return to the initial level even after 12 hours of dim light acclimation (low irradiance: 0.463 ± 0.039; $P < 0.0001$; high irradiance: 0.399 ± 0.073; $P < 0.0001$).

At the temperature of 24°C, the $\Delta F/F_m'$ was also suppressed during the irradiance exposure (low irradiance: from the initial 0.679 ± 0.015 to 0.417 ± 0.021 after 6 hours of exposure; $P < 0.0001$), and the decline was more pronounced under high irradiance (high irradiance: from the initial 0.689 ± 0.007 to 0.216 ± 0.016 after 6 hours of exposure; $P < 0.0001$; Fig. 2C, D). Nevertheless, the $\Delta F/F_m'$ for both irradiance treatments fully or mostly returned to the initial level after 12 hours of dim light acclimation (low irradiance: 0.661 ± 0.025; $P = 0.2148$; high irradiance: 0.603 ± 0.012; $P < 0.0001$).

At the temperature of 32°C, the $\Delta F/F_m'$ declined, but it was well-tolerated under the low irradiance exposure (low irradiance: from the initial 0.683 ± 0.012 to 0.538 ± 0.038 after 6 hours of exposure; P = 0.0028); however, under high irradiance, the decline was significant during the exposure (high irradiance: from the initial 0.684 ± 0.017 to 0.249 ± 0.060 after 6 hours of exposure; P < 0.0001; Fig. 2E, F). Nevertheless, the $\Delta F/F_m'$ for both irradiance treatments fully or mostly returned to the initial level after 12 hours of dim light acclimation (low irradiance: 0.670 ± 0.024; P = 0.6609; high irradiance: 0.521 ± 0.106; P < 0.0001).

The chronic effect of desiccation on the photochemical efficiency under 50% humidity and the potential of recovery after rehydration in seawater

The measured $\Delta F/F_m'$ of the algae exposed to air with 50% humidity were well maintained from the initial state (0.676 ± 0.008 SD) after 10 minutes (0.672 ± 0.015), 30 minutes (0.659 ± 0.019), and 1 hour (0.657 ± 0.011) of exposure; however, it decreased somewhat after 2 hours (0.503 ± 0.069). Thereafter, it quickly decreased and dropped to nearly zero after 5 hours (0.314 ± 0.084 at 3 hours; 0.188 ± 0.108 at 4 hours; 0.086 ± 0.089 at 5 hours; Fig. 3).

After the assigned exposure period, the algae were immediately rehydrated in seawater, and the $\Delta F/F_m'$ was remeasured after 30 minutes and 1 day of rehydration. The $\Delta F/F_m'$ at desiccation treatments of 10-minute, 30-minute, and 1-hour exposures was well maintained after both subsequent 30-minute (0.650 ± 0.019 in 10 minutes; 0.628 ± 0.023 in 30 minutes; 0.638 ± 0.016 in 1 hour) and 1-day rehydration (0.626 ± 0.028 in 10 minutes; 0.641 ± 0.012 in 30 minutes; 0.645 ± 0.020 in 1 hour) in seawater. However, once the $\Delta F/F_m'$ decreased under prolonged exposure, subsequent 30-minute (0.486 ± 0.082 in 2 hours; 0.225 ± 0.145 in 3 hours; 0.154 ± 0.136 in 4 hours; 0.033 ± 0.047 in 5hours) and 1-day rehydration (0.529 ± 0.058 in 2 hours; 0.269 ± 0.189 in 3 hours; 0.128 ± 0.175 in 4 hours' 0.009 ± 0.015 in 5 hours) in seawater never recovered the initial levels. Therefore, scatter plots of $\Delta F/F_m'$ and desiccation periods were almost identical for $\Delta F/F_m'$ measured just after desiccation and $\Delta F/F_m'$ remeasured after 30 minutes and 1 day of rehydration.

The decline in $\Delta F/F_m'$ during desiccation was closely related to the loss of interior water from the algae. The $\Delta F/F_m'$ was well maintained when the relative water content (RWC, %) was above 80% (95.1% after 10 minutes of desiccation – 80.7% after 1 hour of desiccation; Fig. 4A). However, $\Delta F/F_m'$ decreased as RWC decreased below 70% and dropped to nearly zero as RWC decreased below 20%. As $\Delta F/F_m'$ did not recover from the desiccation condition even after 30 minutes and 1-day of immersion, the scatter plots of $\Delta F/F_m'$ and RWC just after the assigned desiccation period were almost identical to those for $\Delta F/F_m'$ measured just after desiccation and re-immersion (Fig. 4B, C).

The chronic effect of desiccation on the photochemical efficiency under saturated humidity (99%) and the potential of recovery after rehydration in seawater

The $\Delta F/F_m'$ of the algae exposed to air with saturated humidity (99%) remained well-maintained from the initial state (0.679 ± 0.016 SD) even after 5 hours (0.702 ± 0.016), 1 day (0.691 ± 0.014), 2 days (0.693 ± 0.016), 3 days (0.671 ± 0.015), and 5 days (0.666 ± 0.024) of exposure (Fig. 5). However, the $\Delta F/F_m'$ of the algae under desiccation conditions slightly decreased after 7 days of exposure (0.509 ± 0.152) and dropped further after a prolonged exposure period of 14 days (0.322 ± 0.082 in 10 days; 0.253 ± 0.247 in 14 days).

Likewise, the $\Delta F/F_m'$ of the algae exposed to desiccation treatments for 5 hours, 1 day, 2 days, 3 days, and 5 days remained well-maintained after both subsequent 30-minute (0.683 ± 0.012 in 5 hours; 0.660 ± 0.014 in 1 day; 0.671 ± 0.012 in 2 days; 0.647 ± 0.026 in 3 days; 0.643 ± 0.016 in 5 days) and 1-day rehydration (0.668 ± 0.014 in 5 hours; 0.663 ± 0.014 in 1 day; 0.654 ± 0.010 in 2 days; 0.585 ± 0.055 in 3 days; 0.572 ± 0.070 in 5 days) in seawater. However, the $\Delta F/F_m'$ after 7, 10, and 14 days of exposure remained suppressed even after subsequent 30-minute (0.574 ± 0.069 in 7 days; 0.299 ± 0.063 in 10 days; 0.236 ± 0.230 in 14 days) and 1-day rehydration (0.515 ± 0.092 in 7 days; 0.280 ± 0.043 in 10 days; 0.255 ± 0.214 in 14 days) in seawater.

In contrast to the desiccation experiment under 50% humidity, the RWC under saturated humidity showed more than 100% after 7 days of exposure (highest: 120.8% ± 5.12 in 5 days of exposure; Fig. 6). However, the RWC decreased to 90.8% and 81.6% after 10 and 14 days of exposure, respectively.

