

Light absorption and impacts of low salinities on photosynthetic behaviour in the epiphytic alga *Neosiphonia savatieri* (Rhodomelaceae, Rhodophyta)

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Abstract Filamentous epiphyte outbreaks, a serious problem in the commercial cultivation of *Kappaphycus/Eucheuma*, are often triggered by large changes in abiotic factors. However, the physiological characteristics of epiphytes are poorly understood. In this study, the main pigment contents of *Neosiphonia savatieri* and its healthy host strains, including green thalli of *Kappaphycus striatum* (G-KS), brown and green thalli of *Kappaphycus alvarezii* (B-KA, G-KA), were quantified. The light absorption capacities of lipid- and water-soluble pigments of these seaweeds were also measured. Photosynthetic parameters, including pigment content and fast chlorophyll *a* fluorescence kinetics, were further studied in *N. savatieri* exposed to different salinities (16–34 psu) for 10 h in an outdoor experiment. The phycobiliprotein (PBP), Chl-*a*, and carotenoid contents of *N. savatieri* were comparatively higher than the hosts, with the values of 2.50, 0.29, and 0.12 mg g⁻¹ (fw), respectively. The mean extinction coefficients of lipid- and water-soluble pigments in *N. savatieri* were both higher than G-KS, B-KA, and G-KA in the 400–700 nm bands. Salinity of 28 psu increased the pigment contents of the epiphyte, and 16–22 psu did not have a negative impact. Polyphasic fluorescence transients revealed that salinities

at 22–28 psu were beneficial to photosynthetic electron transport, mainly attributed to the enhancement of energy absorption per active PSII reaction centre. Given these observations, *N. savatieri* has a higher light-harvesting capacity and better tolerance to low salinities. These two physiological properties made *N. savatieri* well suited to the low light and hypo-saline conditions that are frequently triggered by fluctuations in abiotic factors.

Keywords *Neosiphonia savatieri* · Mean extinction coefficient · Eucheumatoids · Salinity · Fast chlorophyll *a* fluorescence

Introduction

Eucheumatoid species, including *Eucheuma denticulatum* (N.L. Burman) Collins & Harvey, *Kappaphycus alvarezii* (Doty) Doty, and *Kappaphycus striatum* (Schmitz) Doty ex Silva, have been farmed as raw material for the extraction of the valuable hydrocolloid, carrageenan, since the 1970s (Doty and Alvarez 1975; Bixler and Porse 2011). To date, eucheumatoid farming is considered a profitable business uplifting the socioeconomic conditions of families in coastal communities (Ask 2001; Hurtado et al. 2014; Msuya et al. 2014; Pang et al. 2015). Occurrence of epiphytes is a common problem faced in commercial cultivation of the eucheumatoids. Epiphytic filamentous algae (EFA), the dominant species attaching to *K. alvarezii*, have been noticed since early *K. alvarezii* cultivation (Parker 1974). However, this problem has received little attention until the recent increase in epiphyte outbreaks and their negative impact on biomass production and carrageenan quality. Recently, the dominant problem EFA species were identified as *Polysiphonia* sp. (Hurtado et al. 2006) and two strains of *Neosiphonia*,

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Neosiphonia savatieri (Hariot) M. S. Kim & I. K. Lee, and *Neosiphonia apiculata* (Hollenberg) Masuda & Kogame (Vairappan 2006; Vairappan et al. 2008). Hurtado et al. (2006) and Vairappan (2006) recorded that the outbreaks of *Polysiphonia* sp. in the Philippines and *N. savatieri* in Malaysia lowered the production of *K. alvarezii*. Vairappan et al. (2013) found that the infection of *Kappaphycus* spp. by *N. apiculata* reduced carrageenan molecular size and affected the physical properties of the carrageenan in Malaysia. Pang et al. (2011, 2015) also reported that the occurrence of *N. savatieri* resulted in a tremendous decrease in biomass production of *Kappaphycus* spp. in Li'an Bay of China in 2009 and 2010. In 2016, the seaweeds farmed, e.g. *K. alvarezii* and *K. striatum*, experienced die-offs due to the outbreak of *N. savatieri* from March to April in this bay.

Considerable work has been performed on the epiphyte's identity (Kim 2005), symptoms and secondary infection on the host seaweeds (Vairappan et al. 2008), cellular morphology of *Neosiphonia* (Masuda et al. 2001; Pang et al. 2011), and specific elimination methods for *N. savatieri* (Pang et al. 2012). However, little work has been done to investigate the physiological responses of *Neosiphonia* to abiotic factors such as seawater temperature, salinity, nutrient level, and photoperiod. Interestingly, it has been evident from earlier investigations that large changes in the abiotic factors can act as a triggering mechanisms or cues for the epiphytes to infect *Kappaphycus/Eucheuma* in the Philippines, Indonesia, Malaysia, and Tanzania (Mtolera et al. 1996; Hurtado et al. 2006; Vairappan 2006). Our previous study also mentioned that the outbreak of *N. savatieri* correlated with drastic seawater temperature changes (mainly associated with seasonality) and lower salinities attributed to rain, stream flows, or tidal flows (Pang et al. 2015). Actually, the large fluctuations of seawater salinity triggered by occasional, continuous heavy rainfall are prevalent in eucheumatoid farming, especially in the tropics. On the other hand, epiphytic *Neosiphonia* occupies the surface of eucheumatoids and shades their hosts, preventing them from getting enough light. How the EFA and hosts cope with changeable illumination, e.g. the weak light conditions in unfavourable weather, is very important to their health and growth (Critchley et al. 2004; Borlongan et al. 2016). At present, however, studies comparing light absorption efficiencies of epiphytes and their hosts are lacking.

