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Effects of periodical dehydration on biomass yield and biochemical composition of the edible red alga *Pyropia yezoensis* grown at different salinities

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ABSTRACT

Pyropia is an important marine crop and periodical dehydration could help kill fouling organisms during cultivation. However, our understanding regarding the effects of periodical dehydration on biomass yield and biochemical composition of *Pyropia* remains very lacking, particularly for thalli grown at different salinities. In this study, *Pyropia yezoensis* experiencing periodical dehydration or not was cultured at four levels of salinities (17, 25, 32, 39 psu) for 19 days. The highest growth rate was found at salinity 25 psu and lowest was at salinity 39 psu. Dehydration reduced relative growth rate at each salinity except for the insignificant decrease at 39 psu. Dehydration did not affect the maximal photochemical efficiency (Fv/Fm) and the highest salinity decreased it slightly. Dehydration induced more Chl *a* and carotenoids and highest Chl *a* content was found at salinity 39 psu. Dehydration did not affect phycoerythrin or phycocyanin content at salinities 25–39 psu but respectively increased them by 28% and 50% at salinity 17 psu. Dehydration did not affect content of any amino acid while salinity interacted with dehydration on most amino acids. The optimal salinity for the production of total amino acids (AA) , essential AA, umami AA, and sweet AA was 25 psu for the thalli without dehydration; while it shifted to 17 psu for the thalli with dehydration. Dehydration and salinity interacted on content of the fatty acids C12, C18:1(n-9), C18:2(n-6), and C20:2(n-6). For the synthesis of eicosapentaenoic acid (EPA, C20:5(n-3)) and polyunsaturated fatty acids (PUFA) in thalli without dehydration, the optimal salinity was 39 psu, but the effect of salinity disappeared when thalli experienced periodical dehydration. These findings show the interactive effects of periodical dehydration and salinity on biomass yield and biochemical composition in *P. yezoensis* and provide insights into optimizing conditions for nori cultivation.

1. Introduction

Macroalgae, mainly inhabiting the intertidal zones, play a critical role in coastal carbon fixation and sequestration in addition to providing habitats for marine animals and sustaining natural ecosystems [1–3]. Furthermore, they are also economically important in the fields of food, feed, pharmaceuticals, cosmetics, biofuel, etc. [4–6]. The history of humans consuming seaweeds as food can be traced back to the late Pleistocene (between 14,220 and 13,980 years ago) at Monte Verde in southern Chile [7]. Due to high content of dietary fiber, essential amino acids, vitamins and minerals, there is growing interest in treating seaweeds as functional food [6]. Among the edible seaweeds, the red macroalgae *Porphyra/Pyropia* species have been an important marine crop in Asian countries for thousands of years [8]. Due to great demand, *Porphyra/Pyropia* species have been massively cultivated. *Pyropia*, also known as laver, has been recently separated from the genus *Porphyra* [9]. The world production of *Pyropia* in 2018 was up to 2, 873 thousand tons (fresh weight) with the total value being about US$ 2.1 billion [10]. There are three *Pyropia* species that have been commercially cultivated, including *Pyropia yezoensis*, *P. tenera*, and *P. haitanensis*. *Pyropia yezoensis*, is considered as the most lucrative marine crop in the world thanks to its palatable flavor and high nutritional value [8,11]. *P. yezoensis* is commonly used as commercial food; e.g., nori and sushi are very popular in Asian cuisine. Accordingly, cultivation of *P. yezoensis* is extensively conducted in East Asia countries, particularly China, Japan, and Korea [12,13].

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In China, "supporting bamboo pole" and "semi-floating" farming techniques were first used for *Pyropia* cultivation. These two farming techniques work very well in areas with the depth < 15 m [14]. As the cultivation scale expands, available areas have been saturated. *Pyropia* farming has been extended towards deep areas where "supporting bamboo pole" and "semi-floating" fail to function. Therefore, the full-floating system has been developed. For each farming technique, periodical dehydration is suggested although it is difficult for full-floating system to operate [14]. Dehydration during cultivation can kill most fouling organisms, such as diatoms, macroalgal spores, and invertebrate larvae while *Pyropia* can survive due to its high tolerance to desiccation. Therefore, nets seeded with *Pyropia* are raised out of the sea manually for several hours a day, several times a week, particularly in the early stage of growing season [15]. Gao et al. [16] investigated the effect of single dehydration on physiological performance of *P. yezoensis* and found that Fv/Fm of *P. yezoensis* that lost 86% of cellular water could fully restore after 30 min of re-hydration, suggesting a high resilience to dehydration. Wang et al. [43] showed that *P. yezoensis* with periodical drying had higher tolerance to desiccation, as well as higher contents of soluble proteins, chlorophyll *a*, carotenoids and total fatty acids than those without drying.

Salinity is one of the most critical environmental factors that affect growth and development of macroalgae [17–19]. The average salinity of open-ocean surface waters is around 35 psu but it could vary from 10 to 77 psu in coastal waters because of freshwater influxes, evaporation and precipitation [20]. Previous study showed that the hyposaline condition (5 psu) reduced growth rate of *P. yezoensis* by 57% after 28-day culture while the hypersaline condition (55 psu) did not affect the growth rate significantly compared to the control (30 psu); loss of pigmentation at the hyposaline condition was observed, which may lead to the decrease of growth rate [21]. He et al. [22] investigated the response of *P. yezoensis* (Rhodophyta) blades and conchocelis to short-term hypersaline treatments and found that blades had higher Fv/Fm and carbon fixation efficiency when exposed to the high salinity (120 psu) condition. To obtain optimal salinity for growth and cultivation of *P. yezoensis*, further studies with more salinities levels are needed. In addition, content and profile of amino acids and fatty acids affect flavor and food quality of *P. yezoensis* [11]; however, little is known regarding how salinity regulates the synthesis of amino acids and fatty acids in *P. yezoensis*.

Both dehydration and salinity play an essential role in modulating the physiological performance of *Pyropia* species. These two environmental factors simultaneously occur in nature and can be used as regulating methods to optimize *Pyropia* cultivation in terms of biomass yield and biochemical composition. We hypothesized that dehydration and salinity could interact on physiological and biochemical activity of *P. yezoensis* and thus cultured *P. yezoensis* blades at different salinity levels (17–39 psu) with or without periodical dehydration to test our hypothesis. This study can provide helpful insight into how periodical dehydration affects productivity and food quality of the important marine crop *P. yezoensis* grown at different salinities.

2. Materials and methods

2.1. Sample collection and culture conditions

The *P. yezoensis* (CCAJ LL61) was obtained from Center for Collections of Algae in Jiangsu Ocean University, which is originally from the coastal waters (119.49°E, 34.70°N) of the Yellow Sea, Jiangsu province of China. About 0.2 g of healthy thalli with uniform color and intact shape were selected and cultured in 1-L balloon flasks in natural seawater (salinity of 30 psu) with PES medium for one week before the experiment. The culture temperature was 10 °C and the light intensity was 80 μmol with a 12 h: 12 h (light/dark) photoperiod. The temperature and light density were close to the conditions at the sampling site in winter [11]. The cultures were aerated and the seawater was renewed every three days. These conditions were maintained by an intelligent illumination incubator (Jiangnan GXZ-300C, Ningbo, China). After one week of pre-culture, the thalli were transferred to the conditions with four levels of salinity (17, 25, 32, and 39 psu) and two levels of dehydration (dehydration, without dehydration). The conditions of temperature and light were the same as pre-culture. Different salinity levels were achieved by diluting natural seawater or adding NaCl. The range of salinity covers the scenarios of coastal waters suffering freshwater input and intense evaporation. The highest salinity of 39 psu occurs in Haizhou Bay in China, where *P. yezoensis* was sampled and intensively cultivated, in some years when rainfall is rare according to our field investigation. For the treatment of dehydration, it was conducted every two days during the culture period by exposing thalli to air until the water loss reaching 70%. This frequency and intensity were based on the usual operation during *Pyropia* cultivation. The cultures were conducted for 19 days in triplicates. All following physiological and biochemical parameters were measured at the end of the cultivation.