The effect of salinity on the photochemical efficiency

The measured $\Delta F/F_m'$ of the algae cultured under a salinity gradient of 0–80 psu for 1 to 7 days consistently exceeded 0.500 at salinities of 20 and 50 psu in 1-day (0.634 ± 0.020; 0.595 ± 0.026 SD) and 3-day (0.607 ± 0.035; 0.559 ± 0.039) cultures (Fig. 7A, B). However, the range of salinities at which these values were consistently above 0.500 was somewhat narrow for 5-day culture, specifically between 20 and 40 psu (0.551 ± 0.061; 0.633 ± 0.029), and for 7-day culture, specifically between 30 and 40 psu (0.609 ± 0.017; 0.601 ± 0.016; Fig. 7C, D). Outside of these ranges, the measured $\Delta F/F_m'$ values dropped to zero or nearly zero.

Evaluation of the appropriate shipping and preservation environment

The measured $\Delta F/F_m'$ of the algae preserved at 24°C remained stable across the initial state (I; 0.673 ± 0.012 SD), after 1 day of preservation (P; 0.690 ± 0.014), and subsequent rehydration (R; 0.635 ± 0.013) in seawater (Fig. 8). However, for the algae preserved at 4°C, the initial state (I) measurement was 0.666 ± 0.027, which dropped to zero after 1 day of preservation (P; 0.000 ± 0.000) and did not recover even after subsequent rehydration (R; 0.000 ± 0.000) in seawater.

DISCUSSION

In Japan, *C. chemnitzia* var. *laetevirens* is widely distributed along the southern coastlines in Honshu, Shikoku, Kyushu and Ryukyu islands, which are affected by the warm *Kuroshio* Current (Yoshida 1998).

In the present study, we observed that the response of $\Delta F/F_m'$ of PSII at 50 μmol photons m^{-2} s^{-1} (12L12D photoperiod) maintained high values between 24 and 34°C during a 3-day temperature exposure ranging from 8–36°C. However, even though a relatively high tolerance to temperature, the values suddenly dropped above 35°C, suggesting a possible onset of heat stress and PSII deactivation. Thermal stress may be related to structural rearrangement in the thylakoid membranes (Roleda 2009) or to the accumulation of hydrogen peroxide that inhibits *de novo* synthesis of the D_1 protein in PSII (Allakhverdiev and Murata 2004; Allakhverdiev et al. 2008; Takahashi and Murata 2008), resulting in a decline in photochemical efficiency. However, we must exercise caution in interpreting these results since this alga typically matures and disappears in summer (August) when seawater temperatures reach 28°C at the study site (Terada et al. 2016; Borlongan et al. 2017). Additionally, relatively long temperature exposure may negatively affect all metabolic processes in the thalli, particularly at high temperatures (Terada et al. 2021b), and further enhance the decline of the photochemical efficiency of the alga. Therefore, even though the $\Delta F/F_m'$ values remain high at these temperatures, they should be avoided for onshore continuous cultivation during the summer season. Moreover, the growth characteristics of this alga should also be elucidated for a better understanding to optimize the cultural environments in the cultivation.

In contrast, the $\Delta F/F_m'$ were gradually declined along with the decrease of temperature at less than 20°C, suggesting the low capacity of the photochemical efficiency under low temperatures. Given that the seawater temperature in winter generally decreases to 15°C and sometimes reaches 13°C due to the cold weather, winter temperature can be considered to be close to threshold level for survival (Terada et al. 2016; Borlongan et al. 2017). More interestingly, the results of the 6-hour continuous exposure at 16°C under 400 and 1000 μmol photons m^{-2} s^{-1}, where the $\Delta F/F_m'$ did not return to initial levels even after 12-h dim-light acclimation (20 μmol photons m^{-2} s^{-1}), suggested that combined effects of low temperature and irradiance caused the acceleration of photodamage to PSII. Given that the $\Delta F/F_m'$ of the treatments of 24 and 32°C under 400 μmol photons m^{-2} s^{-1} returned to the initial levels after subsequent dim light acclimation, sensitivity of the $\Delta F/F_m'$ seemed to be accelerated even under low light in cold temperature. As similar sensitivities to low temperature–light stress was also observed in our past studies of *C. lentillifera* (Terada et al. 2021b), the fisherman carefully controls the incident sunlight by shading in *C. lentillifera* cultivation in the Ryukyu Islands. As same with this alga, the light and temperature environments for the cultivation of *C. chemnitzia* var. *laetevirens* might also be carefully managed especially in winter.

Desiccation causes significant structural, physiological, and biochemical changes in marine algae (Dring and Brown 1982). These changes include an increase in the ionic concentration of cells due to the loss of liquid water in the fronds, an increase in reactive oxygen species (ROS), inhibition of antioxidant enzymes, cellular alterations, and photosynthetic inactivation (Collén and Davison 1999; Contreras-Porcia et al. 2011; Karsten 2012; Holzinger and Karsten 2013; Flores-Molina et al. 2014). In the present study, we observed that $\Delta F/F_m'$ in desiccation experiments under 50% humidity at 24°C and dim-light (20 μmol

photons m^{-2} s^{-1}) remained relatively stable within 1 hour of aerial exposure. However, with prolonged desiccation, $\Delta F/F_m'$ decreased rapidly and did not return to the initial level even after subsequent 30-minute and 1-day rehydration in seawater. Given that this unicellular alga can be found in the upper subtidal zone around 2–3 m deep, which is rarely exposed in air even in the spring low tide, these results suggest that the alga may be unable to tolerate natural desiccation in the intertidal zone during tidal emersion.

We found that $\Delta F/F_m'$ was well maintained when the relative water content (RWC) was above 80%. However, as RWC once decreased below 80%, $\Delta F/F_m'$ quickly dropped and did not recover even after one day of rehydration in seawater. This suggests that the loss of liquid water from the frond (cytoplasm) causes an irreversible reduction in photochemical efficiency. In our recent study of the subtidal green alga *C. lentillifera*, a similar result was observed where the $\Delta F/F_m'$ declined when the relative water content (RWC) dropped below 90% and could not recover to the initial levels (Terada et al. 2021b). Since these *Caulerpa* species are subtidal algae, they are not expected to have a high capacity for photosynthetic recovery after desiccation compared to intertidal seaweeds (Davison and Pearson 1996; Holzinger and Karsten 2013; Beer et al. 2014). However, our previous studies on two intertidal algae, *Gracilaria vermiculophylla* (Ohmi) Papenfuss (as *Agarophyton vermiculophyllum*, Gracilariales) and *Sargassum fusiforme* (Harvey) Setchell (Fucales), have shown relatively strong tolerance to desiccation. We found that $\Delta F/F_m'$ of two algae were recovered after rehydration in seawater even when nearly 80% of the interior water of the thallus was lost during desiccation (Kameyama et al. 2021; Yonemori et al. 2023). Strong tolerance to desiccation has also been reported in the macroscopic gametophytes of two *Pyropia* species (Bangiales; Terada et al. 2021a; Xu et al. 2021), P. *tanegashimensis* (Shinmura) Kikuchi et Fujiyoshi (Sutherland et al. 2011; = *Phycocalidia tanegashimensis* in Santiañez and Wynne 2020) and *P. yezoensis* f. *narawaensis* Kikuchi, Niwa et Nakada (= *Neopyropia yezoensis* f. *narawaensis* in Kikuchi and Niwa 2020).