In this study, we compared the light absorption capacities of *N. savatieri* and its hosts, green thalli of *K. striatum* (G-KS) and brown and green thalli of *K. alvarezii* (B-KA and G-KA, respectively). In addition, the effects of low salinities on the whole thalli of *N. savatieri* were investigated in an outdoor experiment. The present study aims to explain why the outbreak of epiphytes is more likely to happen during abiotic factor fluctuations.

Materials and methods

Plants and sampling location

Neosiphonia savatieri, healthy green thalli of *Kappaphycus striatum* (G-KS), and brown and green thalli of *Kappaphycus alvarezii* (B-KA, G-KA) were chosen as test samples for light absorption quantification. *Neosiphonia savatieri* was collected in Li'an Bay, Hainan Province, China (18° 27' N, 110° 5' E) during an epiphyte outbreak on 28 March 2016. Surface seawater temperature and salinity, recorded using a mercury thermometer and a hand refractometer, were 19.0 °C and 34 psu, respectively. The *N. savatieri*-infected *Kappaphycus* were transported to a field laboratory, located near Li'an Bay, within 10 min of taking samples. Once in the laboratory, the epiphyte-infected specimens were cleaned carefully with a soft brush to remove dirt and other contaminants. Fresh *N. savatieri* were handpicked from the hosts and then washed repeatedly with seawater. In this process, the parts of *N. savatieri* existing on the surface of its host were collected. Tissues (about 2 g wet weight, 40 portions or parts of thalli) were frozen immediately and kept in liquid nitrogen for pigment measurements.

The outbreak of *N. savatieri* was destructive to the hosts (mainly *Kappaphycus*) in Li'an Bay in March 2016. It was difficult to obtain fresh epiphyte and its healthy hosts simultaneously. Healthy *Kappaphycus* thalli, i.e. G-KS, B-KA, and G-KA, previously cultured were used to measure the light absorption capacity and further compared with *N. savatieri*.

Hypo-saline exposure tests of *N. savatieri*

Attached fresh *N. savatieri* thalli were separated from the hosts using a razor blade under a stereo microscope. Sections of the host (about 1.0 cm in length) were kept to protect the rhizoids of *N. savatieri* and ensure the integrity of epiphyte samples. The whole *N. savatieri* thalli were cultured in a plastic tank (50 × 40 × 40 cm) with 20 L seawater under natural lighting. The solar radiation was measured every hour from 9:30 a.m. to 19:30 p.m. The seawater temperature was maintained at 22 °C (±2) with an automatic heating unit or an ice bath. The seawater salinity was 16, 22, 28, and 34 psu, respectively. Media with lower salinities than normal seawater were prepared by diluting filtered seawater with distilled H₂O. Samples exposed to 34 psu salinity (the salinity of normal seawater) were taken as the control.

Quantification of light absorption

Pigment extracts and the average extinction coefficients were determined according to the method of Li and Meng (2014). Briefly, about 0.1 g of frozen algal tissues was homogenized in a pre-chilled mortar using a pestle. A total of 8 mL of 95%

ethanol was added and incubated overnight at 4 °C to extract Chl-*a* and carotenoids as described by Arnon (1949). The transmittance of ethanol extracts of different optical paths (3.0 and 10 mm) at 400–700 nm were quantified with a dual-beam UV–visible spectrophotometer (Hitachi, U-2900 UV/VIS, Japan) with quartz cell. Similarly, for the light absorption quantification of phycobiliprotein (PBP) extracts, 0.5 g of frozen algal tissues was homogenized in a pre-chilled mortar using a pestle. The homogenate was added to 6 mL of 10 mM CaCl₂ and incubated for 36 h at 4 °C. After incubation, homogenized samples were vortexed and centrifuged at 15,000×*g* for 10 min at 4 °C, and then the supernatants were collected. The transmittances were recorded by scanning the supernatant. The average light transmittance of the material was calculated with the equation below (Li and Meng 2014)

$$\bar{T} = \frac{\sum_{\lambda=\lambda_d}^{\lambda_u} S(\lambda) \times T(\lambda) \times V(\lambda)}{\sum_{\lambda=\lambda_d}^{\lambda_u} S(\lambda) \times V(\lambda)} \quad (1)$$

where \bar{T} (%) represents the average light transmittance. λ_d and λ_u are the lower-limit wavelength (400 nm) and the upper-limit wavelength (700 nm), respectively. $T(\lambda)$ represents the monochromatic light transmittance of each wavelength (%). $S(\lambda)$ is the relative spectral power distribution on the corresponding wavelength (W). $V(\lambda)$ is the efficiency of spectral illumination of the corresponding wavelength. The values of $S(\lambda)$ and $V(\lambda)$ can be obtained from information given by International Commission on Illumination (CIE) and National Standardization Technical Committee (2008). According to CIE, the sampling interval to obtain values of $S(\lambda)$ and $V(\lambda)$ is 5 nm in the range 400–700 nm.