2.2. Measurement of growth

The growth rate of *P. yezoensis* was determined by weighing fresh thalli at the beginning and end of the experiment. Thalli were taken out from the culture flasks and placed on a smooth glass plate. Surface water of the thalli was removed off with tissue paper before weighing. It was deemed as the complete removal of surface water when the color of the tissue paper did not change after wiping the thalli [23].The relative growth rate (RGR) was determined as follows: RGR (% d^{-1}) = [ln(W_2/W_1)]/t × 100, where W_1 and W_2 are the initial and final weight, respectively; t is the number of culture days.

2.3. Assessment of photosynthesis

Maximum photochemical efficiency of PSII was used to represent photosynthesis of thalli. It was measured with a pulse modulation fluorometer (Water-PAM, Walz, Germany). Samples were dark adapted for 15 min to relax photosynthetic activity before the measurement. The saturating pulse was set 5000 μmol photons m^{-2} s^{-1} (0.8 s). The maximal efficiency of PSII photochemistry was determined as the ratio of variable to maximal chlorophyll fluorescence (Fv/Fm), where Fv = (Fm − F_0), Fm and F_0 were the maximal and minimal fluorescence yield, respectively. F_0 was measured by using modulated measuring light (< 0.1 μmol photons m^{-2} s^{-1}) and Fm was determined at a 0.8 s saturating pulse of 5000 μmol photons m^{-2} s^{-1} in dark-adapted thalli.

2.4. Determination of photosynthetic pigments

To measure chlorophyll *a* content, approximately 0.02 g fresh weight of thalli were extracted with 5 mL of absolute methanol at 4 °C for 24 h in darkness. The sample was not grinded as it has demonstrated that the duration of 24 h is enough to extract the pigments completely by comparing the ungrinded data to grinded data in a preliminary experiment. The extracting solution was then centrifuged (5000 *g*, 4 °C, 10 min) and the optical density of the supernatant at 470, 653 and 666 nm was recorded using an nucleic acid and protein analyzer (Ultrospec 3300 pro, Amersham Bioscience, England). The concentrations of Chl *a* and carotenoids (mg g^{-1} FW) were calculated according to Wellburn [24]: Chl *a* = 15.65 × OD666–7.53 × OD653; carotenoids = (1000 × A470 + 1403.57 × A666–3473.87 × A653)/221.

To determine the contents of phycoerythrin and phycocyanin in thalli, about 0.02 g FW per sample were homogenized in 0.1 M phosphate buffer (pH 6.8) at 4 °C with mortar and pestle. The extraction solution was centrifuged (10,000 *g*, 4 °C, 10 min), and then the supernatant was spectrophotometrically scanned (Ultrospec 3300 pro, Amersham Bioscience, England). According to Beer & Eshel [25], phycoerythrin (mg g^{-1}FW) = [(A564 − A592) − (A455 − A592) × 0.2] × 0.12; phycocyanin (mg g^{-1} FW) = [(A564 − A592) − (A455 − A592)

× 0.51] × 0.15.

2.5. Determination of amino acids

The seaweed samples were dried in an oven at 105 °C until constant weight. The dry samples were grinded and about 50 mg powders were placed in a 15 mL tube. Ten mL 6 N HCl were added and hydrolysis was conducted at 110 °C for 22 h. The hydrolyzed sample was filtered (pore size 0.45 μm) and then dried under nitrogen. It was dissolved in 10 mL of 0.2 M sodium citrate buffer (pH 2.2), filtered using a syringe filter (pore size 0.2 μm), and then diluted 40 times with ultrapure water. Samples (20 μL) were injected into an Amino Acid Analyzer (L8900, Hitachi High-Technologies, Japan). Amino acid standard solution (Aladdin, China) was also injected for the system calibration and amino acid quantification. Content of amino acid was expressed as mg g^{-1} DW.

2.6. Determination of fatty acids

The preparation of fatty acid methyl esters (FAME) and determination of fatty acids were carried out according to Gao et al. [6] with some modification. About 50 mg DW thalli were grinded and the powder was placed in a 10 mL centrifuge tube. Then 2 mL of H_2SO_4-CH_3OH were added, and the solution was mixed with a multi-tube vortex mixer (DMT-2500, China) for 10 min and heated in water bath at 80 °C for 1 h. Afterwards 1 mL of deionized water and 2 mL of isooctane were added and the mixture was centrifuged at 1700*g* for 5 min. The supernatant layer containing FAME was collected, filtered through a filter membrane (0.22 μm, Leigu, China) and analyzed with a gas chromatography mass spectrometer (Shimadzu, GCMS-QP2010SE, Japan). Chromatograph peaks were identified based on the retention time of a Supelco 37 component FAME mix (Sigma-Aldrich). Quantification of FA was expressed as a percentage of the total peak areas for total FAME.

2.7. Statistical analysis

Results were expressed as means of replicates ± standard deviation. Data were analyzed using the software SPSS v.23. The data conformed to a normal distribution (Shapiro-Wilk, $P > 0.05$) and the variances could be considered equal (Levene's test, $P > 0.05$). Two-way ANOVA was conducted to assess the effects of dehydration and salinity on relative growth rate, Fv/Fm, Chl *a*, carotenoids, phycoerythrin, and phycocyanin. Two-way multivariate ANOVA (MANOVA) was conducted to assess the effect of dehydration and salinity on the profiles of amino acid and fatty acid. Least-Significant Difference (LSD) was conducted for post hoc investigation. A confidence interval of 95% was set for all tests.

3. Results and discussion

3.1. Effects of dehydration and salinity on growth and photosynthesis of *P. yezoensis*

The relative growth rate of *P. yezoensis* in this study ranged from 19.26 ± 0.46% to 24.24 ± 0.37% d^{-1} (Fig. 1). The highest growth rate was much higher than that (~5% d^{-1}) in Samanta et al [21]'s study but similar to that in Gao et al [11]'s and Ma et al [26]'s studies. The large difference in growth rate might be due to different ecotypes of *P. yezoensis*. The thalli of *P. yezoensis* in Samanta et al [21]'s study was collected in a seaweed farm at Jebu Island (126.62° E, 37.16° N), Korea, while they were collected in a seaweed farm at Lianyungang (119.30° E, 34.50° N), China in our study. Both dehydration and salinity affected relative growth rate of *P. yezoensis* (Table 1). Dehydration reduced relative growth rate by 7–10% although the decrease at 39 psu was not significant. Regardless of dehydration conditions, the highest growth rate occurred at salinity 25 psu, followed by 32 and 17 psu, and the lowest growth rate was found at 39 psu. Compare to salinity 25 psu, the salinity 39 psu decreased growth rate by 18% and 12% compared to

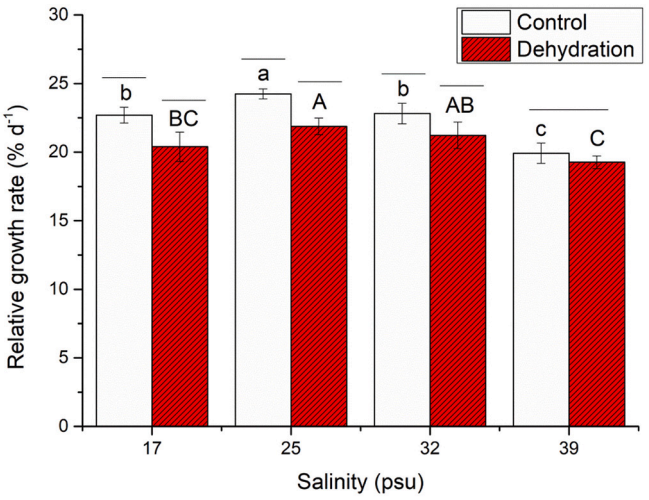

Fig. 1. Relative growth rate of *P. yezoensis* cultured at different salinities with or without periodical dehydration. Control means the treatment without periodical dehydration. The error bars indicate the standard deviations ($n = 3$). Different lowercase and capital letters above the error bars represent significant differences ($P < 0.05$) among salinities without dehydration or with dehydration, respectively. Horizontal short bars represent significant differences ($P < 0.05$) between dehydration treatments.

control (the treatment without periodical dehydration) and dehydration conditions, respectively. The optimal salinity for growth of *P. yezoensis* was 25 psu rather than 32 psu in this study. Wang et al. [27] also found that compared to 29.6 psu *P. katadae* var. *hemiphylla* had higher growth rate at 22.6 psu. It seems that *Pyropia* species prefer the lower salinities less than 30 psu. This was consistent with field scenarios in which *P. yezoensis* inhabits intertidal zones that suffer freshwater input. Wu et al. [28] also found that the growth rate of *P. haitanensis* decreased with the increase of salinity at lower nitrate level. Growth rate of seaweeds is usually reduced in hypersaline water, which could be attributed to both cumulative enzyme effects and reduced turgor pressure that inhibit cell division [29]. Although dehydration and the highest salinity decreased growth of *P. yezoensis* in this study, the decreased extent was not very large. Even the lowest growth rate in this study was above 19% d^{-1}, indicating that *P. yezoensis* has a high tolerance to dehydration and changes of salinity.

