The toxigenic red-tide-forming dinoflagellates Alexandrium leei and Alexandrium catenella differ in terms of the sensitivity to strong light and low temperature of their photosynthetic machinery

Original research article

The toxigenic red-tide-forming dinoflagellates *Alexandrium leei* and *Alexandrium catenella* differ in terms of the sensitivity to strong light and low temperature of their photosynthetic machinery

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Footnotes

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A.S. and Y.N. designed the research. T.S and Y.T prepared the tested organisms. A.S., R.T. and Y.N. performed the research. T.S., K.Y. and P.N. analyzed the data. Y.N. wrote the paper, which all authors approved.

Funding:
This work was supported, in part, by grants from the Japan Society for the Promotion of Science, KAKENHI (grant numbers 21H05305 to Y.N., T.S. and K.Y.; and 22K06259 to Y.N.); by a grant from the Japan Science and Technology Agency (grant number JPMJPF2010 to Y.N.); and by a grant from the Cooperative Research Program of the "Network Joint Research Center for Materials and Devices" (to Y.N.).

Conflict of interest:
The authors know of no conflict of interest associated with this publication.

Keywords:
Alexandrium; Low temperature; Photoinhibition; Photosynthesis; Red tide; Strong light

Abbreviations:
FAME, fatty acid methyl ester; GLC, gas-liquid chromatography; PPFD, photosynthetic photon flux density; PSII, photosystem II; PSI, photosystem I; ROS, reactive oxygen species, SD, standard deviation

Highlights:

- Two noxious *Alexandrium* species differ with respect to the sensitivity of PSII to photoinhibition.
- Photoinhibition of PSII in *A. leei* is resistant to higher temperatures.
- Photoinhibition of PSII in *A. catenella* is resistant to lower temperatures.
- Tolerance of PSII to photoinhibition affects the temperature for cell proliferation.

Abstract

Alexandrium leei and *Alexandrium catenella* are noxious dinoflagellates that form red tides but the two species differ substantially in optimal growth conditions. For formation of blooms in natural environments, *A. leei* favors moderate temperatures (around 20 °C) while *A. catenella* favors much lower temperatures (around 10 °C). We examined the effects of temperature and light on cell proliferation and photosynthesis in cell cultures of the two species. Under light at 100 μmol photons m^{-2} s^{-1}, the growth rate of both species was highest at 20 °C. At 25 °C, *A. leei* continued to proliferate but *A. catenella* did not survive. Conversely, at lower temperatures 12 °C and 15 °C, the growth rate of *A. leei* was very low while that of *A. catenella* remained high. The net photosynthetic activity, as determined in terms of oxygen evolution, was highest at 20 °C in *A. leei*. By contrast, it was highest at 15 °C in *A. catenella*. Illumination of cells with strong light at 1,000 μmol photons m^{-2} s^{-1} decreased the activity of photosystem II (PSII), as determined in terms of Fv/Fm, in both species. In *A. leei*, the activity of PSII declined more rapidly at 12 °C and 15 °C than at 20 °C and 25 °C under strong light. In *A. catenella*, the activity of PSII declined more rapidly at 20 °C than at 12 °C and 15 °C. Thus, *A. catenella* appeared to be more resistant to photoinhibition of PSII at low temperatures than *A. leei*. In addition, the ability to repair photodamaged PSII at 12 °C and 15 °C was greater in *A. catenella* than that in *A. leei*. Thus, the tolerance of PSII to strong light and low temperatures might determine the range of temperatures that supports the formation of blooms of the two *Alexandrium* species.

1. Introduction

Blooms of noxious dinoflagellates have a negative impact on aquaculture and sanitation in coastal areas around the world. Many noxious dinoflagellates form blooms in seawater at relatively high temperatures in summertime, but some noxious species bloom from autumn to spring. In the genus *Alexandrium,* some species appear to favor relatively low temperatures for formation of blooms in coastal waters. For example, blooms of *Alexandrium catenella* (formerly *A. tamarense* genotype 1) and *Alexandrium leei* are not seen in summertime [1, 2].

Blooms of *A. catenella* cause paralytic poisoning of shellfish via the production and food-web transfer of paralytic shellfish toxins [3], and there are reports that the blooms of this species also kill fish [4]. By contrast, *A. leei* produces paralytic shellfish toxins at very low levels, but a bloom of *A. leei* killed aquacultured fishes and, in particular, red sea bream and Greater amberjacks, in Nomi Bay in Japan, recently [2]. Thus, blooms of these two *Alexandrium* species are a threat to the commercial production of both fish and shellfish.

The two species of *Alexandrium* differ substantially in terms of the temperatures that support the formation of blooms. In the coastal waters of Japan, *A. leei* forms blooms at moderate temperatures, around 20 °C [2], while *A. catenella* forms blooms at much lower temperatures, around 10 °C [5-7]. However, the environmental conditions that are responsible for the formation of blooms remain to be clarified. To minimize damage to aquaculture by these noxious species, it is necessary to clarify the mechanisms of bloom formation. Therefore, we decided to examine the effects of temperature and light conditions on specific physiological properties of these species to identify environmental factors that might promote the formation of their respective blooms.