The simplified Lambert–Beer law was adopted to calculate the mean extinction coefficients of the pigment extracts, as shown below

$$\ln(\bar{T}) = 2\ln(1-\bar{r}) - \bar{\alpha} \cdot L \quad (2)$$

where $\bar{\alpha}$ represents the mean extinction coefficient, \bar{r} is the mean reflectance, and L is the optical path of different quartz cells, 3.0 and 10 mm, respectively. The values of $\bar{\alpha}$ and \bar{r} could be obtained from the slope and intercept of a plot of $\ln(\bar{T})$ against L . The final results of $\bar{\alpha}$ were displayed as the absorbance per unit Chl-*a* or PBP ($A \text{ (mg Chl-}a\text{)}^{-1}$ or $A \text{ (mg PBP)}^{-1}$).

Chl-*a* fluorescence measurement

The chlorophyll *a* fluorescence (OJIP) transient measurement was conducted by using a Handy Plant

Efficiency Analyzer (Hansatech Instruments, UK) as described in Zhang et al. (2012). The transient was induced by a red light measuring about 3000 μmol photons m⁻² s⁻¹ provided by an array of three light-emitting diodes (peak 650 nm). All the measurements were performed with dark-adapted (for 15 min) leaf disks at room temperature. The measurement for each treatment group was repeated at least eight times using eight different algal thallus samples.

Analysis of fast Chl-*a* fluorescence kinetics

Each OJIP transient was analyzed according to the JIP test (Strasser et al. 2000, 2004) by using the following original data: (i) F_o , the fluorescence intensity at 20 μs when all of the PSII reaction centres (RCs) are open; (ii) F_J , the fluorescence intensity at a time of 2 ms; (iii) F_K , the fluorescence intensity at 300 μs; and (iv) F_m , the maximal fluorescence intensity, assuming that the excitation intensity is high enough to close all of the RCs of PSII. Based on the above data, the following parameters were then calculated: the maximum PSII photochemical efficiency, $F_v/F_m = 1 - F_o/F_m$; the relative variable fluorescence intensity at the *J*-step, $V_J = (F_J - F_o)/(F_m - F_o)$; the relative variable fluorescence intensity at the *K*-step, $V_K = (F_K - F_o)/(F_m - F_o)$; the photosynthetic performance index, PI_{ABS} ; absorption per cross section of the leaf tissue (CS), $ABS/CS_m = F_m$ (at $t = t_{F_m}$); and the density of reaction centres per CS, $RC/CS_m = [1 - (F_o/F_m)] \times (F_m - F_o) \times (F_K - F_o) \times V_J \times (ABS/CS_m)$.

Quantification of pigments

Chl-*a* and carotenoids (Car) were quantified by modified Arnon (1949) and Jensen (1978) methods, respectively. For the PBP quantification, phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC) were measured by the method of Venkataraman (1983). All the equations are listed in our previous publications (Pang et al. 2011; Li et al. 2016).

Statistics

Statistical analyses were performed using SPSS 17.0 software (SPSS Inc., USA). The variation of each parameter was tested by one-way analysis of variance (ANOVA). Least significant difference (LSD) was used to analyze differences between the measurements, and Tukey’s honestly significant difference (HSD) was used for the multiple comparisons. Differences were considered significant at a probability level of $P < 0.05$.

Results

Pigment contents of the four seaweeds

Figure 1 shows the fluctuation in the solar radiation throughout the testing period at the study site. Pigment contents of epiphytic *N. savatieri* and three healthy hosts are presented in Fig. 2. The PBP, Chl-*a*, and Car contents of *N. savatieri* were comparatively higher than its healthy hosts, with the values of 2.50, 0.29, and 0.12 mg g⁻¹ (fw), respectively. Among the investigated carrageenophyte species, PBP content of B-KA was higher than G-KS and G-KA, which could be quantitatively attributable to higher APC content. There was almost no significant difference of Chl-*a* contents of G-KA, B-KA, and G-KA, ranging from 0.46 to 0.56 mg g⁻¹ (fw). The Car contents of these three seaweeds also had similar levels, which ranged from 0.012 to 0.018 mg g⁻¹ (fw).

Light absorption of lipid- and water-soluble pigments

The light absorption capacities of lipid- and water-soluble pigments of four seaweeds are shown in Table 1. The $\bar{\alpha}$ values of both ethanol and 10 mM CaCl₂ extracts from *N. savatieri* were both higher than those of the other three algae under the photosynthetically active radiation (PAR, 400–700 nm).

