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Photosynthesis and growth of *Ulva ohnoi* and *Ulva pertusa* (Ulvophyceae) under high light and high temperature conditions, and implications for green tide in Japan

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SUMMARY

The macroalga *Ulva ohnoi* constitutes a considerable fraction of green tides in coastal areas of Japan, but little is known about the physiological characteristics of this species. To investigate the environmental factors that promote the formation of green tides, we tested the responses of *U. ohnoi* and another common Japanese species, *Ulva pertusa*, to various levels of irradiance at different water temperatures. Because the two species are morphologically similar, we identified them using the PCR-restriction fragment length polymorphism method. Under laboratory conditions, we evaluated the photosynthetic, dark respiration, and relative growth rate at a range of water temperatures (5 to 35°C) and photosynthetically active radiation (0 to 1000 μmol photons m^{-2} s^{-1}). The maximum gross photosynthetic rate of *U. ohnoi* was larger than that of *U. pertusa*. The dark respiration rates revealed no significant differences among the species and temperature conditions. At 500 μmol photons m^{-2} s^{-1}, the relative growth rate of *U. ohnoi* was larger than that of *U. pertusa* in higher temperature and the difference was the largest at 20°C. The estimated compensation irradiance and estimated saturation irradiance of *U. ohnoi* and *U. pertusa* ranged from 0.709 to 5.510 and 40.530 to 58.674 μmol photons m^{-2} s^{-1}, which were lower than those in other intertidal green macroalgae, from 6 to 11 and 50 to 82 μmol photons m^{-2} s^{-1}, respectively. Thus, *U. ohnoi* which exists as free-floating near the water surface and accumulating inside the green tide can survive extensively in the water column of the intertidal zone, furthermore, the species can maintain rapid growth in this situation. Therefore, as a result of this study, it is suggested that the ecological success of *U. ohnoi* in shallow waters such as the tidal flats, estuarine, and coasts of the inner bay in comparison with *U. pertusa*.

Key words: accumulation of Ulvophyceae, algal bloom, light and dark bottle method, marine macroalgae, photosynthesis-irradiance curve, relative growth rate, Yatsu tidal flat.

INTRODUCTION

In recent years, blooms of green macroalgae, known as 'green tide,' have occurred in eutrophic inner bays around the world (Fletcher 1996). Although several definitions of green tide have been proposed (Fletcher 1996; Ohno 1999; Taylor *et al*. 2001; Nelson *et al*. 2003), in general, it refers to large blooms and accumulation of green macroalgae of the Ulvophyceae (e.g., *Ulva pertusa* Kjellman; Yamochi 2013, *Ulva prolifera* Müller and *Ulva intestinalis* Linnaeus; Wang *et al*. 2012) in shallow waters (e.g., tidal flats, estuaries, and coasts of inner bays). In Japan, green tide has been reported in various locations, such as Tokyo Bay, Osaka Bay, Hiroshima Bay, and Hakata Bay (Hiraoka *et al*. 2004; Uchimura *et al*. 2004; Yabe *et al*. 2009; Yamochi 2013). Green tide adversely affects other primary producers in shallow waters because green macroalgae completely cover these species, inhibiting photosynthesis (Ohno 1999). The anoxic conditions resulting from decomposition of the green macroalgae cause a release of gaseous sulfur compounds such as hydrogen sulfide, carbon disulfide, and exposure to these toxic gases poses health risks to both humans and wildlife (Charlier *et al*. 2007; Peu *et al*. 2011). In addition, the accumulation and degradation of a large amount of *Ulva* blooms have serious impacts on local biodiversity and biogeochemistry (Frankenstein 2000).

Ulva pertusa is a common inhabitant of intertidal areas in Japan and is reported to form green tide (Okamura 1921). Arasaki (1984) reported that an invasive species of *Ulva*, other than *U. pertusa*, is involved in green tide formation. Ohno (1988) investigated which *Ulva* species form green tide in Uranouchi Bay (Kochi Prefecture) and concluded that green tide is mainly composed of a newly invasive *Ulva* species from subtropical marine environments. Hiraoka *et al*. (2004) showed that the species found in Uranouchi Bay is identical to *Ulva ohnoi* Hiraoka et Shimada, based on both morphological characteristics and DNA sequence data. They reported that *U. ohnoi* is also present in coastal areas in Fukuoka and Okinawa. *Ulva ohnoi* is considered to be the major green tide-forming species in the tidal flats of Tokyo Bay (Yabe & Ishii 2015) and Osaka Bay (Yoshimura & Yamochi 2011).