The change (0.54–0.59) of maximal photochemical efficiency (Fv/Fm) of *P. yezoensis* cultured under different conditions was very small (Fig. 2). Dehydration did not have a main effect while salinity had an interactive effect with dehydration (Table 1). For instance, for thalli without dehydration, there was no significant difference between salinities 25 and 39 psu while the salinities of 17 and 32 psu both reduced Fv/Fm by 4% compared to salinity of 25 psu; for thalli with dehydration, there was no significant difference for thalli grown at 17–32 psu but the highest salinity of 39 psu reduced it by 6% compared to salinity of 25 psu. Although salinity interacted with dehydration affected Fv/Fm but the changed extents were very minor, suggesting that dehydration and salinity had very limited effect on Fv/Fm. On the other hand, Li et al. [14] reported that periodical dehydration alone reduced Fv/Fm in *P. yezoensis* by 12%. The different effect of dehydration in their study may be due to the dehydration period. In Li et al. [14]'s study, thalli were dehydrated daily while the dehydration period was every two days in the present study. In addition, a high recovery rate of Fv/Fm was reported in *P. haitanensis* [30], of which the Fv/Fm was completely restored within 30 min after commencement of the re-hydration. On the other hand, the Fv/Fm of *P. katadae* var. *hemiphylla* dropped to near 0 when the water loss of the thalli was 67% and thalli with water loss>45% failed to recover to the initial level even being rehydrated for three days [27]. Our study combined with the previous findings

Table 1
Two-way analysis of variance for the effects of dehydration and salinity on relative growth rate, Fv/Fm, and Chl *a* of *P. yezoensis*. Dehydration*Salinity means the interactive effect of Dehydration and Salinity, df means degree of freedom, F means the value of F statistic, and Sig. means *p*-value.

Source	Relative growth rate			Fv/Fm			Chl *a*		
	df	F	Sig.	df	F	Sig.	df	F	Sig.
Dehydration	1	34.159	<0.001	1	0.30	0.864	1	9.805	0.006
Salinity	3	24.080	<0.001	3	5.929	0.006	3	8.879	0.001
Dehydration * salinity	3	1.817	0.185	3	3.505	0.040	3	0.233	0.872
Error	16			16			16		

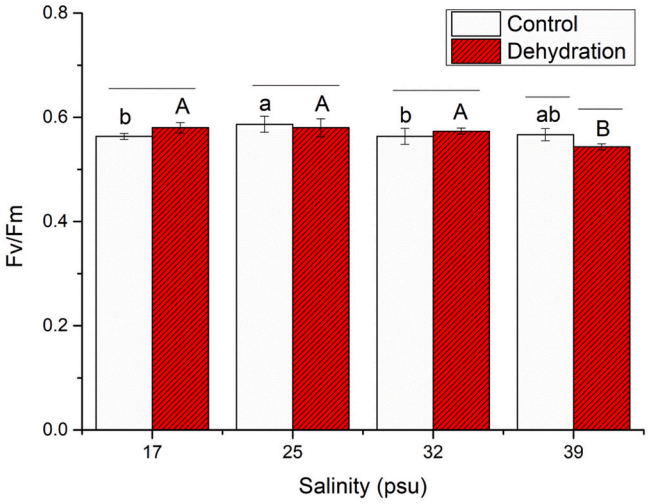

Fig. 2. Maximal photochemical efficiency (Fv/Fm) of *P. yezoensis* cultured at different salinities with or without periodical dehydration. Control means the treatment without periodical dehydration. The error bars indicate the standard deviations ($n = 3$). Different lowercase and capital letters above the error bars represent significant differences ($P < 0.05$) among salinities without dehydration or with dehydration, respectively. Horizontal short bars represent significant differences ($P < 0.05$) between dehydration treatments.

indicates that resilience to dehydration differs among *Pyropia* species. Antidehydration capacities of seaweeds are closed related to their vertical distribution in intertidal zone and the patterns of submersion and emersion. *P. yezoensis* and *P. haitanensis* usually inhabit very high intertidal zones of rocky seashores, exposed to air for a long time and suffering very harsh dehydration. In contrast, *P. katadae* var. *hemiphylla*, are found usually living within water all the time or exposed to air for only a very short time, leading to its vulnerability to dehydration [27,31]. It has been demonstrated that cyclic electron flow in *P. yezoensis* and *P. haitanensis* played a critical role during desiccation and rehydration, which could allow their blades to adapt to changing intertidal environments [16,30].

The changes of salinity (17–39 psu) had a minor effect on Fv/Fm of *P. yezoensis* in this study and it is noticeable only when thalli were cultured under the combined condition of higher salinity with dehydration. Wang et al. [27] also found that there was no significant difference in Fv/Fm of *P. katadae* var. *hemiphylla* among the salinities (15.6, 22.6, 29.6, 36.6, 43.6, 50.6 psu) during 10 days of culture. The dumb effect of salinity (15–45 psu) on PSII activity (electron transport rate) was also reported in *P. haitanensis* [28]. These findings suggest that *Pyropia* species have a high tolerance to hyperosmotic and hypoosmotic environments. The minor effects of dehydration and salinity on Fv/Fm indicate that the negative effects of them on growth may be mainly through other metabolic activities rather than photosynthesis.

3.2. Effects of dehydration and salinity on pigments of P. yezoensis

The content of Chl *a* in *P. yezoensis* varied from 1.09 to 1.66 mg g^{-1} FW (Fig. 3). Both dehydration and salinity affected Chl *a* content (Table 1). Dehydration induced more Chl *a* although the increase was statistically significant only at 39 psu. The salinity of 39 psu induced highest Chl *a* content, while the differences among 17–32 psu were not significant. Samanta et al [21] also showed that higher salinity (55 psu) induced more Chl *a* synthesis in *P. yezoensis* compared to the lower salinity (30 psu). The higher Chl *a* content and unchanged Fv/Fm at 39 psu suggest the acclimation process of thalli to the hypersaline condition, synthesizing more photosynthetic pigments to maintain photosynthetic activity.

Carotenoids in *P. yezoensis* ranged from 0.20 ± 0.01 to 0.31 ± 0.02 mg g^{-1} FW in this study (Fig. 4). Two-way ANOVA showed that dehydration affected carotenoids content and salinity did not have a significant effect (Table 2) although LSD analysis showed that other salinities increased carotenoids content in thalli without dehydration compared to salinity 32 psu. Gao et al. [23] also found that the lower salinities (10 and 20 psu) induced more carotenoids compared to the control (30 psu). Dehydration enhanced carotenoids content although it was not statistically significant at salinity 17 psu. In addition to acting as light-harvesting antennas for photosynthesis, carotenoids could dissipate the excess energy as heat or directly scavenge reactive oxygen species caused by environmental stress [32,33]. The enhanced carotenoids content should be a sign that thalli's defensive response to the harm brought by dehydration. Li et al. [14] also reported that daily dehydration induced more carotenoids in *P. yezoensis* grown in a farm in the south of Shandong province (120.55°E, 35.75°N), China. The stimulated carotenoids synthesis under hypersaline and desiccation conditions in the present study may play an essential role in protecting photosynthetic

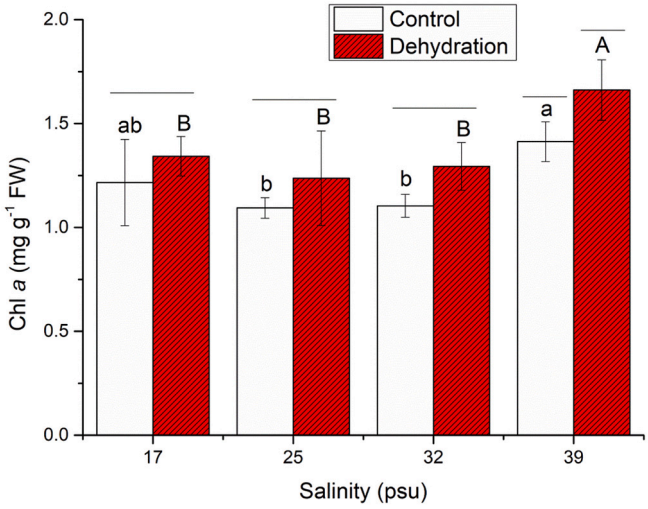

Fig. 3. Chl *a* content of *P. yezoensis* cultured at different salinities with or without periodical dehydration. Control means the treatment without periodical dehydration. The error bars indicate the standard deviations (n = 3). Different lowercase and capital letters above the error bars represent significant differences ($P < 0.05$) among salinities without dehydration or with dehydration, respectively. Horizontal short bars represent significant differences ($P < 0.05$) between dehydration treatments.