Effects of low salinities on pigments of *N. savatieri*

At all tested salinities, no obvious loss of the main pigments was observed (Fig. 3). Chl-*a* and carotenoid contents at 28 psu were both significantly higher than those of the control (34 psu). At 22 and 16 psu, these pigments remained constant. Similarly, PBP of 28 psu exposed thalli was also slightly increased, and 22 or 16 psu treatments did not result in a significantly reduced PBP level.

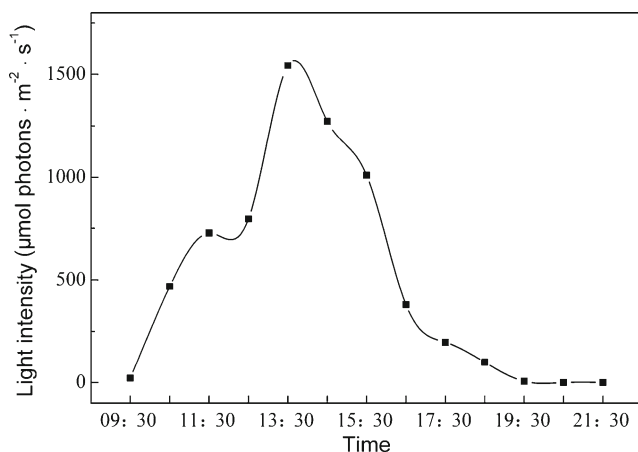


Fig. 1 Characteristic of the solar radiation during the experimental period

Effects of altered salinities on fast Chl-*a* fluorescence kinetics

The effects of salinities (34, 28, 22, or 16 psu) on chlorophyll *a* fluorescence (OJIP) transients in *N. savatieri* are shown in Fig. 4. All the treatments showed the typical OJIP chlorophyll fluorescence transient, and the O, J, I, and P steps were marked.

Changes in the JIP test parameters in *N. savatieri*

In order to unravel the detailed influence of low salinity on PSII behaviour, the main physiological parameters calculated by the JIP test were used to reflect the light absorption and, energy transformation in the thalli (Figs. 5 and 6).

Figure 5 demonstrates that (i) the maximum quantum yield for primary photochemistry (F_v/F_m) was almost constant at all salinities; (ii) performance index (PI_{ABS}) of 22 and 16 psu exposed thalli were markedly higher those exposed to 34 psu (control), and the 28 psu exposure also gave a slightly increased level; (iii) absorption flux per cross section of fronds (ABS/CS_m) was slightly decreased; and (iv) the energy absorption per active PSII reaction centre (ABS/RC) increased sharply at 22 and 16 psu.

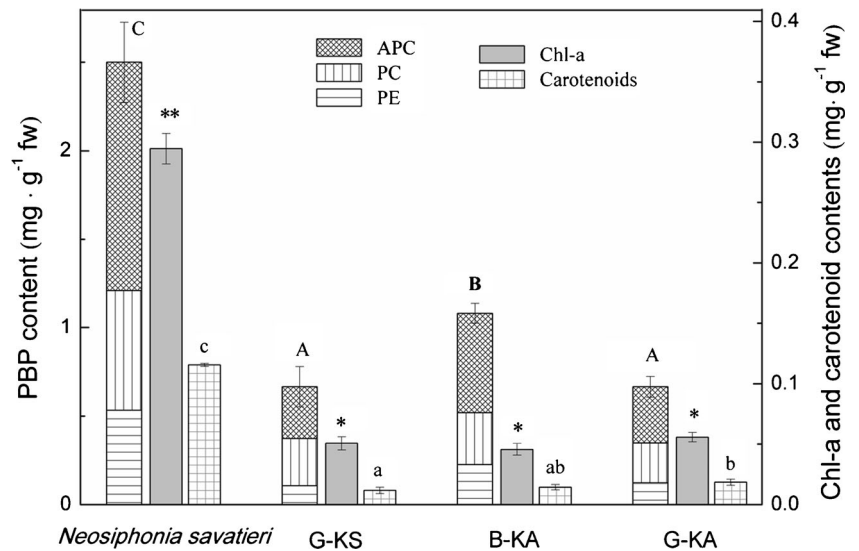
Figure 6 shows the effects of different salinities on the donor side, i.e. the oxygen-evolving complex (OEC) and acceptor side of PSII consisting of Q_A , Q_B , and plastoquinone (PQ). Both parameters remained unchanged for treatments at 34–22 psu. Treatment at 16 psu salinity resulted in an obvious increase of V_K and V_J .

Discussion

Epiphytes are small, often filamentous species that live attached to larger host species. The outbreak of epiphytes on seaweed farms is a complex problem, and the extent of the outbreak often depends on the state of health of the cultivated strain, as well as abiotic parameters, including weather fluctuations. It has been proven that the primary reasons for these outbreaks are extreme changes in seawater temperature and salinity (Vairappan et al. 2013; Pang et al. 2015).