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To clarify the causes of green tide, many studies have investigated the physiological characteristics of green macroalgae under experimental conditions. Some studies have shown that nitrogen application increases both photosynthetic rate and growth of *Ulva* (Sfriso *et al*. 1988; Fong *et al*. 1993; Ménesguen & Piriou 1995; Pedersen 1995), suggesting that nitrogen availability is a major limiting factor in the generation of green tide. In the past 20 years, the nitrogen concentrations of inner bays adjacent to urban areas have decreased in Japan (Kazama & Ando 2010; Tada *et al*. 2010). However, the generation of green tide is still observed in these areas. Thus, we focused on the photosynthesis and growth responses of *Ulva* species to understand recent green tide formation in Japan.

As in the case of *U. pertusa*, the previous study showed the light intensity is one of the most important factors of growth in the species (Floreto *et al*. 1993). However, these characteristics of *U. ohnoi* have not been investigated (Yabe *et al*. 2009), and this lack of basic knowledge hinders our understanding of how *U. ohnoi* has formed green tide in recent years. In green tides, accumulated *Ulva* spp. in the surface layer are exposed to intense light and high temperature, whereas those in the bottom layer are in dark conditions. In this study, we investigated the photosynthesis and growth responses of two common *Ulva* species in Japan, *U. ohnoi* and *U. pertusa*, under experimental conditions, and discussed how *U. ohnoi* becomes a dominant green tide species in these tidal flats.

MATERIALS AND METHODS

Sampling site and stock cultures

Samples were collected from the Yatsu tidal flat (35°40′34.1″N, 140°00′31.8″E) in inner Tokyo Bay. The green tide at this tidal flat was first reported in 1995 and was caused mainly by free-floating *Ulva* (Yabe *et al*. 2009). Samples were stored in a plastic bag and transported directly to the laboratory, where they were immediately briefly rinsed with fresh water to remove any surface contaminants and detritus. Collected *Ulva* was initially cultivated in a 100-L culture tank (SBF-100 TO, Tanaka Sanjiro, Fukuoka, Japan) containing artificial seawater (Marine Art SF-1, Tomita Pharmaceutical, Tokushima, Japan; 36.4 g L^{-1}) and nutrients (KW 21, Daiichi Kogyo, Kumamoto, Japan; 0.5 mL L^{-1}) in a naturally lit glass room controlled at 25°C for 2 weeks according to Kishida and Baba (2003).

Because *Ulva* species forming green tides are morphologically similar, genetic analysis is needed to identify them; yet, the previous studies on the physiological properties of green tide species (Murase *et al*. 1993; Kishida & Baba 2003) have been performed without genetic analysis. In this study, PCR-restriction fragment length polymorphism method (Yabe & Ishii 2015) was used for species identification. After the identification, *U. ohnoi* and *U. pertusa* were cultured separately as described above.

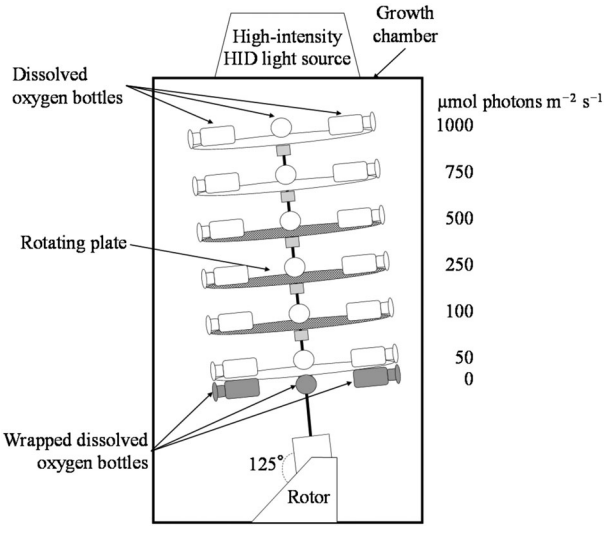


Fig. 1. Multi-step receiver. Light intensities were adjusted by changing the sample distance from the light source placed on top of the growth chamber and utilizing the shading cloth on some rotating plates. The rotor was used RT-50 (TAITEC, Saitama, Japan). The rotating shaft was attached with low-angled rather than vertical against HID light source.

Photosynthesis and respiration rates

Measurements of photosynthesis and respiration were carried out by measuring dissolved oxygen (DO) concentration using a set of three punched round pieces (25 mm in diameter) from each thallus except the parts of the outer edge, scratches, and wrinkles. Fresh weight (gFW) of round pieces of *Ulva* was measured, and three of those were placed in a 100-mL DO bottle filled with artificial seawater. Dark bottles were covered with aluminum foil. Samples were incubated for 2 h at different temperatures 10, 20 and 30°C. A multi-step light receiver (Fig. 1) was placed in a growth chamber (FLI-2000A, Tokyo Rikakikai, Tokyo, Japan) equipped with a high-intensity HID light unit (HLU-50, Tokyo Rikakikai). As shown in Figure 1, bottles were placed on plates at different distances from HID light. Photosynthetically active radiation (PAR) was measured with a quantum sensor (MQ-200, Apogee Instruments, UT, USA), and adjusted PAR 0, 50, 100, 250, 500, 750, and 1000 μmol photons m^{-2} s^{-1}. In this experiment, three DO bottles were attached to a plate that rotated at 5 rpm and was irradiated.