Nonetheless, a similar experiment conducted for up to 14 days with aerial exposure under saturated humidity (99%) in a plastic container revealed that $\Delta F/F_m'$ remained stable for up to 5 days, as the frond's interior water was maintained due to the saturated humidity (Sato et al. 2022). However, the $\Delta F/F_m'$ gradually decreased as the duration of exposure extended, reaching a low point after 14 days. During the 5-day exposure, the RWC in the frond increased up to 120% (Sato et al. 2022). However, for longer durations, it declined to less than 100%, indicating that the saturated humidity resulted in higher water content in the frond, negatively affecting the ionic concentration of cells or above-mentioned changes.

The freshly harvested products of *C. lentillifera* are typically packed in plastic containers with a wet moist sheet to maintain high humidity levels. They are then shipped and handled without seawater at room temperature (i.e., 20–25°C). In a previous study on *C. lentillifera*, it was found that the photochemical efficiency can be maintained for up to 4 to 8 days (Terada et al. 2018). Therefore, in the present study, as *C. chemnitzia* var. *laetevirens* has a similar tolerance for high humidity levels, similar handling method might be available for a harvested product of this alga.

In terms of salinity response under a gradient of 0–80 psu for up to 7 days of culture at 24°C and dim-light (12L12D), $\Delta F/F_m'$ remained steadily high within the salinity range of 30 to 40 psu, but declined beyond this range, indicating a stenohaline characteristic (Shindo et al. 2022b; Ito et al. 2023). Under extreme hypo- or hypersaline stresses, the cytoplasm increases its ionic concentrations and undergoes a change in ion ratios due to selective uptake, which can inhibit photosynthesis and respiration (Kirst, 1990; Karsten, 2012). However, intertidal algae are known to exhibit euryhaline characteristics (Gao et al. 2016). For instance, a previous study on the red alga *G. vermiculophylla* showed that it had a broad photosynthetic tolerance to salinity ranging from 20–60 psu during a 7-day exposure, with $\Delta F/F_m'$ tolerating 0 psu after 3 days, indicating its ability to withstand intertidal and brackish water environments (Kameyama et al. 2021). Another intertidal brown alga, *S. fusiforme*, also exhibits euryhaline characteristics, with $\Delta F/F_m'$ showing good tolerance between 10 and 50 psu during a 10-day exposure (Yonemori et al. 2023). In the present study, the salinity of seawater in the subtidal zone around the study site at Nagashima Island remained relatively stable, ranging from 30 to 34 psu. However, it is important to note that in coastal areas, particularly in rocky intertidal pools and estuaries, there is a possibility of extreme hyposaline environments due to precipitation and exposure to riverine discharge (Karsten 2012). Indeed, knowledge of the salinity and desiccation tolerances of this alga is crucial when selecting potential cultivation sites and other factors to consider for sustainable harvesting. In fact, since onshore *C. lentillifera* cultivation facilities in Ryukyu Islands typically have translucent roofs, there is little direct impact from low salinity due to precipitation. Therefore, a similar facility may also be necessary for *C. chemnitzia* var. *laetevirens* cultivation.

As *Caulerpa* species are typically tropical algae, fresh products of *C. lentillifera* in the Ryukyu Islands are shipped and handled at room temperature (Terada et al. 2018). In the present study, *C. chemnitzia* var. *laetevirens* was found to be intolerant to overnight storage at 4°C (refrigerator temperature) under package treatments, but when kept at 24°C (room temperature), the $\Delta F/F_m'$ were maintained, suggesting that it should also be handled at room temperature to maintain optimal conditions. Therefore, this alga can be cultivated by modifying the techniques used for cultivating Japanese *C. lentillifera* and adjusting its environment accordingly. To ensure appropriate shipping conditions, it is essential to maintain saturated humidity in a plastic container at room temperature.

Declarations

Data availability statement

Datasets generated in the present study are available from the corresponding author upon reasonable request.