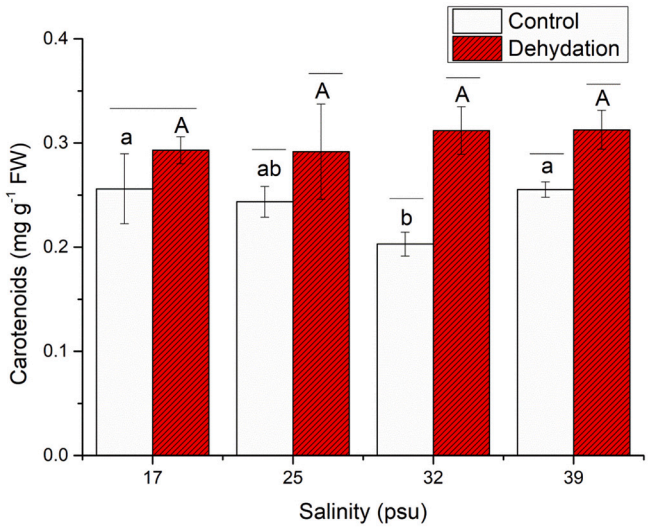

Fig. 4. Carotenoids content of *P. yezoensis* cultured at different salinities with or without periodical dehydration. Control means the treatment without periodical dehydration. The error bars indicate the standard deviations ($n = 3$). Different lowercase and capital letters above the error bars represent significant differences ($P < 0.05$) among salinities without dehydration or with dehydration, respectively. Horizontal short bars represent significant differences ($P < 0.05$) between dehydration treatments.

apparatuses of *P. yezoensis* from the harm of environmental stress.

Phycoerythrin content ranged from 5.35 ± 0.70 to 6.84 ± 0.29 mg g^{-1} FW (Fig. 5). Two-way ANOVA showed that neither dehydration nor salinity affected phycoerythrin content (Table 2) although LSD analysis showed that dehydration induced more phycoerythrin content at salinity of 17 psu. Compared to phycoerythrin, there was a larger variation for phycocyanin content (from 1.12 ± 0.70 to 2.84 ± 0.16 mg g^{-1} FW) (Fig. 6). Two-way ANOVA showed that dehydration did not have a main effect but salinity interacted with dehydration to affect phycocyanin content (Table 2). Phycocyanin content increased with salinity although the increase between salinities 17 and 25 psu for thalli with dehydration was not statistically significant. In terms of the interaction of dehydration and salinity, dehydration did not affect phycocyanin content at salinities 25–39 psu but increased it by 50% at salinity 17 psu. Apart from acting as light-harvesting proteins, phycoerythrin and phycocyanin could deal with ROS as antioxidant compounds [34]. In the present study, higher phycoerythrin and phycocyanin levels in *P. yezoensis* at higher salinities or dehydration conditions may be a defense response to ROS caused by hypersaline and desiccation stress to maintain photosynthetic activity (Fv/Fm).

3.3. Effects of dehydration and salinity on amino acids of *P. yezoensis*

Seventeen amino acids were identified in *P. yezoensis* cultured under different salinity and dehydration conditions (Table 3). Ala, Glu, and Asp were the most abundant AAs among them, which is consistent with the *P. yezoensis* from in a nori farm in at Akashi, Hyogo Prefecture, Japan [35] and the *P. yezoensis* from Jindo, Haenam, and Seochun on the southern and western coasts of Korea [36], indicating that different

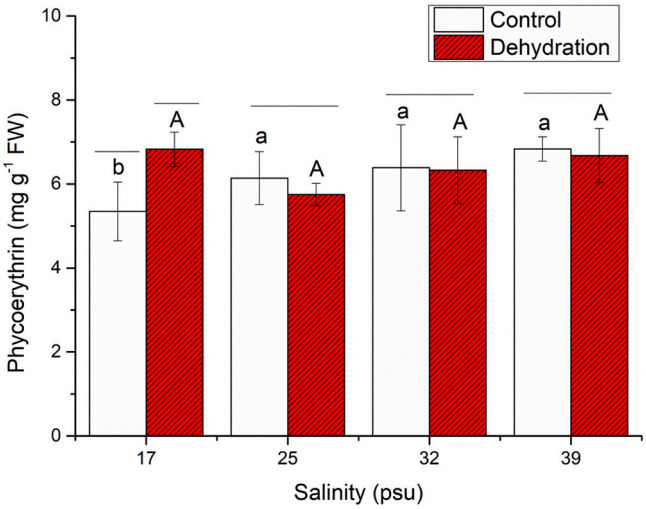

Fig. 5. Phycoerythrin content of *P. yezoensis* cultured at different salinities with or without periodical dehydration. Control means the treatment without periodical dehydration. The error bars indicate the standard deviations ($n = 3$). Different lowercase and capital letters above the error bars represent significant differences ($P < 0.05$) among salinities without dehydration or with dehydration, respectively. Horizontal short bars represent significant differences ($P < 0.05$) between dehydration treatments.

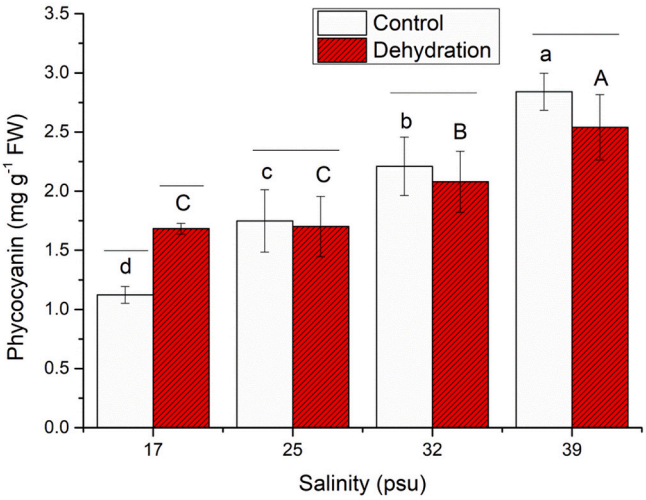

Fig. 6. Phycocyanin content of *P. yezoensis* cultured at different salinities with or without periodical dehydration. Control means the treatment without periodical dehydration. The error bars indicate the standard deviations (n = 3). Different lowercase and capital letters above the error bars represent significant differences ($P < 0.05$) among salinities without dehydration or with dehydration, respectively. Horizontal short bars represent significant differences ($P < 0.05$) between dehydration treatments.

Table 2
Two-way analysis of variance for the effects of dehydration and salinity on carotenoids, phycoerythrin, and phycocyanin of *P. yezoensis*. Dehydration*Salinity means the interactive effect of Dehydration and Salinity, df means degree of freedom, F means the value of F statistic, and Sig. means *p*-value.

Source	Carotenoids			Phycoerythrin			Phycocyanin		
	df	F	Sig.	df	F	Sig.	df	F	Sig.
Dehydration	1	40.733	<0.001	1	0.696	0.416	1	0.054	0.820
Salinity	3	1.288	0.312	3	1.853	0.178	3	40.186	<0.001
Dehydration * salinity	3	2.621	0.086	3	2.633	0.085	3	4.544	0.017
Error	16			16			16		

Table 3
Content of amino acids (mg g^{-1} DW) in *P. yezoensis* cultured at four salinity levels without (17, 25, 32, 39) or with (17D, 25D, 32D, 39D) periodical dehydration. Values are means of three replicates ± standard deviation. Different lowercase and capital letters represent significant differences ($P < 0.05$) among salinities without dehydration or with dehydration, respectively. Asterisks represent significant differences ($P < 0.05$) between dehydration treatments.