Dissolved oxygen concentrations were measured before and after incubation by the light and dark bottle method (Ikusima 1965), as follows. *Ulva* samples were acclimated in the experimental temperatures overnight in the dark to avoid cut influence (Serisawa *et al*. 2001; Watanabe *et al*. 2014). Dissolved oxygen concentration was measured using a fluorescent oxygen sensor and a DO meter (MicroxTX3, PreSens, Bayern, Germany) at the beginning and end of incubation. The net photosynthetic rate (P_n) and dark respiration rate (*Rd*) were normalized in two ways, to dry weight (gDW) and to chlorophyll *a* (Chl *a*) concentration. To obtain the wet/dry weight ratio, dry weight was determined after drying at 80°C for at least 48 h. After drying, samples were kept in a desiccator until it became a constant weight.

To measure Chl a content, other samples from the same thallus used in photosynthetic measurement were cut into circles with an 11 mm diameter, homogenized with a mortar and pestle, and extracted in 10 mL of absolute methanol under refrigeration. After 24 h, the absorbance of the methanol extract was measured using a spectrometer (UV-1800,

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Shimadzu, Kyoto, Japan). Chlorophyll *a* content was calculated using the following equation (Porra *et al.* 1989):

$$\mathrm{Chl\,a}\left(\mathrm{mg\,mL^{-1}}\right) = \frac{16.29(\mathrm{Abs}_{665.2}-\mathrm{Abs}_{750.0})-8.54(\mathrm{Abs}_{652.0}-\mathrm{Abs}_{750.0})}{1000} \quad (1)$$

where $\mathrm{Abs}_{652.0}$, $\mathrm{Abs}_{665.2}$, and $\mathrm{Abs}_{750.0}$ are absorbance values at 652.0, 665.2, and 750.0 nm, respectively.

The P_n was obtained by the following equation (Yamochi 2013):

$$P_n = \frac{C_t - C_0}{t} \times \frac{V}{W} \quad (2)$$

where P_n is the net photosynthetic rate of the sample. C_t is DO after incubation (mg O_2 L^{-1}), C_0 is DO before incubation (mg O_2 L^{-1}), t is the duration of incubation (hours), V is bottle volume (L), and W is the amount of Chl *a* in the explant used for culture (mg Chl *a*) or dry weight (gDW).

To obtain and compare parameters for photosynthesis-irradiance (P-I) curve among different conditions (e.g. two or more species; different temperature conditions), many of earlier studies take a two-step analysis as follow; P-I curve is obtained using the intact data of photosynthetic rate and subsequently perform the analysis of variance on P-I parameters derived from the P-I curve (e.g. Murakami *et al.* 2004; Lideman *et al.*, 2011; Zou & Gao 2014). The two-step analysis requires an assumption that the photosynthetic data are regarded as a paired-samples across light intensity (e.g. a series of samples are taken from the same individual, or the same samples are repeatedly used across light intensity). However, some recent studies take more general solution that can be applied to random independent samples, e.g. fitting the data to a non-linear P-I model and estimating P-I parameters and their differences at a time (e.g. Fujimoto *et al.* 2014; Borlongan *et al.* 2017). The data in our study were not paired and therefore we applied the latter approach by using the following P-I curve (Webb *et al.* 1974; Jassby & Platt 1976; Platt *et al.* 1980; Henley 1993) as the basal equation to fit P_n:

$$P_n = P_{\max}\left(1-\exp\left(-\frac{\alpha}{P_{\max}}Ir\right)\right) - Rd \quad (3)$$

where P_{max} is the maximum gross photosynthetic rate, α is the initial slope of the photosynthesis, Ir is light intensity, and Rd is the dark respiration rate. To analyze photosynthesis rate with the differences between species, among temperature levels and their interactions (i.e., two-way non-linear model of the analysis of covariance), we subdivided each of the parameters in Equation 3 as:

$$P_{\max} = P_{\max}' + a_1 \cdot Sp + a_2 \cdot T_1 + a_3 \cdot T_2 + a_4 \cdot Sp \cdot T_1 + a_5 \cdot Sp \cdot T_2,$$
$$\alpha = \alpha' + b_1 \cdot Sp + b_2 \cdot T_1 + b_3 \cdot T_2 + b_4 \cdot Sp \cdot T_1 + b_5 \cdot Sp \cdot T_2,$$
$$Rd = Rd' + c_1 \cdot Sp + c_2 \cdot T_1 + c_3 \cdot T_2 + c_4 \cdot Sp \cdot T_1 + c_5 \cdot Sp \cdot T_2,$$

Here, we coded the species and temperature as zero and one as follows: *Sp*: *U. pertusa* = 0 and *U. ohnoi* = 1; T_1: 20°C = 1, and 0 for others; T_2: 30°C = 1, and 0 for others. There are 18 parameters to be estimated: P_{max}', α', and Rd' as baseline parameters (*U. pertusa* at 10°C), and their sub-parameters a_k, b_k, and c_k (k = 1–5), which subdivide P_{max}', α', and Rd', respectively. k = 1, 2, and 3 represent difference in each of the parameters between *U. pertusa* and *U. ohnoi*, at 20°C and at 30°C, respectively. k = 4 and k = 5 indicate the interactions for *U. ohnoi* at 20°C and at 30°C conditions, respectively.