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Figures

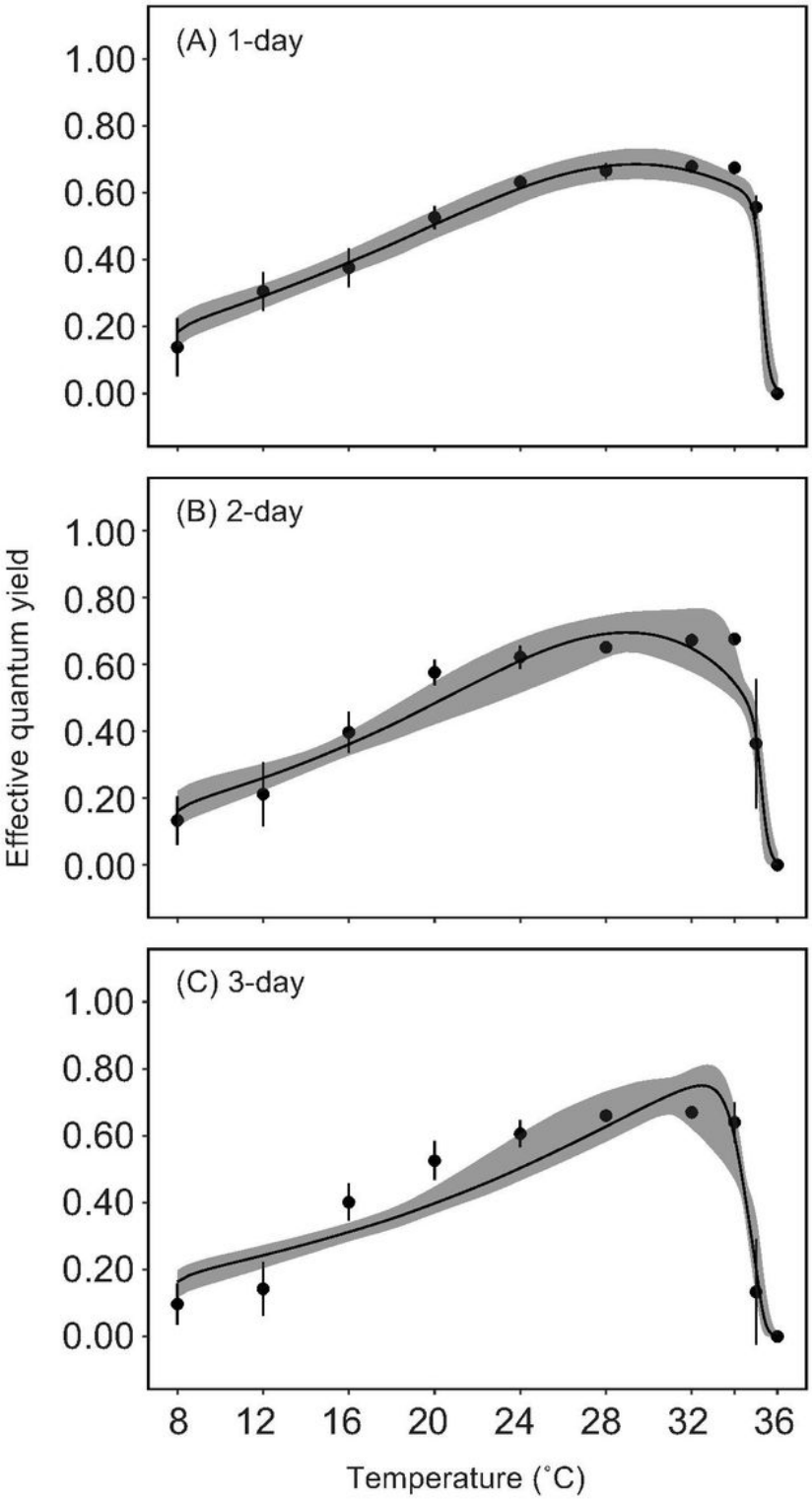

Figure 1

The response of the effective quantum yield ($\Delta F/F_m'$) in *Caulerpa chemnitzia* var. *laetevirens* from Kagoshima, Japan at ten temperature treatments (8, 12, 16, 20, 24, 28, 32, 34, 35, and 36°C) after 1- (A), 2- (B), and 3-d culture (C) under 50 µmol photons m^{-2} s^{-1} (12L12D photoperiod). The dots indicate the average of measured values (n = 10) and error bars indicate standard deviation. The line and the shaded regions indicate the expected value and the 95% highest density credible intervals (HDCI) of the model.

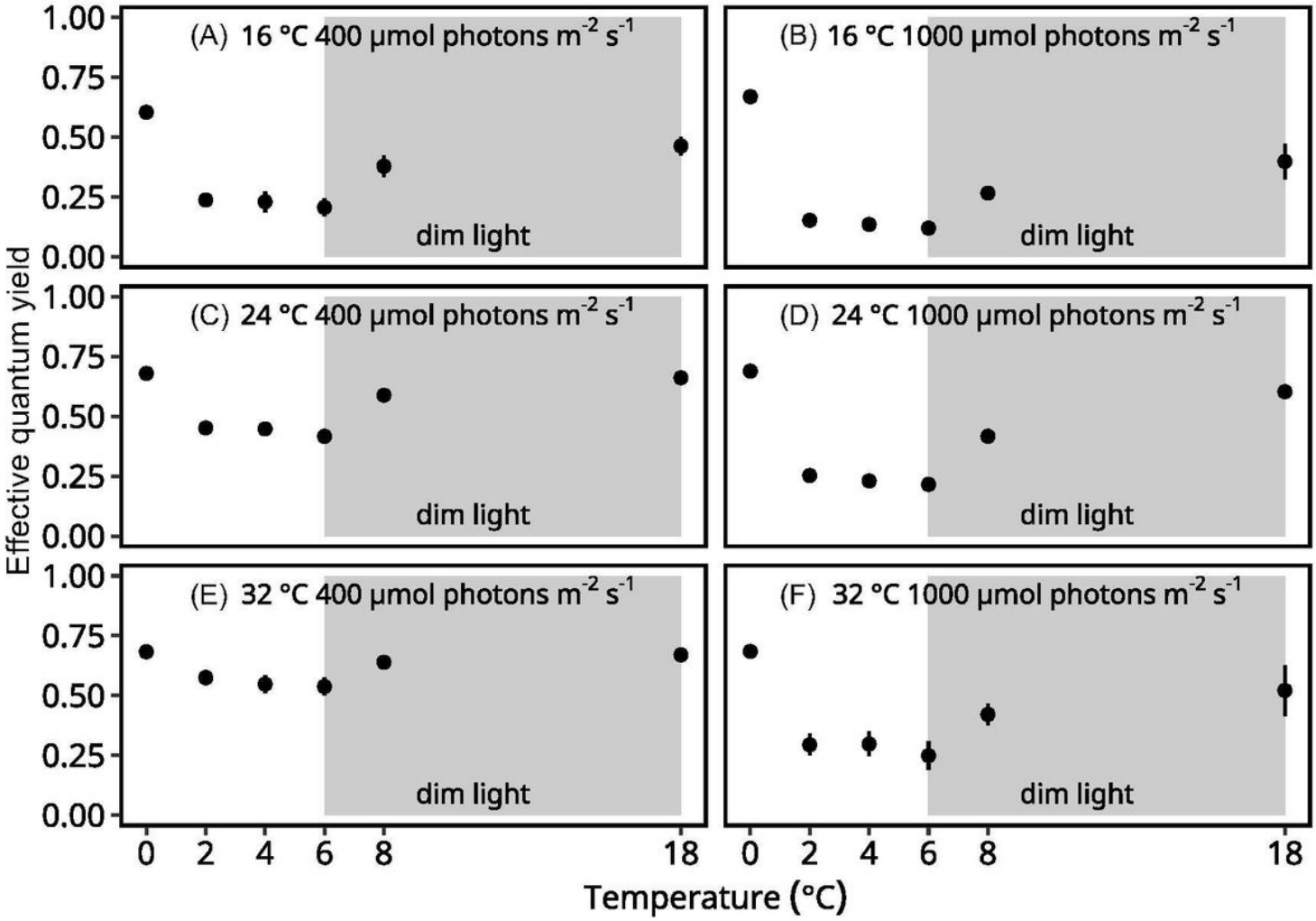

Figure 2

The hourly change of the effective quantum yield ($\Delta F/F_m'$) in *Caulerpa chemnitzia* var. *laetevirens* from Kagoshima, Japan to irradiance at (A, C, E) 400 and (B, D, F) 1000 µmol photons m^{-2} s^{-1} at 16 (A, B), 24 (C, D), and 32°C (D, F). The dots indicate the average of measured values (n = 10), and solid lines indicate standard deviation. The irradiance and temperature exposures were done for 6 hours. Thereafter, the explants were acclimated 12 hours under dim-light (20 µmol photons m^{-2} s^{-1}). The measurements at 8 hours were conducted to confirm the potential of recovery at the level of 2 hours of dim-light acclimation.