In microalgae, photosynthesis mediates conversion of the energy of sunlight into high-energy compounds that are indispensable for growth, such as ATP, NADPH, and sugars. In the photosynthetic machinery, light is converted to chemical energy by two photosystems, namely, photosystem I (PSI) and photosystem II (PSII), both of which are protein-pigment complexes with large molecular masses. In spite of its important role in photosynthesis, PSII is very sensitive to environmental stressors, such as strong light and low and high temperatures [8, 9]. In particular, PSII is susceptible to inactivation by

strong light, and this phenomenon is referred to as photoinhibition of PSII, which often limits the growth of photosynthetic organisms under strong light [10, 11]. Furthermore, the photoinhibition of PSII is accelerated at low temperatures [8, 12, 13], at high temperatures [14, 15], at high concentrations of NaCl [16, 17], by oxidative stress [18, 19], and by nutrient deficiency [20]. Thus, the combination of multiple stressors with strong light rapidly inactivates PSII via acceleration of the photoinhibition of PSII.

In algae and in higher plants, the sensitivity of photosynthesis to low temperatures is associated with changes in levels of unsaturated fatty acids in membrane lipids [21-23]. In general, the degree of unsaturation of fatty acids in membrane lipids is elevated when photosynthetic organisms have acclimated to low temperatures [23].

In the present study, we examined the effects of strong light and low temperatures on PSII in *A. leei* and *A. catenella*. We found that *A. catenella* was more resistant than *A. leei* to the photoinhibition of PSII at low temperatures. In both species, the degree of unsaturation of fatty acids in membrane lipids increased when cells were grown at low temperatures. Our observations suggest that the tolerance of PSII to strong light and low temperatures might be an important factor in determining the range of temperatures that supports the formation of blooms of the two species of *Alexandrium*. We discuss the eco-physiological consideration of temperature sensitivity of the two species in relation to climate change.

2. Materials and methods

2.1. Strains and culture conditions

Cells of clonal axenic strains of *A. leei*, which had been isolated from Nomi Bay, Japan on 10 April 2017 [2], and those of *A. catenella*, which had been isolated from Osaka Bay, Japan on 27 February 2018, were grown at 20 °C and 15 °C, respectively, in 100-mL conical flasks that contained 30 mL of modified SWM-3 medium [24, 25] and were incubated, without shaking, under light at 100 μmol photons m^{-2} s^{-1} from fluorescent lamps (FL15NF; Panasonic Co. Ltd., Osaka, Japan) with 12 h of continuous light and 12 h of darkness daily.

Cells that had been precultured in 30 mL of SWM-3 medium for 14 d at various temperatures (12 °C, 15 °C, 20 °C and 25 °C) were transferred to conical flasks that contained 30 mL of SWM-3 medium at an initial cell density of 3,000-4,000 cells/mL, and they were grown under light at 100 μmol photons m^{-2} s^{-1} for four to five days. Cells were counted under a light microscope (Eclipse E400; Nikon, Tokyo, Japan) in a boarder-line slide glass (S6300; Matsunami, Osaka, Japan).

Cultures with a cell density of 10,000 cells/mL were used for assays. Photosynthetic photon flux densities (PPFDs) inside the incubator were measured with a Quantum Scalar Laboratory PPFD sensor (QSL-2101; Biospherical Instruments Inc., San Diego, CA, USA).

2.2. Measurements of photosynthetic activity

To determine net photosynthetic activity, we monitored the evolution of oxygen in the presence of 5 mM $NaHCO_3$, as electron acceptor, with a Clark-type oxygen electrode (Hansatech Instruments, King's Lynn, UK) during illumination of cells with light at various intensities from a halogen lamp (LA-150 UE; HAYASHI-REPIC, Tokyo, Japan), at the same temperature as that used for growth, as described previously [26, 27]. To determine the photosynthetic parameter Fv/Fm, chlorophyll fluorescence was monitored with a Water-PAM-II fluorometer (Waltz, Effeltrich, Germany) in the presence of 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU; for complete reduction of Q_A) after cells had been incubated in darkness for 10 min. The value of Fv was calculated as Fm – Fo, where Fm and Fo are the maximum and minimum fluorescence, respectively [26]. Fo was determined after exposure of cells to light at a weak measuring light at 630 nm for 40 s, and Fm was determined after subsequent exposure to a 0.8-s flash of saturating light at 10,000 μmol photons m^{-2} s^{-1}.

2.3. Assay of photoinhibition of PSII

Cells were exposed to strong light at 1,000 μmol photons m^{-2} s^{-1} from the halogen lamp (LA-150 UE), at the same temperature as that used for growth, for designated times to induce the photoinhibition of PSII, as described previously [28, 29]. For assays of photodamage, lincomycin was added to the suspension of cells at a final concentration of 200 μg ml^{-1} before the onset of illumination. For assays of the repair of PSII, cells were

exposed to very strong light at 4,500 μmol photons m^{-2} s^{-1} from the halogen lamp, at the same temperature as that used for growth, to decrease the activity of PSII to 5-20% of the initial level, with the exception that, in *A. leei,* PSII activity fell to 58% of the initial level at 25 °C. Then cells were transferred to light at 100 μmol photons m^{-2} s^{-1} from the halogen lamp and incubated at the same temperature as that used for growth. The activity of PSII was monitored in terms of Fv/Fm, as described above.