Previous studies have concluded that an *N. savatieri* covering on the surface of a seaweed results in severe competition for light absorption (Pang et al. 2011, 2015). Vairappan (2006) recorded that the maximum density of *N. savatieri* on *K. alvarezii* fronds was about 50 epiphytes cm⁻² during an outbreak in Malaysia. The main axis diameter of *N. savatieri* is ~60 μm. It can be calculated that the area of carrageenophytes constantly occupied by *N. savatieri* only accounts for 0.14% of the total host surface. Given this, competition between *N. savatieri* and its hosts for incident sunlight would seem to be nonlethal for carrageenophytes. However,

Fig. 2 The pigment contents in *Neosiphonia savatieri* and carrageenophytes. Phycobiliproteins (PBP) include phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE). G-KS, green thalli of *Kappaphycus striatum*; B-KA, brown thalli of *Kappaphycus alvarezii*; G-KA green thalli of *K. alvarezii*. Data with different letters (i.e. a, b and A, B) and different symbol amounts (i.e. * and **) are significantly different ($P < 0.05$)



N. savatieri is a plant with vertical axes and a height of 4–20 mm. The shaded surface of eucaumatoids can be up to 12–60% during peak seasons, when the epiphytes move with the flow of the water, especially for rapid water motion. This is not a trivial feature because it would lead to a sharp decrease in the light energy reaching the host surface. Moreover, it can also be assumed that any light insufficiency from poor light conditions would be further aggravated by the filaments of *N. savatieri* covering the surface of the seaweed, which at times can be lethal to the hosts. The current study found that the pigment contents of *N. savatieri* was considerably higher than the commercially exploited agarophytes, e.g. *Gracilaria*, *Gelidium* and *Gelidiella* (Baghel et al. 2014) and carrageenophytes including *Kappaphycus* (Fig. 2) and *Eucauma* (Ralph and Clinton 1976). Findings further revealed that the mean extinction coefficients of both lipid- and water-soluble pigments of *N. savatieri* under PAR bands were higher than G-KS, B-KA and G-KA (Table 1). These results suggest that *N. savatieri* might have a higher light-harvesting capacity than its host and that this characteristic contributed to its colonization and growth under poor light conditions. Light harvesting might be another competitive pathway between *N. savatieri* and *Kappaphycus*. Similar survival strategies have also been reported for other red algae,

e.g. *Gracilaria lemaneiformis* (Xu and Gao 2009) and *Gracilaria bursa-pastoris* (Marinho-Soriano 2012), and

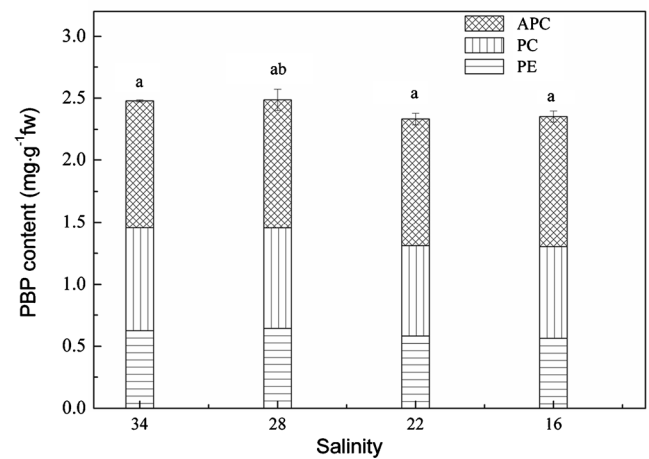
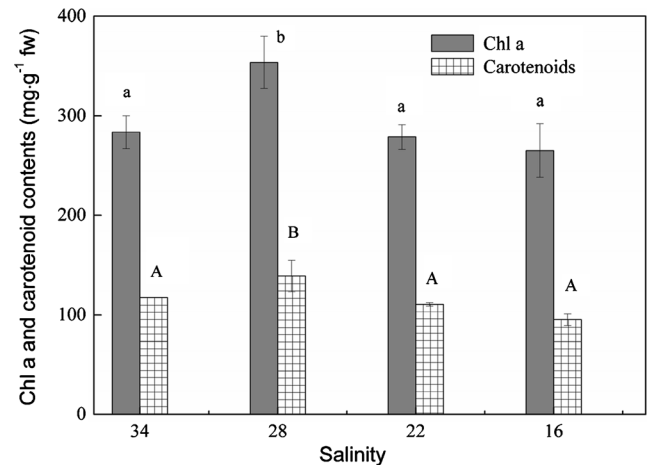


Table 1 The mean extinction coefficient ($\bar{\alpha}$) of different extracts for four seaweeds

Seaweeds	Ethanol extracts	10 mM CaCl ₂ extracts
	$\bar{\alpha}$ (A/mg Chl-a)	$\bar{\alpha}$ (A/mg PBP)
<i>Neosiphonia savatieri</i>	2.65	0.80
G-KS	2.22	0.69
B-KA	2.12	0.66
G-KA	2.16	0.70

Fig. 3 The variation of Chl-a, carotenoids, and phycobiliproteins in *Neosiphonia savatieri* exposed to low salinities. Values are averages of four replicates \pm SEM. Data with different letters (i.e. a, b and A, B) are significantly different ($P < 0.05$)