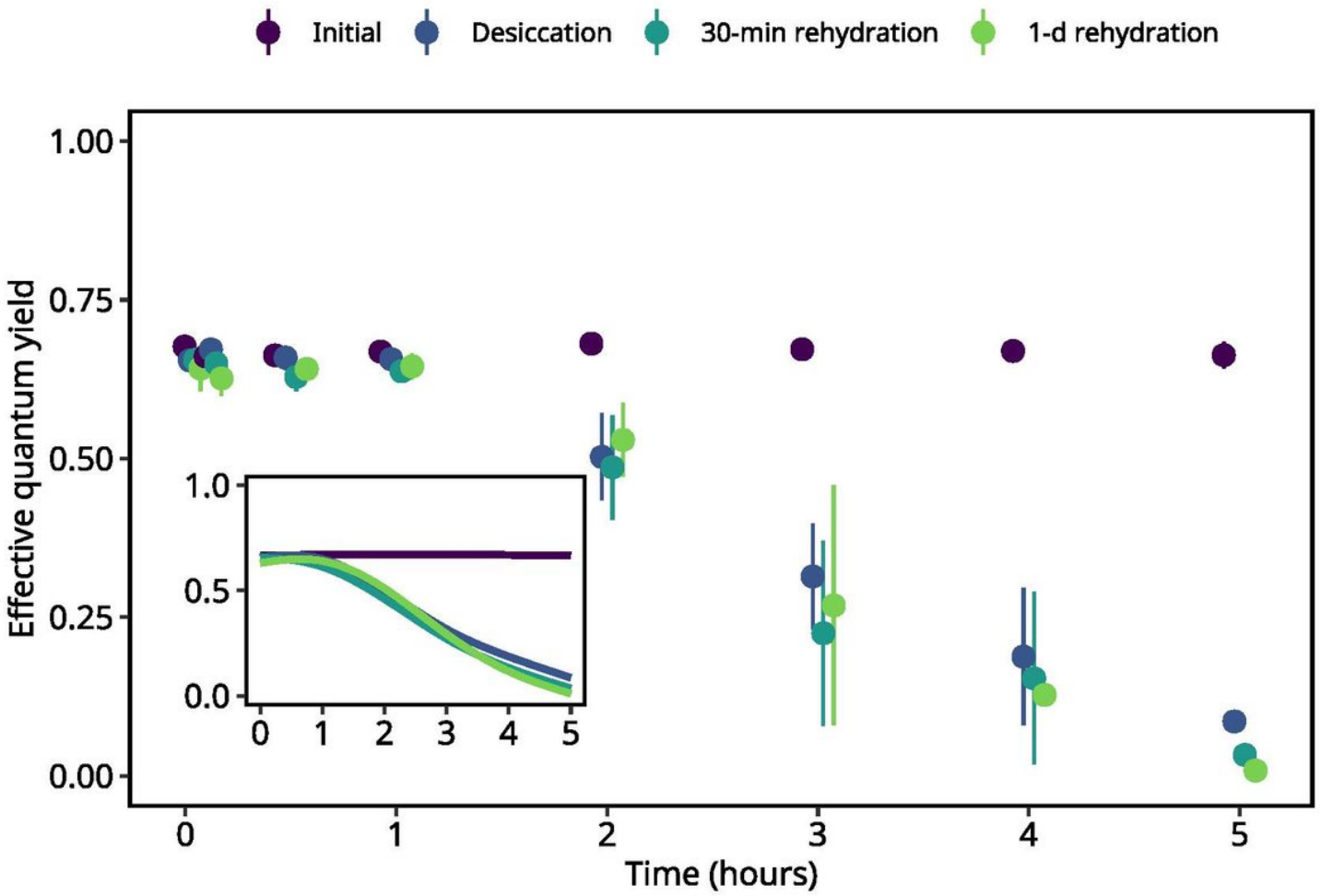

Figure 3

The chronological response of the effective quantum yield ($\Delta F/F_m'$) in *Caulerpa chemnitzia* var. *laetevirens* from Kagoshima, Japan to aerial exposure (0, 0.17, 0.5, 1, 2, 3, 4, and 5 hours) at 24°C and the humidity of 50% under the irradiance of 20 µmol photons m^{-2} s^{-1} (dim-light condition). The dots indicate the average of measured values (n = 50), and error bars indicate standard deviation. The inset shows the trends for a Generalized Additive Model (GAM) fitted to the expected values. The dots were offset to enhance clarity. The graphs indicate measurements of the initial value (purple), those exposed in air (blue), those in subsequent 30-minute (green) and 1-day rehydration in seawater (light green), respectively.