Amino Acid	17	25	32	39	17D	25D	32D	39D
Asp	$23.52 \pm 1.45^{ab*}$	$26.84 \pm 2.81^{a*}$	24.92 ± 3.15^{ab}	21.22 ± 2.22^{b}	29.44 ± 1.45^{A}	21.25 ± 1.02^{B}	23.08 ± 2.94^{B}	22.57 ± 3.91^{B}
Thr	12.33 ± 0.94^{a}	$13.9 \pm 0.83^{a*}$	13.76 ± 1.89^{a}	12.46 ± 1.28^{a}	14.63 ± 0.52^{A}	11.39 ± 0.62^{B}	12.81 ± 1.51^{AB}	13.07 ± 2.30^{AB}
Ser	11.56 ± 1.05^{a}	$13.15 \pm 0.99^{a*}$	12.83 ± 1.36^{a}	11.53 ± 1.24^{a}	13.64 ± 0.43^{A}	10.71 ± 0.58^{B}	12.18 ± 1.12^{AB}	12.91 ± 2.66^{AB}
Glu	$27.59 \pm 1.54^{ab*}$	$31.01 \pm 3.9^{a*}$	26.84 ± 3.38^{ab}	24.3 ± 2.56^{b}	32.94 ± 1.69^{A}	23.78 ± 1.3^{B}	25.29 ± 2.41^{B}	25.14 ± 4.28^{B}
Gly	$13.4 \pm 1.13^{a*}$	$15.54 \pm 0.83^{a*}$	15.21 ± 1.51^{a}	13.69 ± 1.32^{a}	15.94 ± 0.67^{A}	12.71 ± 1.09^{B}	14.38 ± 1.17^{AB}	14.73 ± 2.63^{AB}
Ala	25.81 ± 1.52^{b}	$32.96 \pm 4.16^{a*}$	32.6 ± 3.07^{a}	31.66 ± 3.57^{a}	29.97 ± 1.43^{AB}	25.71 ± 1.25^{B}	31.39 ± 3.14^{AB}	32.24 ± 5.58^{A}
Cys	$0.32 \pm 0.12^{a*}$	0.51 ± 0.09^{a}	0.53 ± 0.34^{a}	0.42 ± 0.06^{a}	0.82 ± 0.14^{A}	0.46 ± 0.08^{B}	0.5 ± 0.14^{B}	0.55 ± 0.17^{AB}
Val	$13.64 \pm 1.48^{a*}$	$15.24 \pm 0.57^{a*}$	14.86 ± 2.05^{a}	13.38 ± 1.08^{a}	16.34 ± 0.52^{A}	12.54 ± 0.46^{B}	13.89 ± 1.74^{AB}	14.11 ± 2.54^{AB}
Met	4.65 ± 0.54^{a}	4.04 ± 0.90^{a}	3.16 ± 1.71^{a}	3.33 ± 0.41^{a}	5.66 ± 0.20^{A}	3.49 ± 0.64^{B}	3.3 ± 0.74^{B}	3.73 ± 1.34^{B}
Ile	8.47 ± 0.94^{a}	$9.72 \pm 0.42^{a*}$	9.82 ± 1.36^{a}	8.83 ± 0.80^{a}	10.11 ± 0.41^{A}	8.06 ± 0.36^{B}	9.15 ± 1.10^{AB}	9.11 ± 1.47^{AB}
Leu	$17.22 \pm 1.73^{a*}$	$19.24 \pm 0.88^{a*}$	18.92 ± 2.49^{a}	17.1 ± 1.60^{a}	20.6 ± 0.91^{A}	15.73 ± 0.46^{B}	17.52 ± 2.19^{AB}	17.66 ± 3.11^{AB}
Tyr	6.04 ± 1.27^{a}	6.59 ± 0.84^{a}	5.96 ± 2.66^{a}	5.86 ± 0.48^{a}	8.14 ± 0.40^{A}	5.72 ± 0.35^{B}	5.98 ± 1.11^{AB}	6.37 ± 1.56^{AB}
Phe	$9.2 \pm 0.93^{a*}$	$10.36 \pm 0.46^{a*}$	10.29 ± 1.37^{a}	9.17 ± 0.86^{a}	10.98 ± 0.48^{A}	8.49 ± 0.39^{B}	9.49 ± 1.16^{AB}	9.62 ± 1.69^{AB}
Lys	$12.6 \pm 1.10^{a*}$	$14.05 \pm 0.73^{a*}$	14.29 ± 1.11^{a}	12.52 ± 1.13^{a}	15.36 ± 0.56^{A}	11.66 ± 0.54^{B}	13.1 ± 1.67^{B}	13.26 ± 2.17^{AB}
His	4.62 ± 0.10^{a}	4.65 ± 0.44^{a}	4.43 ± 0.44^{a}	4.48 ± 0.38^{a}	4.80 ± 0.68^{A}	3.86 ± 0.25^{B}	4.15 ± 0.30^{AB}	4.26 ± 0.74^{AB}
Arg	$13.44 \pm 1.36^{a*}$	$14.89 \pm 0.94^{a*}$	14.37 ± 2.09^{a}	13.23 ± 1.26^{a}	16.31 ± 0.57^{A}	12.13 ± 0.23^{B}	13.29 ± 1.64^{B}	13.78 ± 2.55^{AB}
Pro	10.54 ± 0.99^{a}	$11.87 \pm 0.67^{a*}$	11.33 ± 1.60^{a}	10.32 ± 0.95^{a}	12.17 ± 0.58^{A}	9.52 ± 0.46^{B}	10.56 ± 1.27^{AB}	10.69 ± 1.94^{AB}
TAA	$214.95 \pm 18.15^{a*}$	$244.57 \pm 19.16^{a*}$	234.13 ± 31.45^{a}	213.5 ± 20.95^{a}	257.84 ± 9.46^{A}	197.19 ± 7.89^{B}	220.05 ± 25.09^{AB}	223.81 ± 40.54^{AB}
EAA	$96.17 \pm 9.11^{a*}$	$106.09 \pm 5.36^{a*}$	103.90 ± 14.47^{a}	94.50 ± 8.63^{a}	114.79 ± 4.77^{A}	87.34 ± 2.76^{B}	96.71 ± 12.01^{AB}	98.60 ± 17.84^{AB}
Umami AA	$51.12 \pm 2.98^{ab*}$	$57.85 \pm 6.65^{a*}$	51.76 ± 6.53^{ab}	45.52 ± 4.77^{b}	62.38 ± 3.11^{A}	45.04 ± 2.29^{B}	48.37 ± 5.32^{B}	47.71 ± 8.19^{B}
Sweet AA	61.31 ± 4.68^{a}	$73.52 \pm 6.54^{a*}$	71.97 ± 7.49^{a}	67.21 ± 7.04^{a}	71.71 ± 2.95^{A}	58.64 ± 3.30^{B}	68.50 ± 6.59^{AB}	70.57 ± 12.79^{AB}

TAA, total amino acids. EAA, essential amino acids: His, Thr, Arg, Val, Met, Phe, Ile, Leu, and Lys. NEAA, non-essential amino acids: Asp, Glu, Ser, Gly, Ala, Tyr, Cys, Trp, and Pro. Umami amino acids: Aps and Glu. Sweet amino acids: Gly, Pro, Alan, and Ser.

locations did not affect the dominant proportions of these amino acids. The total AA content ranged from 197.19 to 244.57 mg g^{-1} DW and EAA accounted for nearly half of it. Umami AA and sweet AA also had a noticeable proportion, with the range of 21–24% and 28–32%, respectively. Dehydration did not affect content of any amino acid (Table S1). Salinity affected the contents of Asp, Glu, Met, and umami AA (Table S1). Dehydration and salinity interacted on the contents of Asp, Thr, Ser, Glu, Gly, Cys, Val, Ile, Leu, Phe, Lys, Arg, TAA, EAA, umami AA, and sweet AA (Table S1). For instance, the salinity of 25 psu induced the highest Asp, Glu and umami AA content in thalli without dehydration while it the optimal salinity was 17 psu for thalli with dehydration. In thalli without dehydration, there was no difference in contents of Thr, Ser or Gly, Cys, Val, Ile, Leu, Phe, Lys, Arg, TAA, EAA, and sweet AA among the salinities but the salinity of 17 psu induced the highest contents of Thr, Ser, Gly, Cys, Val, Ile, Leu, Phe, Lys, Arg, TAA, EAA, and sweet AA content for thalli with dehydration.