2.4. Analysis of fatty acid composition

The analysis of fatty acids was performed as described previously [30]. Freeze-dried cells (5 mg) were placed in a 15-mL screw-capped glass test tube and mixed with 5 mL of chloroform/methanol (2:1, v/v) that contained 100 μL of 10 mg/mL heptadecanoic acid as the internal standard. The mixture was sonicated in an ultrasonic cleaner for 10 min. The upper layer was removed with an aspirator, and the lower layer was filtered through a cotton plug and transferred to a 15-mL tube. Then the solvents were removed by centrifugation in a centrifugal concentrator (VC-96N; TAITEC, Koshigaya, Japan). The extracted lipids were trans-methylated by saponification and then subjected to boron trifluoride-catalyzed (BF_3-catalyzed) methylation. In brief, extracted lipids were hydrolyzed in a screw-capped glass tube with 750 μL of 0.5 M potassium hydroxide and 100 μL of 10 mg/mL heptadecanoic acid (internal standard) in methanol at 100 °C for 9 min. The reaction mixture was mixed with 1 mL of 14% BF_3 in methanol, at 100 °C for 7 min. Subsequently, 3 mL of hexane and 2.5 mL of a saturated solution of NaCl were added to the mixture, which was then centrifuged at 2500 x g for 10 min. The upper layer, containing fatty acid methyl esters (FAMEs), was loaded onto a Sep-Pak silica column (630 mg; Waters) that had been pre-washed with n-hexane, and FAMEs were eluted with 8 mL of n-hexane/diethyl ether (96:4, v/v). The eluent was evaporated in the centrifugal concentrator, and FAMEs were dissolved in 100 μL of acetone for analysis by gas-liquid chromatography (GLC). The GLC system consisted of a gas chromatograph (GC14A; Shimadzu Corporation, Japan) equipped with a flame ionization detector and capillary column (TC-70, 60 m × 0.25 mm i.d.; GL Science, Tokyo, Japan). The column temperature was programmed to increase linearly from 180°C to 230 °C at a rate of 1 °C/min. The temperature of the injection and detector port was maintained at 250 °C.

Nitrogen, as carrier gas, was passed over the column at a rate of 5 mL/min. FAMEs were identified on chromatograms by reference to the retention times of standards.

2.5. Statistical analysis

For determinations of cell number, measurements of photosynthetic activity, and analysis of fatty acid composition, mean values and standard deviations were calculated from triplicate assays. The significance of differences between two data points was examined with Student's *t*-test. When we compared multiple data points, we determined the significance of differences by one-way analysis of variance (ANOVA) using Tukey's HSD test. Values of $P < 0.05$ were taken as evidence of significance. Analyses were performed with Microsoft Excel (Microsoft, Redmond, WA, USA) and Minitab Statistical Software (Minitab Inc., State College, PA, USA).

3. Results

3.1. A. catenella proliferates more rapidly than A. leei at low temperatures

We grew cells of *A. leei* and *A. catenella* photoautotrophically at various temperatures under 12 h of continuous light at 100 μmol photons m^{-2} s^{-1} and 12 h of darkness daily (Supplemental Fig. S1). The growth rate of *A. leei* was highest at 20 °C, among the temperatures that we tested (Fig. 1A). At 25 °C, the growth rate was 23% lower than at 20 °C, while at 15 °C and at 12 °C, the growth rates were 35% and 60% lower than at 20 °C, respectively. The growth rate of *A. catenella* was also highest at 20 °C (Fig. 1B). At 25 °C, cells did not proliferate. By contrast, at 15 °C and at 12 °C, the growth rates were only 14% and 27% lower than that at 20 °C, respectively. Thus, it appeared that *A. leei* tolerated higher temperatures than *A. catenella* in terms of proliferation and, conversely, that *A. catenella* might tolerate lower temperatures.

3.2. Net photosynthesis is more efficient at low temperatures in A. catenella than in A. leei

We monitored the effects of temperature on net photosynthetic activity in cells of *A. leei* and *A. catenella*. After cells had been grown at various temperatures under light

at 100 μmol photons m^{-2} s^{-1} for 14 days, we measured net photosynthetic activity, at the same temperature as that used for growth under light at various intensities. In cells of *A. leei* grown at 20 °C, net photosynthetic activity increased as the intensity of measuring light was raised, reaching a plateau with light at 750 μmol photons m^{-2} s^{-1} (Fig. 2A). In cells grown at 25 °C and 15 °C, the activities yielded similar light curves, with maximum activities being approximately 30% lower than at 20 °C. In cells grown at 12 °C, the activity reached a plateau with light at 100 μmol photons m^{-2} s^{-1}, with maximum activity being half that in cells grown at 20 °C. In *A. catenella*, the maximum net photosynthetic activity at 15 °C was 26% higher than that at 20 °C, while that at 12 °C was 25% lower than that at 20 °C, although the shapes of the light curves were similar among cell cultures that had been grown at all three temperatures (Fig. 2B). Thus, it appeared that net photosynthetic activity in *A. catenella* might be higher at low temperatures than that in *A. leei*.

3.3. Photoinhibition of PSII at low temperatures is mitigated in A. catenella

We examined the effects of strong light and low temperatures on the activity of PSII. After cells had been grown at various temperatures under light at 100 μmol photons m^{-2} s^{-1} for 14 days, cells were exposed to strong light at 1,000 μmol photons m^{-2} s^{-1} for designated times at the same temperature as that used for growth, and the activity of PSII was monitored in terms of Fv/Fm. In cells of *A. leei* grown at 20 °C and 25 °C, the activity of PSII declined at a similar rate, reaching around 82% of the initial activity after 120 min under strong light (Fig. 3A). In cells grown at 15 °C, the activity of PSII declined to 59% of the initial level in 120 min, suggesting the typical photoinhibition of PSII, while in cells grown at 12 °C, the activity of PSII declined still further, to 43% of the initial level. In cells of *A. catenella* grown at 20 °C, the activity of PSII had fallen to 51% of the initial level after 120 min under strong light (Fig. 3B). By contrast, the activity remained high, at approximately 75% of the original activity, after 120 min both at 15 °C and 12 °C. Thus, photoinhibition of PSII was accelerated at 15 °C and 12 °C in *A. leei*, whereas it was mitigated at the low temperatures in *A. catenella*.