Since Rd is the dark respiration rate and only additive in Equation 3, Rd' and its sub-parameters (c_k) should be estimated solely based on the data at 0 μmol photons m^{-2} s^{-1}, separately from the other parameters (particularly α), to avoid correlated estimations. Therefore, we estimated the P-I parameters by using the following two-parts of models. The two-way model of the analysis of variance was used for Rd' and its sub-parameters (c_k), based on the data at 0 μmol photons m^{-2} s^{-1} with the differences of species and temperature including their interactions. Adding the separately estimated Rd, we fitted the data to Equation 3. Equation 3 was fitted for both of the P_n per dry weight and Chl a content of *Ulva* species. The saturation irradiance (Ik) and compensation irradiance (Ic) were also calculated as P_{max}/α and P_{max} log (P_{max} / (P_{max} – Rd))/α, respectively (Schubert *et al.* 2006).

Relative growth rate

Measurements of growth were performed using these round pieces taken from each thallus in the same way as the experiments for measurement of photosynthesis and respiration described above. Samples from stock cultures were preincubated under each experimental condition overnight. Samples were placed into a culture bottle (CB-2, AS ONE Co., Osaka, Japan) containing 300 mL of artificial seawater. Culture bottles were randomly placed in the incubation chambers and cultured in triplicate for 7 days at low and high PAR (60 and 500 μmol photons m^{-2} s^{-1}, respectively; a photoperiod of 12 : 12 h LD) and several water temperatures (60 μmol photons m^{-2} s^{-1}: 5 to 35°C at an interval 5°C, 500 μmol photons m^{-2} s^{-1}: 10 to 30°C at an interval 10°C). For low PAR experiments, a temperature gradient growth chamber (TG-280CCFL-5LD, NK system, Osaka, Japan) with LED light source was used and the other growth chamber (FLI-2000A) was used for the high PAR experiments. The samples were manually stirred three times daily during experiments.

The fresh weight of samples was measured at the beginning and end of the culture period, and growth was calculated as the relative growth rate (RGR) (Takada *et al.* 2011):

$$\mathrm{RGR} = \frac{\mathrm{Ln}(W_2) - \mathrm{Ln}(W_1)}{7} \times 100 \quad (4)$$

where W_2 is the final fresh weight (gFW), W_1 is the initial fresh weight (gFW) and 7 is the number of days of culture. The RGRs were analyzed using a quadratic model of the analysis of covariance (ANCOVA) with species as the factor and temperature as the covariance each for the two light levels (60 and 500 μmol photons m^{-2} s^{-1}).

Statistical assumptions and computations

We performed a nonlinear least-squares estimation via a Gauss-Newton algorithm using 'nls' function of R statistics to fit Equation 3, which were successfully converged. The R code to fit Equation 3 is shown in Appendix S1. The datasets of P-I and RGR are also shown in Appendix S2 and S3, respectively. Normality assumptions were checked graphically

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with quantile-quantile plots of residuals against fitted values. The points distributed around a diagonal line, indicating normality. All of the statistical analyses were carried out by using R version 3.6.1 (R Development Core Team 2019).

RESULTS

Effect of temperature on P-I curves

The P-I curves and parameters of P-I curves in *U. ohnoi* and *U. pertusa* grown at different temperatures are presented as per gDW (Fig. 2 and Tables 1 and 2). The P_{max} changed with temperature and was the highest at 20°C (Tables 1 and 2). The P_{max} of *U. ohnoi* were larger than that of *U. pertusa* under all temperature conditions, and the species difference in P_{max} was the largest at 20°C and still large at 30°C but marginal at 10°C (Table 1). The slope α was significantly larger than zero and at the largest at 20°C, but there were no other significant differences among the species and temperature conditions (Table 1). For the *Rd*, there were also no significant differences among the species and temperature conditions (Table 1). The *Ic* of *U. ohnoi* and *U. pertusa* ranged from 1.291 to 3.040 and 3.476 to 4.345 μmol photons m^{-2} s^{-1}, respectively (Table 2). Moreover, the estimated *Ik* of *U. ohnoi* and *U. pertusa* were 48.205 to 60.697 and 37.077 to 49.318 μmol photons m^{-2} s^{-1}, respectively (Table 2). These results show that the P_{max} reached its saturation around 50 μmol photons m^{-2} s^{-1} in both species regardless of growth temperature.