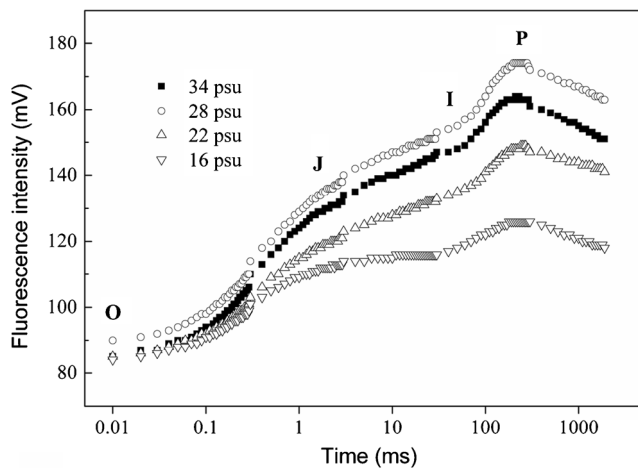


Fig. 4 OJIP Chl-*a* fluorescence induction transients of *Neosiphonia savatieri* treated with low salinities

seagrass *Thalassia testudinum* (Enríquez 2003). In this context, these advantages, i.e. ecological dominance on the surface of hosts and a higher extinction coefficient, could help the epiphytes to capture more light energy for survival and growth under persistent weak light conditions during unfavourable weather, e.g. showers and overcast skies.

Interestingly, results also showed that the $\bar{\alpha}$ of *N. savatieri* ethanol extracts and water soluble pigments were both higher than those of G-KS, B-KA and G-KA (Table 1); however, the proportions of the main pigments were similar (Fig. 2). What caused the difference in extinction coefficients? It is highly likely that some bromophenols (Fan et al. 2003; Xu et al. 2003) and flavonoids (Shi et al. 2011), present in other rhodomelaceans, but absent in the eucheumatoids, play an important role in light absorption and, in the end, make the extinction coefficients different. This phenomenon is an interesting subject that needs to be investigated further.

Many studies have reported that some red algae under hypo-saline conditions (15 psu and below) became white due to a severe decrease of pigments and died after a few days (Macler 1988; Dawes et al. 1999). Hayashi et al. (2011) recorded the disorganization of the cell ultrastructure and the disruption of turgid chloroplasts in the cortical and subcortical cells of *K. alvarezii* cultured at 15 psu. Pigment loss, ‘ice–ice’ whitening and fragmentation of *K. alvarezii* and *E. denticulatum* exposed to a salinity of 20 psu or lower have been reported in many publications (Largo et al. 1995; Hayashi et al. 2011; Yong et al. 2014). The photosynthetic apparatus of *K. alvarezii* incubated at 22 psu was irreversibly damaged, and salinities of 18 psu or below were lethal (Huang et al. 2010). In contrast, outbreaks of *Neosiphonia* were more

Fig. 5 The changes of F_v/F_m , PI_{ABS} , ABS/CS_m , and ABS/RC in *Neosiphonia savatieri* exposed to low salinities. Values are averages of eight replicates \pm SEM. Data with different letters (i.e. a, b, c) are significantly different ($P < 0.05$)