In photosynthetic cells, PSII is damaged by light and it is repaired, simultaneously, by a rapid and efficient repair system that requires the synthesis *de novo* of proteins that

comprise the reaction center of PSII, such as the D1 protein [10]. In the presence of lincomycin, an inhibitor of protein synthesis, photodamage to PSII can be monitored exclusively and in the absence of any repair of PSII [31]. We monitored photodamage to PSII in the presence of lincomycin under the same conditions as those that we used for assays of photoinhibition of PSII. In *A. leei*, photodamage to PSII did not differ significantly among cells grown at various temperatures (Fig. 4A). By contrast, in *A. catenella*, lower temperatures of 15 °C and 12 °C accelerated photodamage to PSII (Fig. 4B). These observations suggest that the mitigation of photoinhibition of PSII at 15 °C in *A. catenella* might have been due to an elevated capacity for the repair of PSII.

3.4. The repair of PSII is enhanced at low temperatures in A. catenella

To examine the capacity for the repair PSII, we exposed cells, after growth at various temperatures, to very strong light at 4,500 μmol photons m^{-2} s^{-1} for 30 or 45 min at the same temperature as that used for growth. This photoinhibitory treatment decreased the activity of PSII to 5-20% of the initial levels, except in the case of a decrease in activity to 58% at 25 °C in *A. leei* (Fig. 5). Then we transferred cells to growth light at 100 μmol photons m^{-2} s^{-1} and incubated cells at the same respective temperatures. In cells of *A. leei* grown at 20 °C, the activity of PSII fell to 20% of the initial level after illumination with very strong light at 20 °C and recovered to 83% of the initial level, after the transfer of cells to growth light, in 60 min (Fig. 5A). The initial rate of recovery of PSII activity at 15 °C was lower than the rate at 20 °C, and the recovery rate at 12 °C was even lower than the rate at 15 °C (Fig. 5A, 5C). By contrast, in *A. catenella*, the recovery rates at 15 °C and even at 12 °C were higher than the rate at 20 °C (Fig. 5B, 5D). Thus, *A. catenella* seemed able to repair PSII at low temperatures more efficiently than *A. leei*.

3.5. Low temperature increases the unsaturation of fatty acids in membrane lipids

In photosynthetic organisms, sensitivity to low temperatures is associated with relative levels of unsaturated fatty acids in membrane lipids [21-23]. We examined the effects of various temperatures on the composition of fatty acids in membrane lipids. In *A. leei*, major fatty acids were 16:0 (palmitic acid), 20:5 (n-3) (eicosapentaenoic acid, EPA), and 22:6 (n-3) (docosahexaenoic acid, DHA), with relative levels of 24.5%, 21.7%

and 23.4%, respectively, in cells grown at 20 °C (Fig. 6A). Relative levels of unsaturated fatty acids in cells grown at 12 ºC and 15 °C were 71.3% and 68.1%, respectively, which were higher than that (62.7%) in cells grown at 25 °C.

In *A. catenella*, major fatty acids were 14:0 (myristric acid), 16:0, 18:4 (n-3) (stearidonic acid), 20:5 (n-3), 22:1 (n-9) (erucic acid), and 22:6 (n-3), with relative levels of 8.1%, 20.8%, 5.0%, 9.3% and 21.2%, respectively, in cells grown at 15 °C (Fig. 6B). The composition of major fatty acids was more diverse than that in *A. leei*. In cells grown at 12 °C, the relative level of unsaturated fatty acids was 73.0%, which was higher than that (68.9%) in cells grown at 20 °C. Thus, growth at low temperatures increased the degree of unsaturation of fatty acids in membrane lipids both in *A. leei* and *A. catenella*. In addition to the diversity of major fatty acids, two other significant differences, in terms of fatty acid composition, between the two species were the relative levels of 14:0 and 16:0. However, the sum of relative levels of saturated fatty acids did not differ significantly between *A. catenella* and *A. leei* when cells were grown at the same temperature.

4. Discussion

4.1. Effects of strong light and low temperature on PSII

In the present study, we compared *A. leei* and *A. catenella* in terms of the sensitivity of photosynthetic activity to strong light and low temperature. The net photosynthetic activity in *A. leei* was maximal at 20 °C under light at a wide range of intensities, while that in *A. catenella* was maximal at 15 °C (Fig. 2), suggesting that all aspects of photosynthesis in *A. catenella* might be more efficient at low temperatures than in *A. leei*.

In general, the combination of strong light and low temperature has the most deleterious effect on the photosynthetic machinery and, in particular, on PSII [8, 9]. This phenomenon is referred to as the photoinhibition of PSII at low temperature. Although details of the underlying mechanism remain to be clarified, it is likely to involve an imbalance between the rate of photosynthetic electron transport and the activity of the Calvin-Benson cycle at low temperatures [9]. It seems plausible that the accumulation of

excess electrons on the acceptor side of PSI, due to the suppression of the Calvin-Benson cycle at low temperature, might promote the production of reactive oxygen species (ROS) from PSI, for example, the superoxide anion radical and hydrogen peroxide, which accelerate the photoinhibition of PSII via suppression of the repair of PSII [9]. It has been proposed that ROS are major factors that inhibit the repair of PSII via suppression of the synthesis *de novo* of proteins that are required for the repair of PSII, such as the D1 protein [32-34]. In *A. leei*, low temperatures, namely 12 °C and 15 °C, accelerated the photoinhibition of PSII. By contrast, in *A. catenella*, the low temperatures of 12 °C and 15 °C mitigated the photoinhibition of PSII (Fig. 3). Thus, *A. catenella* appeared to be more resistant to the photoinhibition of PSII at low temperatures than *A. leei*. Conversely, *A. catenella* might be more sensitive to the photoinhibition of PSII at high temperatures than *A. leei*.