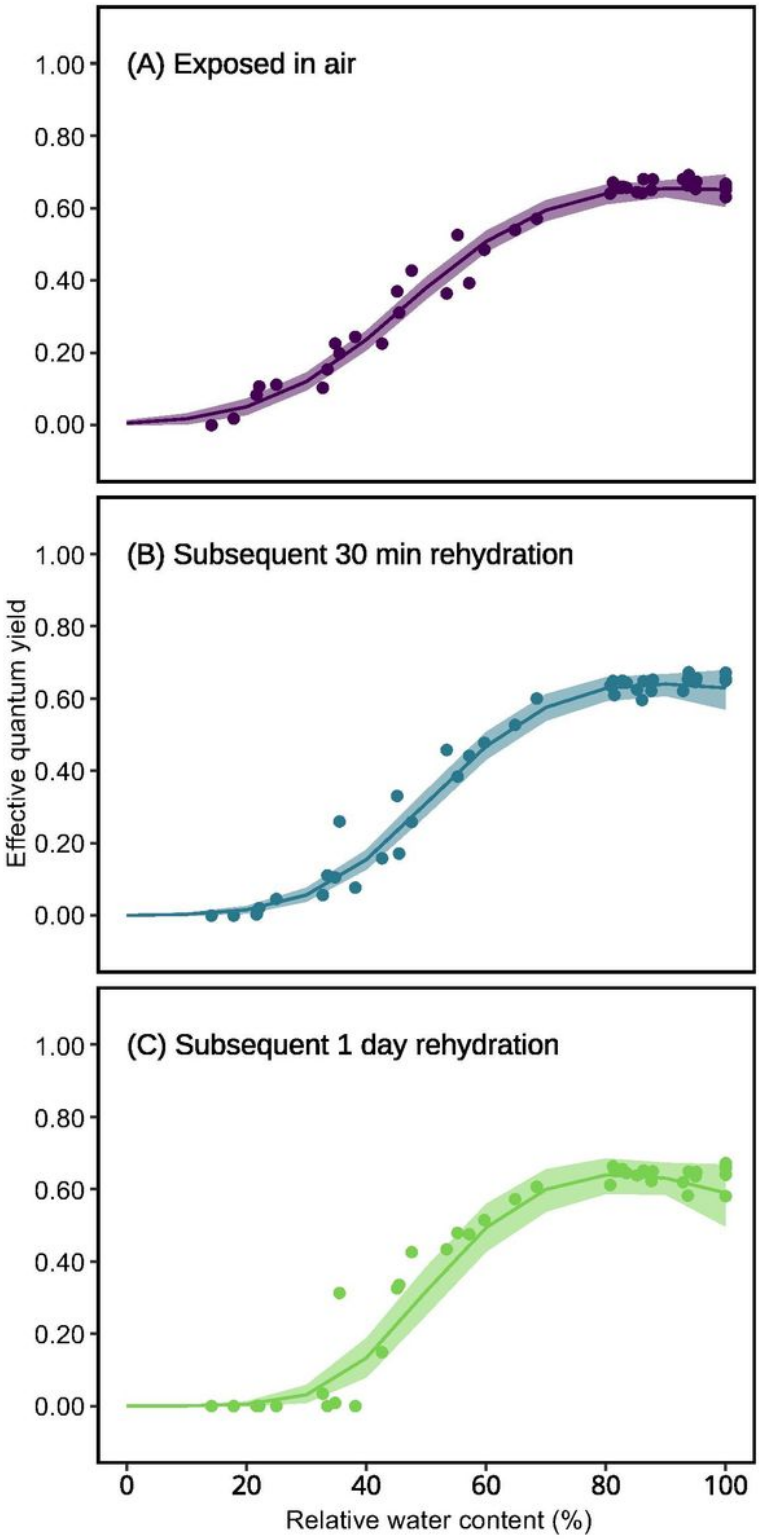

Figure 4

The relationship between the effective quantum yield ($\Delta F/F_m'$) and the relative water content (RWC, %) in *Caulerpa chemnitzia* var. *laetevirens* from Kagoshima, Japan to aerial exposure at 24°C and the humidity of 50% under the irradiance of 20 µmol photons m^{-2} s^{-1} (dim-light condition). The graphs indicate measurements exposed in air (A), those in subsequent 30-minute (B) and 1-day rehydration in seawater (C), respectively. The dots indicate the average of measured values (n = 10), the lines indicate the

expected value, and shaded regions indicate the 95% highest density credible intervals (HDCI) of the model.

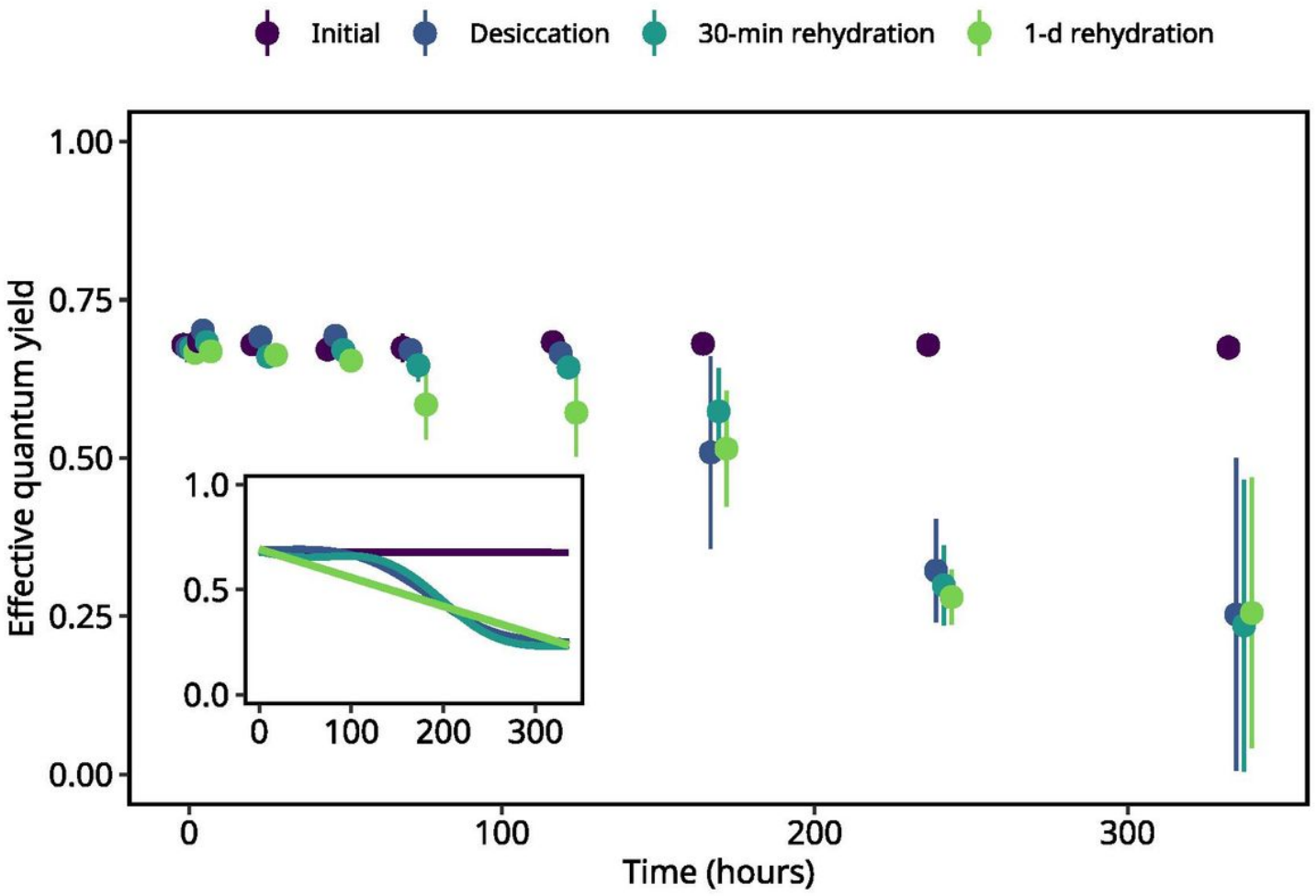

Figure 5

The chronological response of the effective quantum yield ($\Delta F/F_m'$) in *Caulerpa chemnitzia* var. *laetevirens* from Kagoshima, Japan to aerial exposure (0, 0.21, 1, 2, 3, 5, 7, 10, and 14 days) at 24°C and the humidity of 99% under the dim light (20 µmol photons m^{-2} s^{-1}; 12L12D photoperiod). The dots indicate the average of measured values (n = 50), and error bars indicate standard deviation. The inset shows the trends for a Generalized Additive Model (GAM) fitted to the expected values. The dots were offset to enhance clarity. The graphs indicate measurements of the initial value (purple), those exposed in air (blue), those in subsequent 30-minute (green) and 1-day rehydration in seawater (light green), respectively.