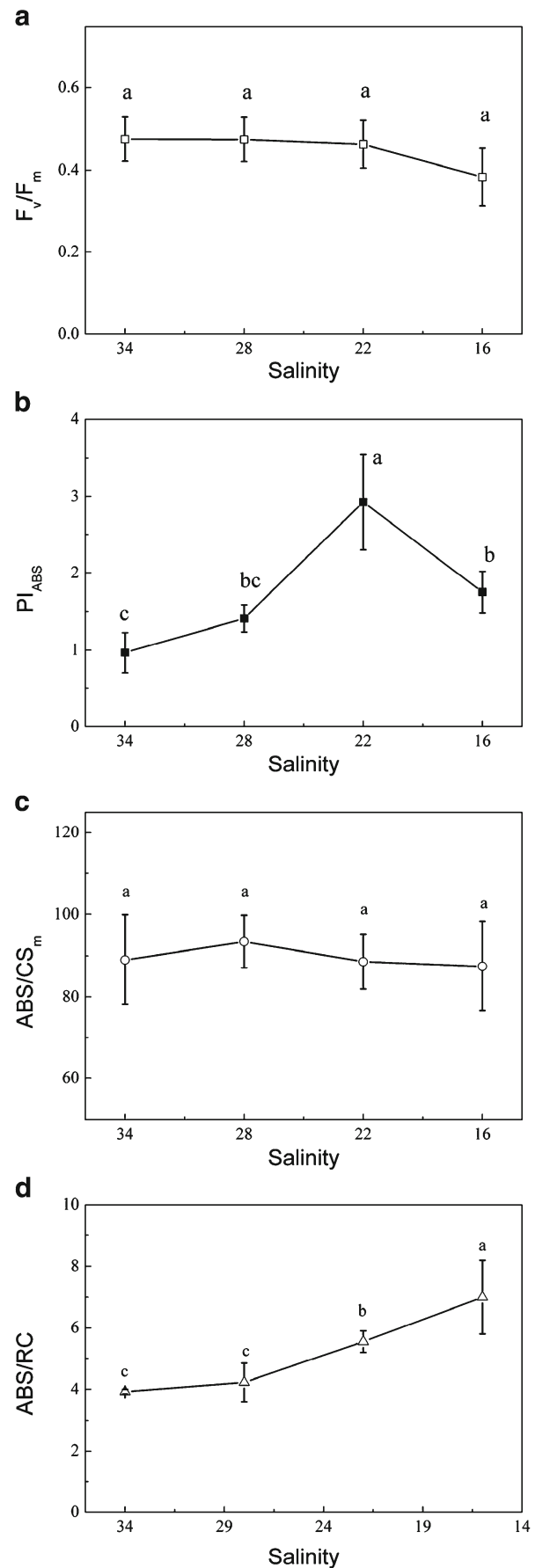
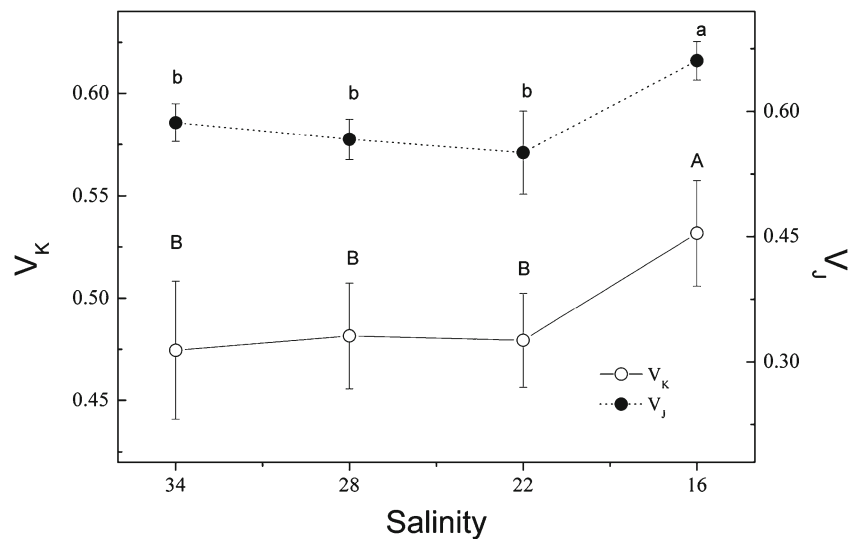


Fig. 6 Donor side parameter (V_K) and acceptor parameter (V_J) of PSII in living tissues of *Neosiphonia savatieri* treated with seawater of low salinities. Values are averages of eight replicates \pm SEM. Data with different letters (i.e. a, b and A, B) are significantly different ($P < 0.05$)



likely to occur at low salinities (Vairappan 2006; Borlongan et al. 2011). In Malaysia, epiphyte outbreaks occurred during the rainy months of September to November, when salinity decreased from 29 to 27 psu (Vairappan 2006). Our results indicate that the photosynthetic performances of *N. savatieri* displayed a high tolerance to hypo-saline conditions. At a salinity of 28 psu, the pigment content of this epiphyte increased. No obvious decrease in pigment content (Fig. 3) and F_v/F_m (Fig. 5a) was observed at salinities down to 16 psu. The increase of PI_{ABS} further indicated that 16–28 psu benefitted *N. savatieri* (Fig. 5b). ABS/CS_m changed little at all salinities (Fig. 5c); however, the energy absorption per active PSII reaction centre was increased. It should be noted that the V_K and V_J of thalli at 16 psu were both higher than those at 22–34 psu (Fig. 6). An increase of V_K is an indicator of damage to the OEC in other plants (Strasser

1997). An obvious increase in V_K was observed at 16 psu, which indicated that the OEC of *N. savatieri* was partly damaged. In addition, a higher value of V_J , which represents the excessive accumulation of Q_A^- , indicated that the linear electron transport chain of *N. savatieri* was inhibited at 16 psu salinity, although the effects were not lethal. In tropical areas, fresh water from rain or stream flows may drastically lower seawater salinity. Notably, the outbreak of epiphytes always correlated with extreme changes in physical abiotic factors (Mtolera et al. 1996; Hurtado et al. 2006; Vairappan 2006; Pang et al. 2015). Results of the current study indicated that low salinities of 28–16 psu benefit a typical epiphyte, *N. savatieri*. Nevertheless, eucheumatoids are not healthy at these salinities, and thus, epiphyte infection is more likely to happen.