Assays of photodamage to PSII, which was examined separately from the repair of PSII in the presence of lincomycin, revealed that the low temperatures of 12 °C and 15 °C did not affect photodamage to PSII in *A. leei* to any significant extent and actually accelerated photodamage to PSII in *A. catenella* (Fig. 4). Therefore, the mitigated photoinhibition of PSII in *A. catenella* at 15 °C might be attributable to the enhanced capacity to repair PSII. Indeed, the rates of repair of PSII at 12 ºC and 15 °C were higher than the rate at 20 °C in *A. catenella* (Fig. 5), suggesting that *A. catenella* might have a high capacity for the repair of PSII at low temperatures. What mechanism(s) might be responsible for this high repair capacity?

4.2. Mechanistic insights into the tolerance of PSII to strong light and low temperature

Our analysis of the fatty acid composition of membrane lipids revealed that the low temperatures of 12 ºC and 15 °C increased the relative levels of unsaturated fatty acids in both *A. leei* and *A. catenella* (Fig. 6). The increased levels of unsaturated fatty acids at low temperatures in *A. catenella* were also observed previously [*A. tamarense*, Korean isolate; 35]. This phenomenon is widely observed in photosynthetic organisms that have acclimated to low temperature and has been attributed to increases in membrane fluidity at low temperatures [23, 36]. In the cyanobacterium *Synechococcus elongatus* PCC 7942, genetic modification with the *desA* gene for the Δ12 desaturase from another

cyanobacterium (*Synechocystis* sp. PCC 6803) resulted in increased unsaturation of fatty acids in membrane lipids, with resultant mitigation of the photoinhibition of PSII at low temperatures via enhancement of the repair of PSII [13, 21, 37]. The unsaturation of fatty acids in membrane lipids might facilitate the turnover of the D1 protein, which constitutes the reaction center of PSII, during the processing of the precursor to D1 at low temperature [38]. Thus, it is likely that increased membrane fluidity, as a result of the unsaturation of fatty acids in membrane lipids, might protect PSII from photoinhibition at low temperatures, via enhancement of the repair of PSII, in *A. leei* and *A. catenella*. However, significant changes in terms of the degree of unsaturation of fatty acids were only observed in 16:0 and 22:6 in *A. leei*, and in 16:0 and 22:1 in *A. catenella*. The roles of other major fatty acids in the acclimation of both species to low temperature remain to be elucidated. In addition, the sum of the relative levels of unsaturated fatty acids in cells grown at the same temperature did not differ significantly between *A. leei* and *A. catenella*, even though the composition of fatty acids differed between the two species. It remains to be determined whether specific fatty acids in membrane lipids contribute to the superior ability of *A. catenella* to repair PSII at low temperature.

4.3. Eco-physiological considerations related to the sensitivity of PSII to strong light and low temperature

The intensity of sunlight in natural environments changes with time of day and seasons of the year. Thus, the species of *Alexandrium* that we used in the present study are exposed to variable and fluctuating illumination, from weak to strong, that can reach approximately 2,000 μmol photons m^{-2} s^{-1} just beneath the surface of the sea. Hence, the sensitivity of photosynthesis to strong light, in other words, the photoinhibition of PSII, might influence cell proliferation more significantly than other physiological parameters, such as the optimal temperature for growth under weak light. The superior ability of *A. catenella* to minimize the photoinhibition of PSII at 12 °C and 15 °C appears to be reflected in the formation of blooms and the depth of water column at which blooms are formed at low temperatures, such as 10 °C, in natural environments. By contrast, the superior ability of *A. leei* to minimize the photoinhibition of PSII at higher temperatures might be reflected in the formation of blooms at moderate temperatures in nature.

However, we cannot exclude the possibility that other eco-physiological traits, such as the capacity for vertical migration, might also modify rates of cell proliferation in response to strong light and low temperatures.

The water temperature was about 18 °C when the red tide of *A. leei* developed in Nomi Bay, Japan, at the beginning of April 2017 and killed aquacultured fishes, perhaps via the actions of unidentified lytic compounds [2]. It is possible that the production of such toxins by *A. leei* might be affected by temperature since it has already been demonstrated that the production of saxitoxins by *A. catenella* is induced by low temperatures [39]. With climate change, the temperature of seawater appears to be rising on a global scale, and this trend is likely to change the distribution and timing of red tides of *Alexandrium* species. For precise forecasting of red tides, to minimize the impact on commercial aquaculture, we need a fuller understanding of the environmental factors responsible for the formation of algal blooms by these noxious species.

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Figure legends

Fig. 1. Effects of various temperatures on the growth rate of cells. Cells of *A. leei* (A) and *A. catenella* (B) were grown at various temperatures and the growth rate was measured. N.D., Not detectable. Values are means ± S.D. (bars) of results from three independent experiments. Different letters denote significant differences between strains ($P < 0.05$; one-way ANOVA).