It was reported that *P. haitanensis* preferred to recover the expression levels of proteins relating to photosynthesis and energy metabolisms firstly and then other proteins to restart its normal life activities after rehydration [37]. That may explain why dehydration did not affect content of any amino acid in this study because there were enough time for thalli to recover the synthesis of amino acids after rehydration. Jung et al. [36] investigated the amino acid profile of *P. yezoensis* collected from different locations and found those at the locations with lower salinities had higher content of Asp and Glu, which is consistent with our study. The induction of amino acid by lower salinity may be related to the change of osmotic pressure which triggered the amino acid-based defense [29]. The potential mechanisms of interaction between dehydration and salinity on amino acid content remain unknown and need to explore in future studies. The changing levels of umami and sweet amino acids at different salinities indicate that lower salinity could alter the flavor of nori, like previous finding for ocean acidification [11]. The optimal salinity for total AA , EAA, umami AA, and sweet AA production was 25 psu for thalli without dehydration; while it changed to 17 psu for thalli with dehydration. This shift should be considered when nori cultivation was conducted with periodical dehydration.

3.4. Effects of dehydration and salinity on fatty acids of *P. yezoensis*

The most abundant FA in *P. yezoensis* is C16, accounting for about half of the total FA (Table 4). The dominant proportion of C16 was also found in red macroalgae *P. umbilicalis* [38], *P. amplissima* [39], *Gracilaria gracilis* [38], *Ceramium virgatum* [38], green macroalgae, *Codium fragile* [38] and *Ulva rigida* [6], and brown macroalgae *Laminaria japonica* [40], *Fucus serratus* [38] and *Cystoseira nodicaulis* [38], indicating that this pattern may exist in all seaweeds. C20:5(n-3) was the second abundant fatty acid in *P. yezoensis*, ranged from 30.65 to 37.06% (Table 4). More than half of the FA was SFA, and PUFA also had a considerate proportion (36.38–43.71%), with the proportion of MUFA less than 3% (Table 4). Compared to n-6 FA, there were much more n-3 FA, leading to the ratio of n-6 to n-3 lower than 0.25 (Table 4). Dehydration and salinity interacted on C12, C18:1(n-9), C18:2(n-6), and C20:2(n-6) (Table S2). For instance, dehydration did not affect C12 at salinities 25, 32 or 39 psu but decreased it at salinity 17 psu. Dehydration did not affect C18:1(n-9), C18:2(n-6), or C20:2(n-6) at any salinity except for 17 psu at which it increased them. Dehydration increased C18:3(n-6), C20:4(n-6) and n-6/n-3 (Table S2). Salinity affected C15, C18:3(n-6), C20:1(n-9), C20:3(n-6), C20:4(n-6), MUFA, and FA(n-6) (Table S2). Lower salinities (17 and 25 psu) induced more C15, C18:3(n-6) compared to the salinity of 39 psu (Table 4). The highest salinity had lower content of C20:1(n-9) and MUFA, while there were no differences among the other salinities (17–32 psu). The content of C20:3(n-6) decreased with the increase of salinity although the difference between 17 and 25 psu was not significant (Table 4). The content of C20:4(n-6) increased with the increase of salinity and the difference between 32 and 39 psu was not significant. The lowest salinity of 17 psu induced less FA(n-6) compared to other salinities (25–39 psu).

In higher plants, unsaturated fatty acids are deemed to play an essential role in strengthening cells' tolerance to drought [41,42]. For instance, the increase in α-linolenic acid (18:3, LA) levels in *Nicotiana tabacum* cells improved plant's tolerance to drought stress [42]. In our study, the contents of MUFA and PUFA were higher in thalli with dehydration than those without dehydration but this increase was only statistically significant for the salinity of 17 psu. This may be attributed to the short-time dehydration and long-time rehydration, which diluted the effect of dehydration. Thus the effect of dehydration was only

Table 4
Content of fatty acids (% of total fatty acid methyl esters) in *P. yezoensis* cultured at four salinity levels without (17, 25, 32, 39) or with (17D, 25D, 32D, 39D) periodical dehydration. Values are means of three replicates ± standard deviation. Different lowercase and capital letters represent significant differences ($P < 0.05$) among salinities without dehydration or with dehydration, respectively. Asterisks represent significant differences ($P < 0.05$) between dehydration treatments.

Fatty Acid	17	25	32	39	17D	25D	32D	39D
C12	$0.47 \pm 0.18^{a*}$	0.10 ± 0.03^{b}	0.23 ± 0.14^{b}	0.13 ± 0.03^{b}	0.13 ± 0.04^{A}	0.19 ± 0.06^{A}	0.22 ± 0.08^{A}	0.19 ± 0.11^{A}
C14	$0.60 \pm 0.08^{a*}$	0.53 ± 0.05^{ab}	0.46 ± 0.02^{b}	0.45 ± 0.04^{b}	0.45 ± 0.04^{A}	0.47 ± 0.05^{A}	0.49 ± 0.17^{A}	0.40 ± 0.03^{A}
C15	0.43 ± 0.08^{a}	0.38 ± 0.09^{a}	0.33 ± 0.05^{ab}	0.24 ± 0.04^{b}	0.32 ± 0.03^{A}	0.35 ± 0.07^{A}	0.30 ± 0.12^{A}	0.24 ± 0.03^{A}
C16	$50.57 \pm 1.72^{a*}$	49.07 ± 2.46^{a}	48.38 ± 0.39^{a}	48.15 ± 1.55^{a}	47.89 ± 1.06^{A}	47.51 ± 0.72^{A}	49.13 ± 0.77^{A}	47.56 ± 1.57^{A}
C17	0.10 ± 0.00^{a}	0.10 ± 0.04^{a}	0.08 ± 0.02^{a}	0.07 ± 0.01^{a}	0.15 ± 0.03^{A}	0.11 ± 0.03^{AB}	0.09 ± 0.05^{B}	0.08 ± 0.02^{B}
C18	6.79 ± 2.80^{a}	5.79 ± 0.62^{ab}	3.92 ± 0.77^{b}	4.63 ± 0.89^{ab}	5.64 ± 1.54^{A}	4.67 ± 1.95^{A}	6.00 ± 2.37^{A}	4.98 ± 0.59^{A}
C18:1(n-9)	$0.59 \pm 0.03^{b*}$	1.06 ± 0.15^{a}	1.03 ± 0.10^{a}	0.59 ± 0.12^{b}	0.97 ± 0.19^{B}	1.23 ± 0.01^{A}	0.97 ± 0.07^{B}	0.70 ± 0.14^{C}
C18:2(n-6)	$0.95 \pm 0.14^{b*}$	1.24 ± 0.03^{a}	$1.34 \pm 0.10^{a*}$	1.06 ± 0.05^{b}	1.22 ± 0.06^{A}	1.22 ± 0.11^{A}	1.15 ± 0.05^{AB}	1.02 ± 0.06^{B}
C18:3(n-6)	0.22 ± 0.05^{a}	$0.20 \pm 0.04^{a*}$	0.18 ± 0.03^{a}	0.12 ± 0.02^{b}	0.17 ± 0.02^{A}	0.15 ± 0.01^{A}	0.14 ± 0.02^{A}	0.14 ± 0.02^{A}
C20:1(n-9)	$1.34 \pm 0.10^{ab*}$	1.69 ± 0.55^{a}	1.40 ± 0.20^{ab}	1.00 ± 0.17^{b}	2.00 ± 0.55^{A}	1.57 ± 0.04^{AB}	1.44 ± 0.01^{B}	1.03 ± 0.22^{B}
C20:2(n-6)	$0.69 \pm 0.03^{a*}$	0.72 ± 0.16^{a}	0.79 ± 0.12^{a}	0.68 ± 0.11^{a}	0.96 ± 0.07^{A}	0.70 ± 0.05^{B}	0.73 ± 0.05^{B}	0.64 ± 0.02^{B}
C20:3(n-6)	1.30 ± 0.10^{a}	$1.42 \pm 0.15^{a*}$	1.12 ± 0.08^{b}	0.82 ± 0.03^{c}	1.33 ± 0.10^{A}	1.22 ± 0.09^{A}	0.95 ± 0.08^{B}	0.84 ± 0.12^{B}
C20:4(n-6)	2.58 ± 0.27^{c}	3.42 ± 0.35^{b}	4.09 ± 0.41^{a}	4.42 ± 0.40^{a}	2.10 ± 0.10^{C}	3.25 ± 0.40^{B}	3.90 ± 0.30^{A}	4.02 ± 0.11^{A}
C20:5(n-3)	$30.65 \pm 6.18^{b*}$	33.74 ± 0.38^{ab}	35.40 ± 0.99^{a}	36.50 ± 1.56^{a}	35.75 ± 1.84^{A}	36.65 ± 1.46^{A}	33.55 ± 2.21^{A}	37.06 ± 1.95^{A}
SFA	$58.97 \pm 4.52^{a*}$	55.96 ± 1.78^{ab}	53.40 ± 1.18^{b}	53.68 ± 2.10^{b}	54.57 ± 2.67^{A}	53.29 ± 2.44^{A}	56.23 ± 2.03^{A}	53.45 ± 1.80^{A}
MUFA	$1.93 \pm 0.08^{b*}$	2.75 ± 0.67^{a}	2.43 ± 0.19^{ab}	1.59 ± 0.26^{b}	2.96 ± 0.74^{A}	2.80 ± 0.03^{A}	2.41 ± 0.08^{AB}	1.73 ± 0.37^{B}
PUFA	$36.38 \pm 6.02^{b*}$	40.74 ± 0.81^{ab}	42.92 ± 1.41^{a}	43.60 ± 2.03^{a}	41.53 ± 1.86^{A}	43.19 ± 2.08^{A}	40.43 ± 2.33^{A}	43.71 ± 1.90^{A}
FA(n-6)	5.73 ± 0.17^{b}	7.00 ± 0.48^{a}	7.52 ± 0.55^{a}	7.09 ± 0.50^{a}	5.78 ± 0.15^{B}	6.54 ± 0.62^{A}	6.88 ± 0.41^{A}	6.66 ± 0.30^{A}
FA(n-3)	$30.65 \pm 6.18^{b*}$	33.74 ± 0.38^{ab}	35.40 ± 0.99^{a}	36.50 ± 1.56^{a}	35.75 ± 1.84^{A}	36.65 ± 1.46^{A}	33.55 ± 2.21^{A}	37.06 ± 1.95^{A}
n-6/n-3	0.19 ± 0.05^{a}	0.21 ± 0.01^{a}	0.21 ± 0.01^{a}	0.19 ± 0.01^{a}	0.16 ± 0.01^{B}	0.18 ± 0.01^{AB}	0.21 ± 0.02^{A}	0.18 ± 0.01^{AB}