The results of P-I curves and estimated P-I curve parameters normalized to Chl *a* are also shown in Appendix S1 (Fig. S1, Tables S1 and S2).

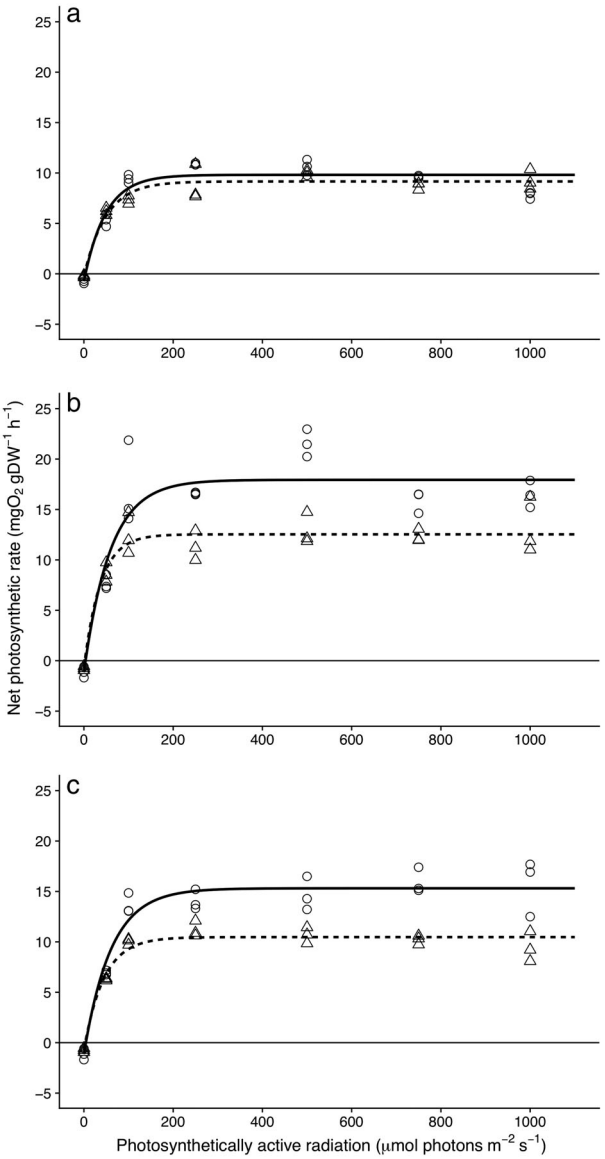


Fig. 2. Photosynthesis-irradiance (P-I) relationships for *Ulva ohnoi* (circles) and *Ulva pertusa* (triangles) at different water temperatures: (a) 10°C; (b) 20°C; (c) 30°C. Solid and dashed lines indicate the fitted P-I curve for *Ulva ohnoi* and for *Ulva pertusa*, respectively.

Effect of temperature on relative growth rate

We compared the RGR of *U. ohnoi* and *U. pertusa* at different growth temperatures and a low and high PAR (Fig. 3a,b). Under the low light experiment (60 μmol photons m^{-2} s^{-1}), the RGR showed a convex pattern with its maximum around 20°C, however the species difference in RGR pattern was negligible (Table 3). No increase in weight was observed in either species at 5 and 35°C (Fig. 3a). Under the high PAR experiment (500 μmol photons m^{-2} s^{-1}), the RGR also showed a convex pattern with its maximum around 20°C, though the RGR of *U. ohnoi* was larger than that of *U. pertusa* in higher temperature and the difference was the largest at 20°C (Fig. 3b and Tables 3 and 4).

DISCUSSION

Photosynthetic features of *U. ohnoi* and *U. pertusa* based on the functional form

Littler and Arnold (1982) studied the primary productivity of 62 species of macroalgae and classified them into six functional form groups. All *Ulva* species were classified into the thin, tubular and sheet-like group, which showed the highest productivity among the six groups. In this study, P_{max} values of *U. ohnoi* (19.09 O_2 mg gDW^{-1} h^{-1}) and *U. pertusa* (16.92 mg O_2 mg gDW^{-1} h^{-1}) were higher than those of intertidal macroalgae species such as *Chaetomorpha linum* (8.14 mg O_2 gDW^{-1} h^{-1}), *Gracilaria verrucosa* Hudson (coarsely-branched group; 13.58 mg O_2 gDW^{-1} h^{-1}), and *Ulva* sp. (16.54 mg O_2 gDW^{-1} h^{-1}) (Menéndez *et al*. 2001). In addition, the *Ic* and *Ik* of *U. ohnoi* and *U. pertusa* ranged from 1.291 to 4.345 and 37.077 to 60.697 μmol photons m^{-2} s^{-1}, respectively. *Ulva* species is well known to adapt to low irradiance of 0.6 to 60 μmol photons m^{-2} s^{-1} (Fortes & Lüning 1980; Vermaat & Sand-Jensen 1987; Sand-Jensen & Borum 1988; Pérez-Lloréns *et al*. 1996; Taylor *et al*. 2001). Furthermore, Merceron *et al*. (2007) concluded that *Ulva*