Fig. 2. Effects of various temperatures on net photosynthetic activity. Cells of *A. leei* (A) and *A. catenella* (B) were grown at various temperatures and then net photosynthetic activity was measured, in terms of the evolution of oxygen in the presence of 5 mM $NaHCO_3$, at the same temperature as that used for growth and under light at various intensities. Values are means ± S.D. (bars) of results from three independent experiments. Different letters denote significant differences between strains ($P < 0.05$; one-way ANOVA).

Fig. 3. Effects of various temperatures on photoinhibition of PSII. Cells of *A. leei* (A) and *A. catenella* (B) were grown at various temperatures and then exposed to strong light at 1,000 μmol photons m^{-2} s^{-1} for the indicated times at the same temperature as that used for growth. The activity of PSII was monitored in terms of Fv/Fm, the maximum quantum yield of PSII. The values of Fv/Fm taken as 100% for cells of *A. leei* grown at 12 °C, 15 °C, 20 °C and 25 °C were 0.650 ± 0.005, 0.710 ± 0.005, 0.693 ± 0.015, and 0.692 ± 0.010, respectively. The values of Fv/Fm taken as 100% for cells of *A. catenella* grown at 12 °C, 15 °C and 20 °C were 0.773 ± 0.012, 0.764 ± 0.006, and 0.727 ± 0.001, respectively. Values are means ± S.D. (bars) of results from three independent experiments. Different letters denote significant differences between strains ($P < 0.05$; one-way ANOVA).

Fig. 4. Effects of various temperatures on photodamage to PSII. Cells of *A. leei* (A) and *A. catenella* (B) were grown at various temperatures and then exposed to strong light at 1,000 μmol photons m^{-2} s^{-1} for the indicated times, at the same temperature as that used for growth, in the presence of 200 μg/ mL lincomycin. The activity of PSII was monitored

in terms of Fv/Fm. The values of Fv/Fm taken as 100% for cells of *A. leei* grown at 12 °C, 15 °C, 20 °C and 25 °C were 0.726 ± 0.002, 0.677 ± 0.037, 0.735 ± 0.009, and 0.757 ± 0.010, respectively. The values of Fv/Fm taken as 100% for cells of *A. catenella* grown at 12 °C, 15 °C and 20 °C were 0.804 ± 0.019, 0.777 ± 0.025, and 0. 0.783 ± 0.007, respectively. Values are means ± S.D. (bars) of results from three independent experiments. Different letters denote significant differences between strains ($P < 0.05$; one-way ANOVA).

Fig. 5. Effects of various temperatures on the repair of PSII. Cells of *A. leei* (A) and *A. catenella* (B) were grown at various temperatures and then exposed to very strong light at 4,500 μmol photons m^{-2} s^{-1} for the indicated times, at the same temperature as that used for growth, to decrease the activity of PSII. Then cells were transferred to weak light at 100 μmol photons m^{-2} s^{-1} and incubated for the indicated times at the same temperature as that used for growth. The values of Fv/Fm taken as 100% were the same as in the legend for Figure 4. The initial rates of the recovery of PSII in cells of *A. leei* (C) and *A. catenella* (D) were calculated from the data shown in (A) and (B). Values are means ± S.D. (bars) of results from three independent experiments. Different letters denote significant differences between strains ($P < 0.05$; one-way ANOVA).

Fig. 6. Effects of various temperatures on fatty acid composition of membrane lipids. Lipids extracted from cells of *A. leei* (A) and *A. catenella* (B) were trans-methylated by saponification and then subjected to BF_3-catalyzed methylation, as described in the text. The fatty acid methyl esters (FAMEs) were analyzed by gas-liquid chromatography. Values are means ± S.D. (bars) of results from three independent experiments. Asterisks indicate statistically significant differences ($P < 0.05$; Student's *t*-test).