SFA, saturated fatty acids. MUFA, monounsaturated fatty acids. PUFA, polyunsaturated fatty acids. FA(n-6), total Omega-6 fatty acids. FA(n-3), total Omega-3 fatty acids. n-6/n-3, the ratio of total Omega-6 fatty acids to total Omega-3 fatty acids.

obvious when it was combined with low salinity. The change of unsaturation fatty acid content could regulate the permeability and mobility of blade membrane and contributes to their osmotic acclimation to the intertidal habitat. For eicosapentaenoic acid (EPA, C20:5(n-3)) and PUFA, the optimal salinity was 39 psu for thalli without dehydration and the lowest salinity reduced them compared to higher salinities, but the effect of salinity disappeared when thalli experienced periodical dehydration. This may be attributed to the simulating effect of dehydration at the lowest salinity. Therefore, it suggests that thalli can be cultivated in a wide range of salinity without affecting EPA and PUFA production when thalli were periodically dehydrated.

4. Conclusions

Periodical dehydration plays an essential role in *Pyropia* cultivation in terms of killing fouling organisms. Salinity can also regulate growth and metabolisms of *Pyropia*. The present study demonstrates that periodical dehydration could reduce relative growth rate of *P. yezoensis* and highest growth rate occurred at salinity 25 psu. The negative effect of dehydration on growth should not be through photosynthesis because it did not show any harm to Fv/Fm. Dehydration induced more Chl *a*, carotenoids at most salinities and more phycoerythrin, phycocyanin, MUFA and PUFA at salinity 17 psu. Higher salinities also stimulated the synthesis of phycocyanin. These changes may be the defensive response of thalli to environmental stress, which effectively protected photosynthesis but did not completely eliminate the negative effects of dehydration and hypersaline stress on growth. Dehydration and salinity interacted on the contents of most amino acids, leading to the shift of optimal salinity for total AA , EAA, umami AA, and sweet AA production from 25 to 17 psu when thalli experienced periodical dehydration. For EPA and PUFA production, the optimal salinity was 39 psu for thalli without dehydration, whereas there were no significant differences among salinities for thalli with periodical dehydration. These findings show that periodical dehydration could lead to the decline in biomass yield of *P. yezoensis* in spite of its function on removing fouling organisms. Therefore, future work should assess the tradeoff between dehydration and biofouling. The interaction of periodical dehydration and salinity should also be considered when culturing *P. yezoensis* at different salinity environments. For instance, to obtain optimal amino acid production, the optimal salinity is 25 psu for thalli without experiencing dehydration while it is 17 psu for thalli exposed to periodical dehydration.

CRediT author contributions statement

Xinshu Li: Conceptualization, Methodology, Writing- Original draft preparation, Writing- Reviewing and Editing, Supervision, Funding acquisition. Xin Sun: Investigation, Methodology, Formal analysis, Visualization. Lin Gao: Formal analysis, Visualization. Juntian Xu: Conceptualization, Methodology. Guang Gao: Conceptualization, Formal analysis, Visualization, Writing- Original draft preparation, Writing- Reviewing and Editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

Appendix A. Supplementary data

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References

[1] G. Gao, A.S. Clare, C. Rose, G.S. Caldwell, *Ulva rigida* in the future ocean: potential for carbon capture, bioremediation, and biomethane production, Global Change Biol. Bioenerg. 10 (1) (2018) 39–51.

[2] D. Krause-Jensen, C.M. Duarte, Substantial role of macroalgae in marine carbon sequestration, Nat. Geosci. 9 (10) (2016) 737–742.

[3] A. Ortega, N.R. Geraldi, I. Alam, A.A. Kamau, S.G. Acinas, R. Logares, J.M. Gasol, R. Massana, D. Krause-Jensen, C.M. Duarte, Important contribution of macroalgae to oceanic carbon sequestration, Nat. Geosci. 12 (9) (2019) 748–754.

[4] C. Couteau, L. Coiffard, Seaweed application in cosmetics, in: J. Fleurence, I. Levine (Eds.), Seaweed in Health and Disease Prevention, Elsevier Inc, Amsterdam, the Netherlands, 2016, pp. 423–441.

[5] G. Gao, J.G. Burgess, M. Wu, S. Wang, K. Gao, Using macroalgae as biofuel: current opportunities and challenges, Bot. Mar. 63 (4) (2020) 355–370.

[6] G. Gao, A.S. Clare, E. Chatzidimitriou, C. Rose, G.S. Caldwell, Effects of ocean warming and acidification, combined with nutrient enrichment, on chemical composition and functional properties of *Ulva rigida*, Food Chem. 258 (2018) 71–78.

[7] T.D. Dillehay, C. Ramírez, M. Pino, M.B. Collins, J. Rossen, J.D. Pino-Navarro, Monte verde: seaweed, food, medicine, and the peopling of South America, Science 320 (5877) (2008) 784–786.

[8] T. Bito, T. Fei, F. Watanabe, Bioactive compounds of edible purple laver *Porphyra* sp. (Nori), J. Agric. Food Chem. 65 (49) (2017) 10685–10692.

[9] J.E. Sutherland, S.C. Lindstrom, W.A. Nelson, J. Brodie, M.D.J. Lynch, M.S. Hwang, H.G. Choi, M. Miyata, N. Kikuchi, M.C. Oliveira, T. Farr, C. Neefus, A. Mols-Mortensen, D. Milstein, K.M. Muller, A new look at an ancient order: generic revision of the Bangiales (Rhodophyta), J. Phycol. 47 (5) (2011) 1131–1151.

[10] FAO, The state of world fisheries and aquaculture 2020, Sustainability in action, Rome. (2020), https://doi.org/10.4060/ca9229en.