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Table 1. Parameter estimates of the photosynthesis-irradiance (P-I) curve per gDW for *Ulva ohnoi* and *Ulva pertusa* at different temperatures

Parameter		Estimate	SE	*t*-value	*P*-value
Basal parameters	P_{max}'	9.404	0.460	20.434	<0.001***
	α'	0.191	0.043	4.459	<0.001***
	Rd'	0.243	0.202	1.201	0.253
Sub-parameters of P_{max}'	a1 (Species: *U. ohnoi*)	1.147	0.650	1.764	0.080
	a2 (Temperature: 20°C)	3.872	0.641	6.036	<0.001***
	a3 (Temperature: 30°C)	1.809	0.647	2.794	0.006**
	a4 (Species: *U. ohnoi* × Temperature 20°C)	4.654	0.918	5.067	<0.001***
	a5 (Species: *U. ohnoi* × Temperature 30°C)	4.089	0.923	4.429	<0.001***
Sub-parameters of α'	b1 (Species: *U. ohnoi*)	0.028	0.061	0.459	0.647
	b2 (Temperature: 20°C)	0.167	0.079	2.124	0.036*
	b3 (Temperature: 30°C)	0.061	0.065	0.932	0.353
	b4 (Species: *U. ohnoi* × Temperature 20°C)	−0.063	0.096	−0.658	0.512
	b5 (Species: *U. ohnoi* × Temperature 30°C)	−0.009	0.085	−0.104	0.917
Sub-parameters of *Rd*'	c1 (Species: *U. ohnoi*)	0.497	0.286	1.736	0.108
	c2 (Temperature: 20°C)	0.497	0.286	1.736	0.108
	c3 (Temperature: 30°C)	0.491	0.286	1.716	0.112
	c4 (Species: *U. ohnoi* × Temperature 20°C)	−0.094	0.405	−0.233	0.820
	c5 (Species: *U. ohnoi* × Temperature 30°C)	−0.094	0.405	−0.233	0.820

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.
Ulva pertusa at 10°C was used as the basal group for intercept and slope. P_{max}', α', and *Rd'* as basal parameters (*U. pertusa* at 10°C), and their sub-parameters a_k, b_k, and c_k (k = 1–5), which subdivide P_{max}', α', and *Rd'*, respectively. k = 1, 2, and 3 represent difference in each of the parameters between *U. pertusa* and *U. ohnoi*, at 20°C, and at 30°C, respectively. k = 4 and k = 5 indicate the interactions for *U. ohnoi* on 20°C and on 30°C conditions, respectively. Estimated, estimated P-I curve parameters; SE, standard error.

Table 2. Extended P-I curve parameters per gDW of *Ulva pertusa* and *Ulva ohnoi* at different temperatures

Species	Temperature	P_{max}†	*Rd*†	α†	*Ik*‡	*Ic*‡
U. pertusa	10	9.404	0.191	0.243	49.318	1.291
U. ohnoi		10.551	0.219	0.734	48.205	3.476
U. pertusa	20	13.276	0.358	0.740	37.077	2.126
U. ohnoi		19.076	0.323	1.136	59.086	3.629
U. pertusa	30	11.212	0.252	0.740	44.546	3.040
U. ohnoi		16.448	0.271	1.136	60.697	4.345

†mg O_2 gDW^{-1} h^{-1}.
‡μmol photons m^{-2} s^{-1}.
Ic, compensation irradiance; *Ik*, saturation irradiance; P_{max}, maximum gross photosynthetic rate; *Rd*, dark respiration rate; α, initial slope.

species can survive even low light conditions. Therefore, the low *Ic* values seem to be adapted to the low light condition by accumulating in green tide.

In the case of other intertidal green macroalgae (Chlorophyta), *Ic*, and *Ik* values in *C. linum* Müller (delicately-branched group), *U. intestinalis*, *U. lobata* Kützing, and *U. rigida* C. Agardh have been studied in the literature, and shown that *Ic* and *Ik* in these species are, 6 to 11 and 50 to 82 μmol photons m^{-2} s^{-1}, respectively (Arnold & Murray 1980). In the case of other intertidal red macroalgae (Rhodophyta), *Ic* and *Ik* values in *Pyropia tenera* Kjellman (thin, tubular and sheet-like group) were 9 and 46 μmol photons m^{-2} s^{-1}, respectively (Watanabe *et al.* 2014). Here, we show that *U. ohnoi* and *U. pertusa* have lower *Ic* and *Ik* in comparison to other intertidal macroalgae species, suggesting that *U. ohnoi* and *U. pertusa* can maintain the sufficient primary production for their survival even if they are accumulated on top of each other in green tide.