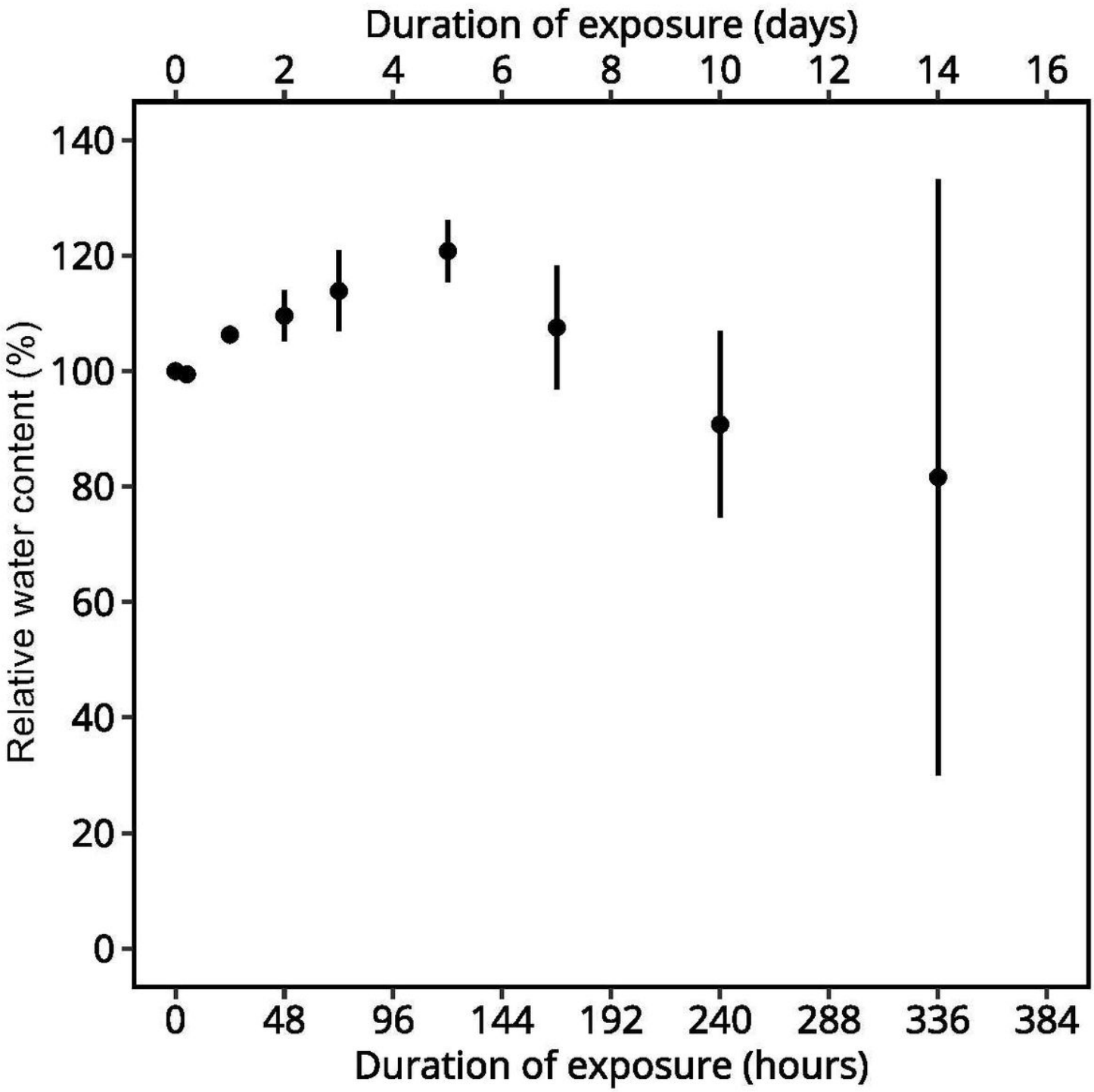

Figure 6

The chronological response of the relative water content (RWC, %) in *Caulerpa chemnitzia* var. *laetevirens* from Kagoshima, Japan to aerial exposure (0, 0.21, 1, 2, 3, 5, 7, 10, and 14 days) at 24°C and the humidity of 99% under the dim light (20 µmol photons m^{-2} s^{-1}; 12L12D photoperiod). The dots indicate the average of measured values (n = 5), and solid lines indicate standard deviation.