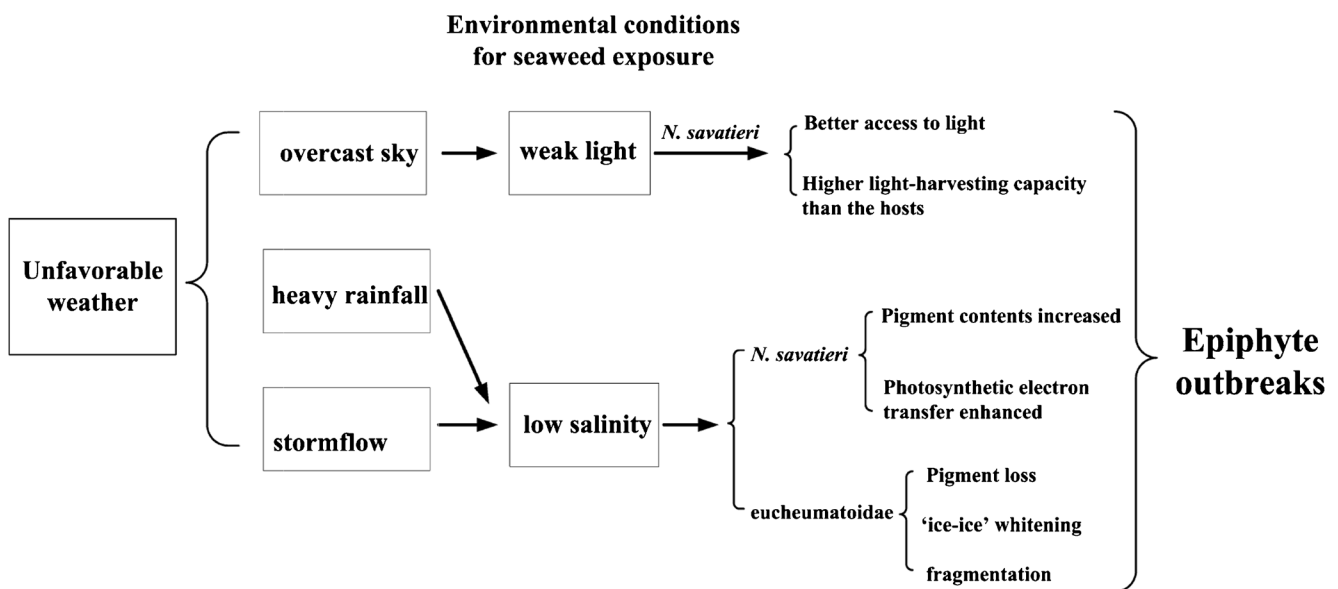


Fig. 7 The influence model of environmental conditions in unfavourable weather on the characteristics of *Neosiphonia savatieri* and its hosts

To date, considerable work has been documented to investigate the tolerance strategies of halophytes living at high salinities, e.g. ion metabolism and organic osmotic solutes (Parihar et al. 2015). However, few reports have focused on the tolerance mechanism of a thalassophyte under low salinities. For higher plants, Zhao et al. (2003) reported that the euhalophyte *Suaeda salsa* grew best under 100 mM NaCl treatment rather than without NaCl. *Suaeda salsa* treated with NaCl retained Na⁺ and Cl⁻ in the vacuoles to lower the plant and maintain life. However, *S. salsa* under PEG treatment alone could not accumulate sufficient ions for osmotic adjustment, which led to growth decrease and death in the end. The activities of tonoplast H⁺-ATPase and H⁺-PPase were both lowered under water stress. For seaweeds, some red algae under hypo-saline conditions (15 psu and below) became white and died after a few days, accompanied by a significant decrease of pigments (Macler 1988; Dawes et al. 1999). *Gracilaria corticata* (J. Agardh) J. Agardh exposed to 15 psu showed lowered thallus rigidity after 9 days of exposure (Kumar et al. 2010). For *Gelidium amansii*, salinities at 15 psu and below induced a greater inhibition on the PSII reaction centres and acceptor side than those at 35 psu (Li et al. 2016). However, integrative studies addressing morphological characteristics and physiological responses to low salinity stress of seaweeds are lacking. Our results indicated that *N. savatieri* has higher tolerance to hypo-salinity than its hosts. Further investigation of cell ultrastructure, osmotic adjustment ability and antioxidative system may help to explain the ecological dominance of *N. savatieri* under low salinity. In addition, climate change has important effects on regional water resources (Menzel and Burger 2002; Muzik 2002). In the 1990s, it was becoming clear that climate change would have a major influence in the tropics and cause deleterious changes to agricultures in those areas (Crabbe 2009). This investigation list a potential risk of the regular outbreaks and economical loss caused by epiphytes in extreme weather such as heavy rainfall.

Based on the above results, we have drawn a model to summarize the competition strategies of *N. savatieri* (Fig. 7). It is important to mention that the temperature fluctuations and high nutrient levels of seawater might also act as triggers for a serious outbreak of the epiphyte. Therefore, further studies are needed to gain insights into the epiphyte's physiological processes under the single or joint influences of salinity, temperature and nutrient uptake.

In conclusion, the light absorption capacities of *N. savatieri* water- and lipid-soluble pigments at wavelengths between 400 and 700 nm were higher than those of green thalli of *K. striatum* and both brown and green thalli of *K. alvarezii*, which contributed to its energy capture under weak light conditions. At salinities in the range of 16–28 psu, the pigment contents and photosynthetic electron transport in *N. savatieri* were increased. Higher light absorption capacities under low

light and higher tolerance to hypo-saline conditions are both conducive to the survival of *N. savatieri*, and in the end, make this alga a 'winner' under conditions having strong abiotic factor fluctuations.

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Contributions Yongfu Li and Jianguo Liu designed the study and wrote the manuscript; Tong Pang collected the materials for the experiment; and Yongfu Li and Litao Zhang performed the experiments and analyzed the data.

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