It is noteworthy to write that *U. ohnoi* and *U. pertusa* showed the low *Ic*, and the P_{max} of *U. ohnoi* and *U. pertusa* were higher than other intertidal algae. These indicate that *U. ohnoi* and *U. pertusa* survive under the lower light condition inside the green tide and maintained the higher primary productivity under high light conditions near the water surface, compared to other intertidal macroalgae.

Interaction among species and growth temperature based on P-I curve parameters and relative growth rate

It is noteworthy that we succeed in showing that the RGR of *U. ohnoi* at temperatures higher than 20°C are significantly higher than those of *U. pertusa*, because the previous study only showed the intertidal macroalgae can maintain their

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Table 3. Analysis of covariance on the effect of species and temperature on the relative growth rate (RGR) against irradiance of *Ulva pertusa* and *Ulva ohnoi*

Parameter	Sum Sq	Df	*F*-value	*P*-value
Irradiance: 60 μmol photons m^{-2} s^{-1}				
Species: *U. ohnoi*	176.038	1	15.771	<0.001***
Temperature	1383.172	1	123.920	<0.001***
Temperature^2	1537.598	1	137.755	<0.001***
Species: *U. ohnoi* × Temperature	0.985	1	0.088	0.768
Species: *U. ohnoi* × Temperature^2	21.497	1	1.926	0.174
Residuals	401.825	36		
Irradiance: 500 μmol photons m^{-2} s^{-1}				
Species: *U. ohnoi*	52.956	1	38.988	<0.001***
Temperature	436.419	1	321.305	<0.001***
Temperature^2	339.585	1	250.013	<0.001***
Species: *U. ohnoi* × Temperature	11.999	1	8.834	0.012*
Species: *U. ohnoi* × Temperature^2	8.438	1	6.212	0.028*
Residuals	16.299	12		

*$P < 0.05$.
***$P < 0.001$.
Ulva pertusa at 10°C was used as the basal group for intercept and slope. Df, degree of freedom; temperature, first-order terms of quadratic function; Sum sq., Type II sum of squares; Temperature^2, second-order terms of quadratic function.

Table 4. Parameter estimates on quadratic models of the analysis of covariance on the relative growth rate (RGR) against irradiance of *Ulva pertusa* and *Ulva ohnoi*

Parameter	Estimate	SE	*t*-value	*P*-value
Irradiance: 60 μmol photons m^{-2} s^{-1}				
(Intercept)	−13.409	3.006	−4.461	<0.001**
Species: *U. ohnoi*	−1.272	4.251	−0.299	0.767
Temperature	2.784	0.345	8.082	<0.001**
Temperature^2	−0.078	0.008	−9.281	<0.001**
Species: *U. ohnoi* × Temperature	−0.145	0.487	−0.297	0.768
Species: *U. ohnoi* × Temperature^2	0.017	0.012	1.388	0.174
Irradiance: 500 μmol photons m^{-2} s^{-1}				
(Intercept)	−28.416	2.933	−9.688	<0.001**
Species: *U. ohnoi*	−11.013	4.148	−2.655	0.021*
Temperature	3.521	0.333	10.573	<0.001**
Temperature^2	−0.078	0.008	−9.418	<0.001**
Species: *U. ohnoi* × Temperature	1.400	0.471	2.972	0.012*
Species: *U. ohnoi* × Temperature^2	−0.029	0.012	−2.492	0.028*

*$P < 0.05$.
**$P < 0.001$.
Ulva pertusa at 10°C was used as the basal group for intercept and slope. Estimated, estimated P-I curve parameters; SE, standard error; temperature, first-order terms of quadratic function; Temperature^2, second-order terms of quadratic function.

photosynthetic and respiratory activity at high water temperature measured in their natural habitat (Maegawa & Sugiyama 1995). Taken together, these results indicate that *U. ohnoi* can adapt successfully to high water temperature than *U. pertusa*. This indication may support a possible distribution of *Ulva* species in Japan studied by Hiraoka *et al*. (2004). They described that *U. ohnoi* mainly forms green tide in warm-temperate coasts of the southwestern region, while *U. pertusa* forms green tide on a little more north-eastern region of Japan.