[11] G. Gao, Q. Gao, M. Bao, J. Xu, X. Li, Nitrogen availability modulates the effects of ocean acidification on biomass yield and food quality of a marine crop *Pyropia yezoensis*, Food Chem. 271 (2019) 623–629.

[12] Z. Feng, T. Zhang, J. Wang, W. Huang, R. Wang, J. Xu, G. Fu, G. Gao, Spatio-temporal features of microplastics pollution in macroalgae growing in an important mariculture area, China, Sci. Total Environ. 719 (2020), 137490.

[13] T. Zhang, J. Li, F. Ma, Q. Lu, Z. Shen, J. Zhu, Study of photosynthetic characteristics of the *Pyropia yezoensis* thallus during the cultivation process, J. Appl. Phycol. 26 (2) (2014) 859–865.

[14] X.L. Li, W.J. Wang, F.L. Liu, Z.R. Liang, X.T. Sun, H.Q. Yao, F.J. Wang, Periodical drying or no drying during aquaculture affects the desiccation tolerance of a sublittoral *Pyropia yezoensis* strain, J. Appl. Phycol. 30 (1) (2018) 697–705.

[15] N.A. Blouin, J.A. Brodie, A.C. Grossman, P. Xu, S.H. Brawley, *Porphyra*: a marine crop shaped by stress, Trends Plant Sci. 16 (1) (2011) 29–37.

[16] S. Gao, G.C. Wang, The enhancement of cyclic electron flow around photosystem I improves the recovery of severely desiccated *Porphyra yezoensis* (Bangiales, Rhodophyta), J. Exp. Bot. 63 (12) (2012) 4349–4358.

[17] G. Gao, L. Qu, T. Xu, J.G. Burgess, X. Li, J. Xu, Future CO_2-induced ocean acidification enhances resilience of a green tide alga to low-salinity stress, ICES J. Mar. Sci. 76 (7) (2019) 2437–2445.

[18] H.D. Nielsen, S.L. Nielsen, Effects of temperature and salinity on germling development and fitness in the brown macroalga *Fucus serratus L*, Int. J. Aquat. Sci. 3 (2) (2012) 3–12.

[19] J.P. Rodríguez, J. Terrados, S. Rosenfeld, F. Méndez, J. Ojeda, A. Mansilla, Effects of temperature and salinity on the reproductive phases of *Macrocystis pyrifera* (L.) C. Agardh (Phaeophyceae) in the Magellan region, J. Appl. Phycol. 31 (2) (2019) 915–928.

[20] C.L. Hurd, P.J. Harrison, K. Bischof, C.S. Lobban, Seaweed ecology and physiology, Biol. Plant. 6 (2014) 524.

[21] P. Samanta, S. Shin, S. Jang, J.K. Kim, Comparative assessment of salinity tolerance based on physiological and biochemical performances in *Ulva australis* and *Pyropia yezoensis*, Algal Res. 42 (2019), 101590.

[22] L. He, A. Huang, J. Niu, X. Lu, X. Xie, G. Wang, Photosynthetic and metabolic analyses reveal a higher resistance to salinity in the *Pyropia yezoensis* (Rhodophyta) blades than in the conchocelis, Algal Res. 40 (2019), 101483.

[23] G. Gao, Z. Zhong, X. Zhou, J. Xu, Changes in morphological plasticity of *Ulva prolifera* under different environmental conditions: a laboratory experiment, Harmful Algae 59 (2016) 51–58.

[24] A.R. Wellburn, The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution, J. Plant Physiol. 144 (3) (1994) 307–313.

[25] S. Beer, A. Eshel, Determining phycoerythrin and phycocyanin concentrations in aqueous crude extracts of red algae, Mar. Freshw. Res. 36 (6) (1985) 785–792.

[26] J. Ma, T. Xu, M. Bao, H. Zhou, T. Zhang, Z. Li, G. Gao, X. Li, J. Xu, Response of the red algae *Pyropia yezoensis* grown at different light intensities to CO_2-induced seawater acidification at different life cycle stages, Algal Res. 49,101950 (2020).

[27] W.J. Wang, X.T. Sun, F.L. Liu, Z.R. Liang, J.H. Zhang, F.J. Wang, Effect of abiotic stress on the gameophyte of *Pyropia katadae* var. *hemiphylla* (Bangiales, Rhodophyta), J. Appl. Phycol. 28 (1) (2016) 469–479.

[28] H. Wu, G. Ding, Z. Xu, Effects of salt stress on growth and photosynthesis of *Pyropia haitanensis* (Rhodophyta) cultured under different nitrogen conditions, Oceanologia et Limnologia Sinica 46 (5) (2015) 1210–1217.

[29] M. Kumar, P. Kumari, C.R.K. Reddy, B. Jha, Salinity and desiccation induced oxidative stress acclimation in seaweeds, Adv. Bot. Res. 71 (4) (2014) 91–123.

[30] S. Gao, J.F. Niu, W.Z. Chen, G.C. Wang, X.J. Xie, G.H. Pan, W.H. Gu, D.L. Zhu, The physiological links of the increased photosystem II activity in moderately desiccated *Porphyra haitanensis* (Bangiales, Rhodophyta) to the cyclic electron flow during desiccation and rehydration, Photosynth. Res. 116 (1) (2013) 45–54.

[31] T. Uji, Y. Gondaira, S. Fukuda, H. Mizuta, N. Saga, Characterization and expression profiles of small heat shock proteins in the marine red alga *Pyropia yezoensis*, Cell Stress Chaperones 24 (1) (2019) 223–233.

[32] N.K. Choudhury, R.K. Behera, Photoinhibition of photosynthesis: role of carotenoids in photoprotection of chloroplast constituents, Photosynthetica 39 (4) (2001) 481–488.

[33] K. Das, A. Roychoudhury, Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants, Front. Environ. Sci. 2 (2014) 53.

[34] J. Cao, J. Wang, S. Wang, X. Xu, *Porphyra* species: a mini-review of its pharmacological and nutritional properties, J. Med. Food 19 (2) (2016) 111–119.

[35] K. Niwa, H. Furuita, T. Yamamoto, Changes of growth characteristics and free amino acid content of cultivated *Porphyra yezoensis* Ueda (Bangiales Rhodophyta) blades with the progression of the number of harvests in a nori farm, J. Appl. Phycol. 20 (5) (2008) 687–693.

[36] S.M. Jung, S.G. Kang, J.S. Son, J.H. Jeon, H.J. Lee, H.W. Shin, Temporal and spatial variations in the proximate composition, amino acid, and mineral content of *Pyropia yezoensis*, J. Appl. Phycol. 28 (6) (2016) 3459–3467.

[37] K. Xu, Y. Xu, D. Ji, J. Xie, C. Chen, C. Xie, Proteomic analysis of the economic seaweed *Pyropia haitanensis* in response to desiccation, Algal Res. 19 (2016) 198–206.

[38] M. Schmid, F. Guihéneuf, D.B. Stengel, Fatty acid contents and profiles of 16 macroalgae collected from the Irish coast at two seasons, J. Appl. Phycol. 26 (1) (2014) 451–463.

[39] N. Blouin, B.L. Calder, B. Perkins, S.H. Brawley, Sensory and fatty acid analyses of two Atlantic species of *Porphyra* (Rhodophyta), J. Appl. Phycol. 18 (1) (2006) 79–85.

[40] M. Honya, T. Kinoshita, M. Ishikawa, H. Mori, K. Nisizawa, Seasonal variation in the lipid content of cultured *Laminaria japonica*: fatty acids, sterols, β-carotene and tocopherol, J. Appl. Phycol. 6 (1) (1994) 25–29.

[41] R.G. Upchurch, Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress, Biotechnol. Lett. 30 (6) (2008) 967–977.

[42] M. Zhang, R. Barg, M. Yin, Y. Gueta-Dahan, A. Leikin-Frenkel, Y. Salts, S. Shabtai, G. Ben-Hayyim, Modulated fatty acid desaturation via overexpression of two distinct ω-3 desaturases differentially alters tolerance to various abiotic stresses in transgenic tobacco cells and plants, Plant J. 44 (2005) 361–371.

[43] W.J. Wang, X.L. Li, T.Q. Sun, Z.R. Liang, F.L. Liu, X.T. Sun, F.J. Wang, Effects of periodical drying and non-drying on nutrient content and desiccation tolerance of an intertidal Pyropia yezoensis strain subject to farming conditions, J. Appl. Phycol. 31 (3) (2019) 1897–1906.