Fig. 1

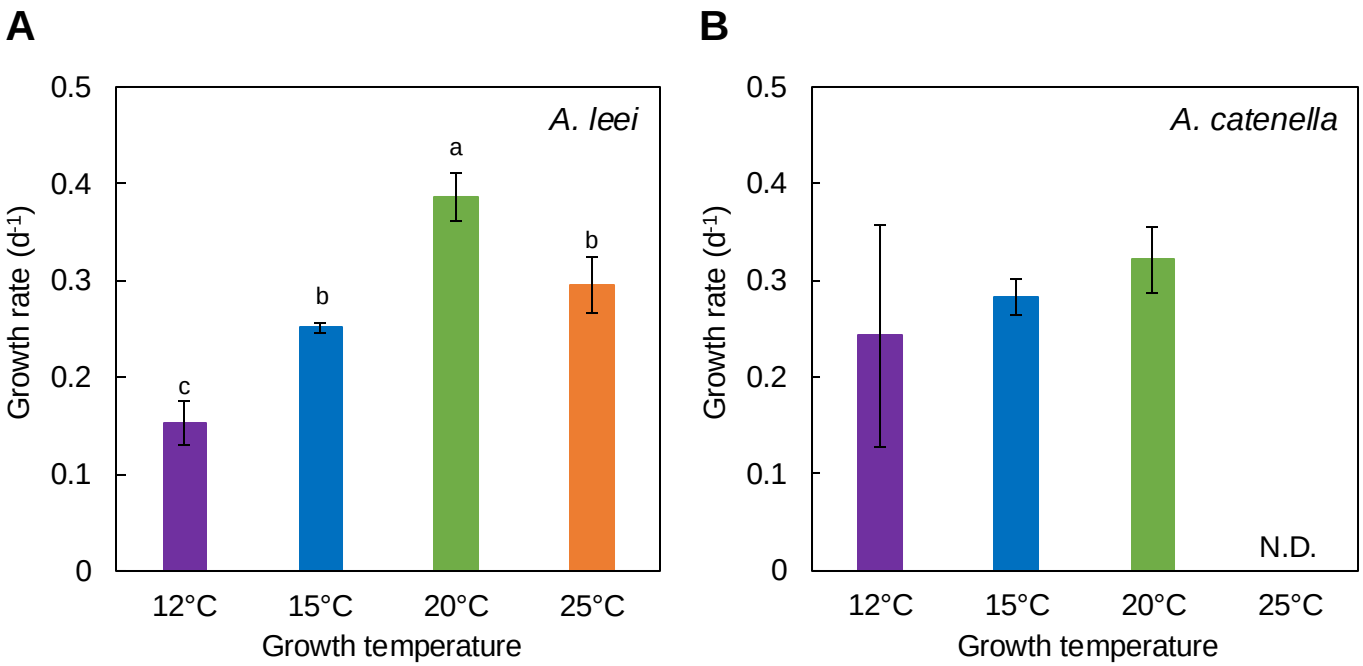

A
B
A. leei
A. catenella
Growth rate (d-1)
0.5
0.4
0.3
0.2
0.1
0
a
b
b
c
N.D.
12°C
15°C
20°C
25°C
Growth temperature

Fig. 2

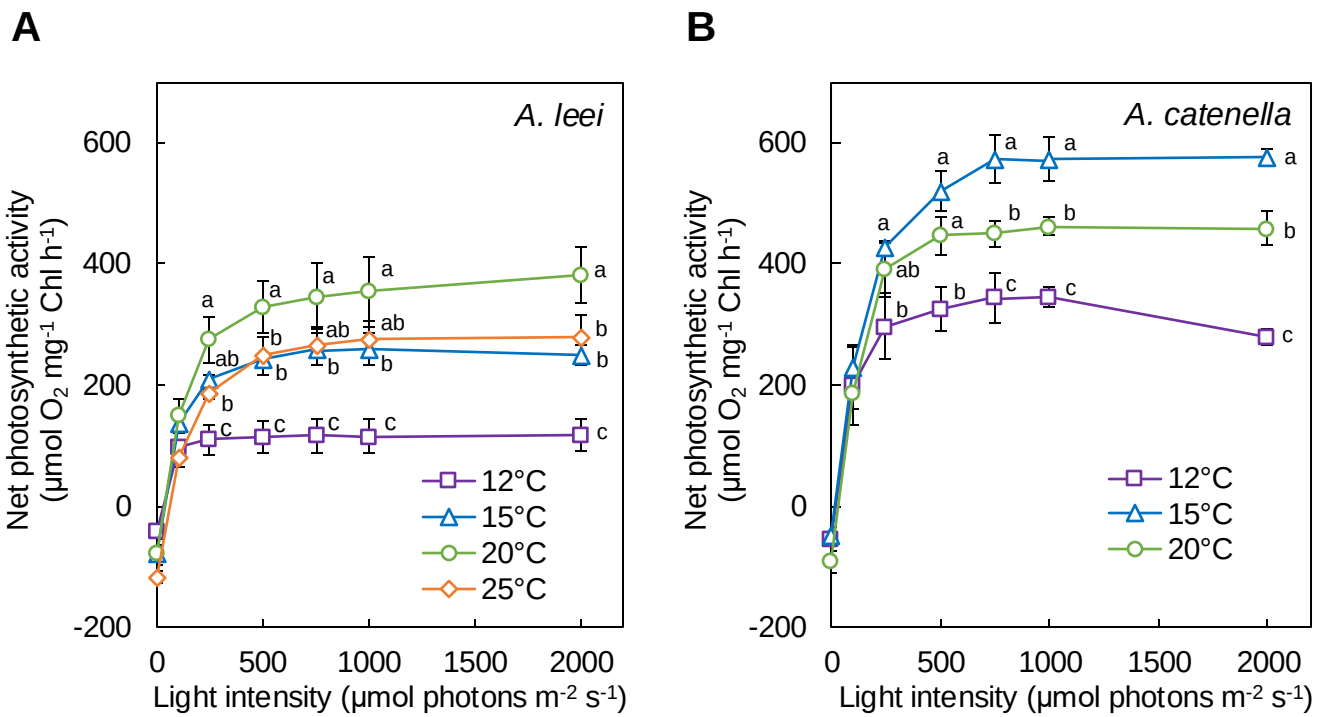

A
A. leei
Net photosynthetic activity (μmol O2 mg-1 Chl h-1)
600
400
200
0
-200
0
500
1000
1500
2000
Light intensity (μmol photons m-2 s-1)
12°C
15°C
20°C
25°C
B
A. catenella
Net photosynthetic activity (μmol O2 mg-1 Chl h-1)
Light intensity (μmol photons m-2 s-1)
12°C
15°C
20°C