In this study, we showed that the P_{max} value and RGR of *U. ohnoi* were significantly higher than that of *U. pertusa*. The results indicate that *U. ohnoi* has larger rates of photosynthesis and growth than *U. pertusa*. This suggests that *U. ohnoi* may survive, grow extensively and dominate in the natural habitats with severe environmental conditions, such as shallow tidal flats. Moreover, it is also suggested that *U. pertusa* may only weakly respond to temperature changes as well as *U. ohnoi*, because the RGR curve for *U. pertusa* was flatter than that of *U. ohnoi* in the high PAR experiment. Both channels and waterways distributed on tidal flats seem to be good habitats for *U. pertusa* because there are no severe environmental conditions for the species. The co-occurrence of two *Ulva* species was also observed in the Yellow Sea: the floating-form of *U. prolifera* and the attached-form of *U. intestinalis* (Wang *et al*. 2012).

In the near future, water temperatures and nutrients are expected to increase in the shallow waters of Japan according to global climate change forecasts, which are suitable physiological conditions for the expansion of green tide (Wells *et al*.

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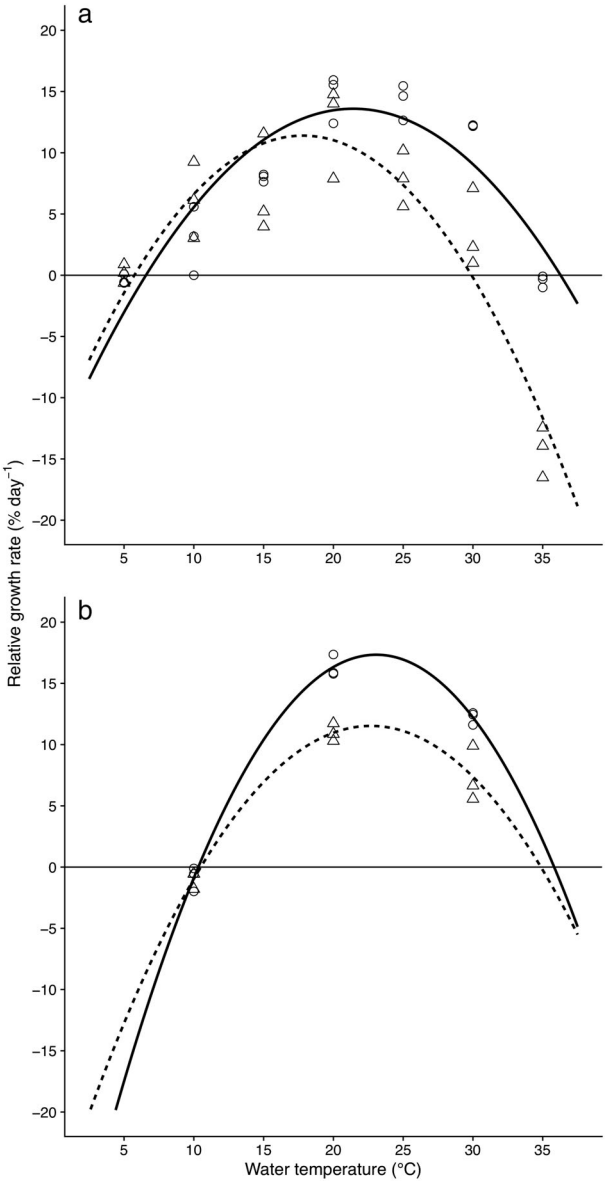


Fig. 3. Relative growth rate (RGR) of *Ulva ohnoi* (circles) and *Ulva pertusa* (triangles) at different water temperatures. (a) RGR at seven water temperatures under 60 μmol photons m^{-2} s^{-1}. (b) RGR at three temperatures under 500 μmol photons m^{-2} s^{-1}. Solid and dashed lines indicate the fitted growth curve for *Ulva ohnoi* and for *Ulva pertusa*, respectively.

2015), and the expansion is expected to be caused by *U. ohnoi*. Nonetheless, there are many other factors that can affect the expansion of green tide including phenology of *Ulva* spp., competition with other primary producers and herbivory in natural habitat. Therefore, further studies including field manipulative experiments and nutrient uptake experiments are also needed to project future expansions in green tide.

In conclusion, we showed that *U. ohnoi* which exists as free-floating near the water surface and accumulating inside the green tide can survive extensively in the water column of the intertidal zone, and the species maintains rapid growth in this situation. Therefore, this study suggests that *U. ohnoi* will be more ecologically successful than *U. pertusa*, in shallow waters such as tidal flats, estuaries, and the coastal areas of the inner parts of bays. Saco *et al.* (2018) observed similar phenomena in two benthic green algae (*Monostroma angicava* Kjellman and *Protomonostroma undulatum* Vinogradova), which coexist in intertidal habitats but express different photosynthetic characteristics. Characteristics of the dominant species, *U. ohnoi*, clarified in this study may help to suppress green tide formation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. The R code to fit Equation 3.

Table S1. Parameter estimates of the P-I curve per Chl a.

Table S2. Extended parameters of the P-I curve per Chl a.

Fig. S1. P-I curves per Chl a.

Appendix S2. The data of photosynthesis rate.

Appendix S3. The data of relative growth rate.

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