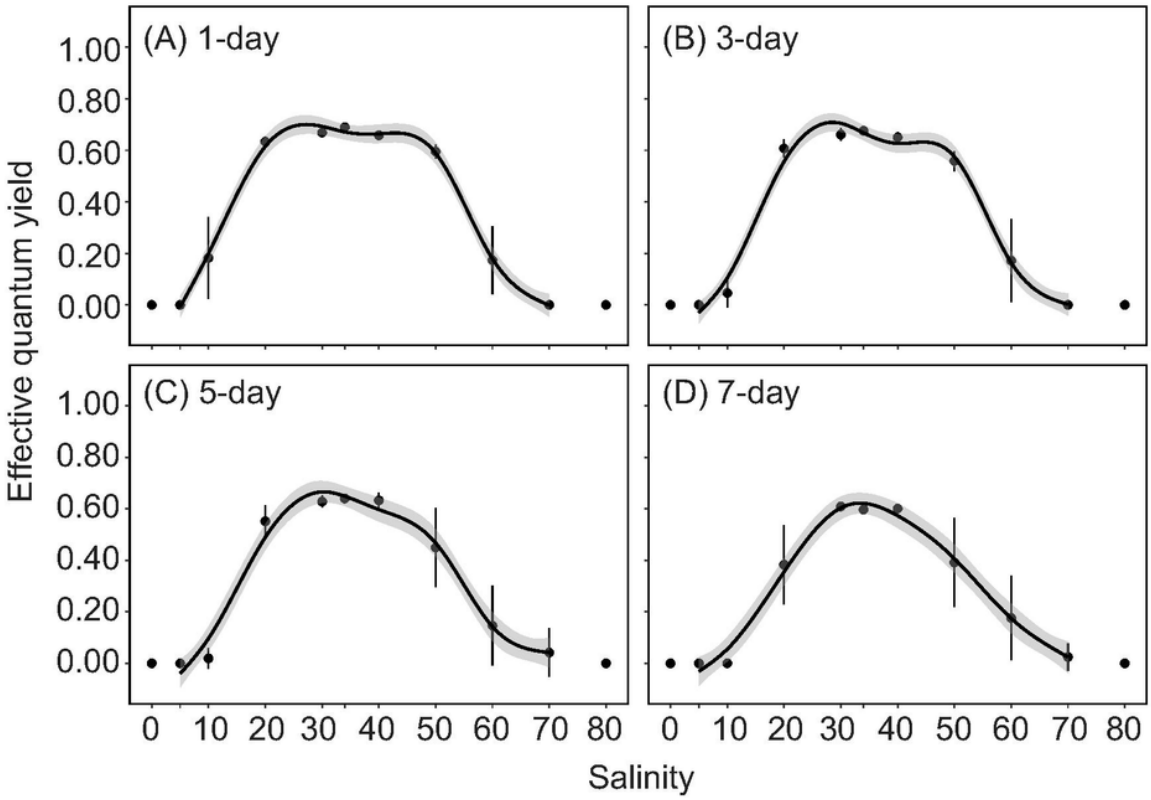

Figure 7

The response of the effective quantum yield (*ΔF/Fm'*) in *Caulerpa chemnitzia* var. *laetevirens* from Kagoshima, Japan to salinity gradients (0, 5, 10, 20, 30, 34, 40, 50, 60, 70, and 80 psu) at 1- (A), 3- (B), 5- (C), and 7-day culture (D) at 24°C under the dim-light (20 µmol photons m-2 s-1; 12L12D photoperiod). The dots indicate the average of measured values (*n* = 10), and error bars indicate standard deviation. The line indicates the expected values of a Generalized Additive Model (GAM) that is fitted to the data from 5 to 70 psu, and the shaded region is the 95% highest density credible intervals (HDCI) of the model.

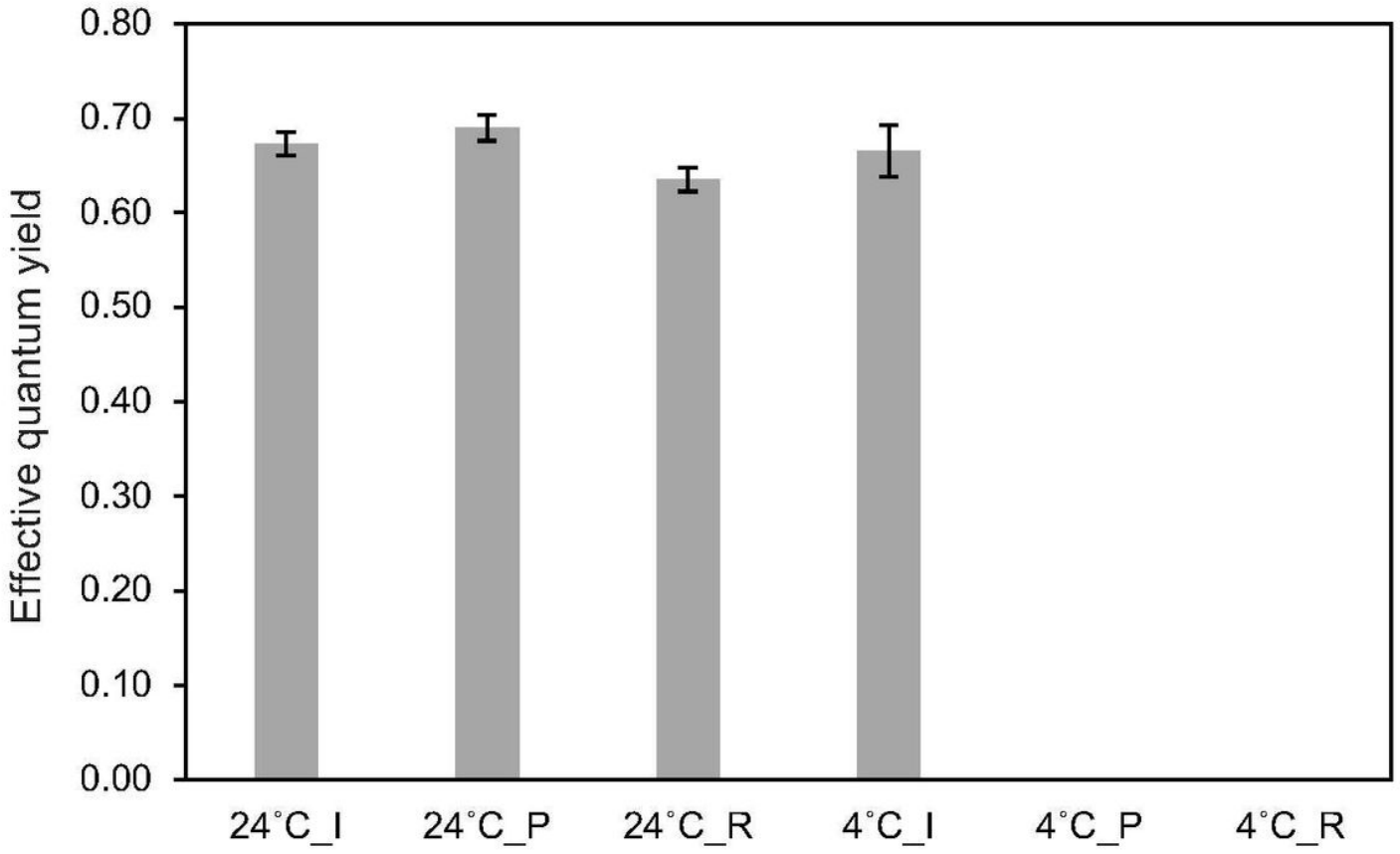

Figure 8

The effective quantum yield (*ΔF/Fm'*) in *Caulerpa chemnitzia* var. *laetevirens* from Kagoshima, Japan after 1-day preservation without seawater at 24 and 4°C under the humidity of 99% and the dim-light (20 µmol photons m-2 s-1; 12L12D; *n*=50). Abbreviations of I, P, and R indicate the initial value (I), those after 1-day preservation (P), and those after subsequent 1-day rehydration (R) in seawater, respectively. The bars indicate the average of measured values (*n* = 10), and solid lines indicate standard deviation.