Fig. 3

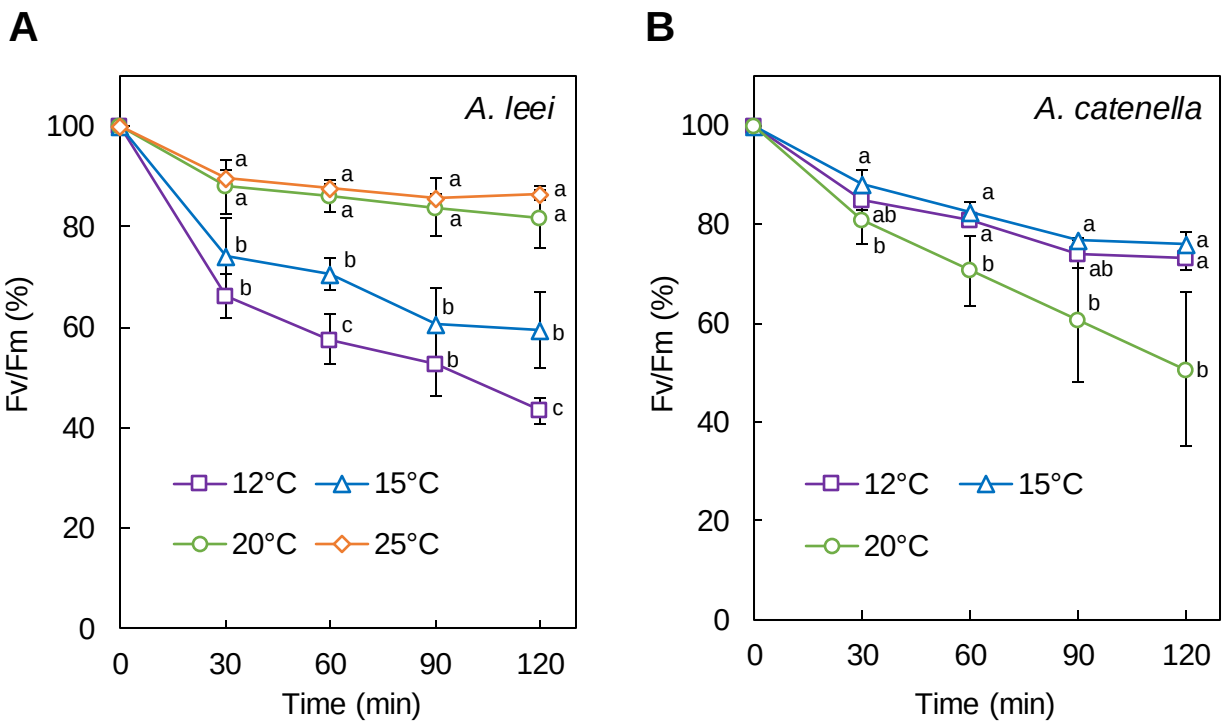

A
A. leei
Fv/Fm (%)
Time (min)
12°C
15°C
20°C
25°C
B
A. catenella
Fv/Fm (%)
Time (min)
12°C
15°C
20°C

Fig. 4

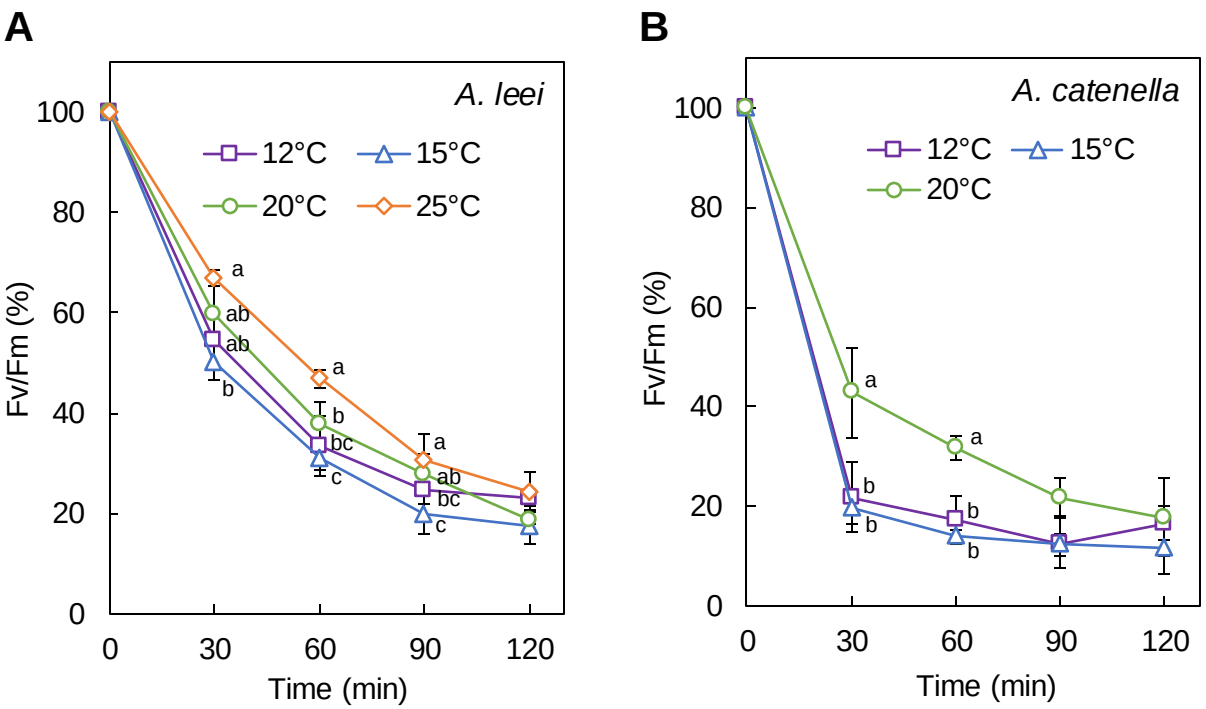

A
A. leei
12°C
15°C
20°C
25°C
Fv/Fm (%)
Time (min)
B
A. catenella
12°C
15°C
20°C
Fv/Fm (%)
Time (min)

Fig. 5

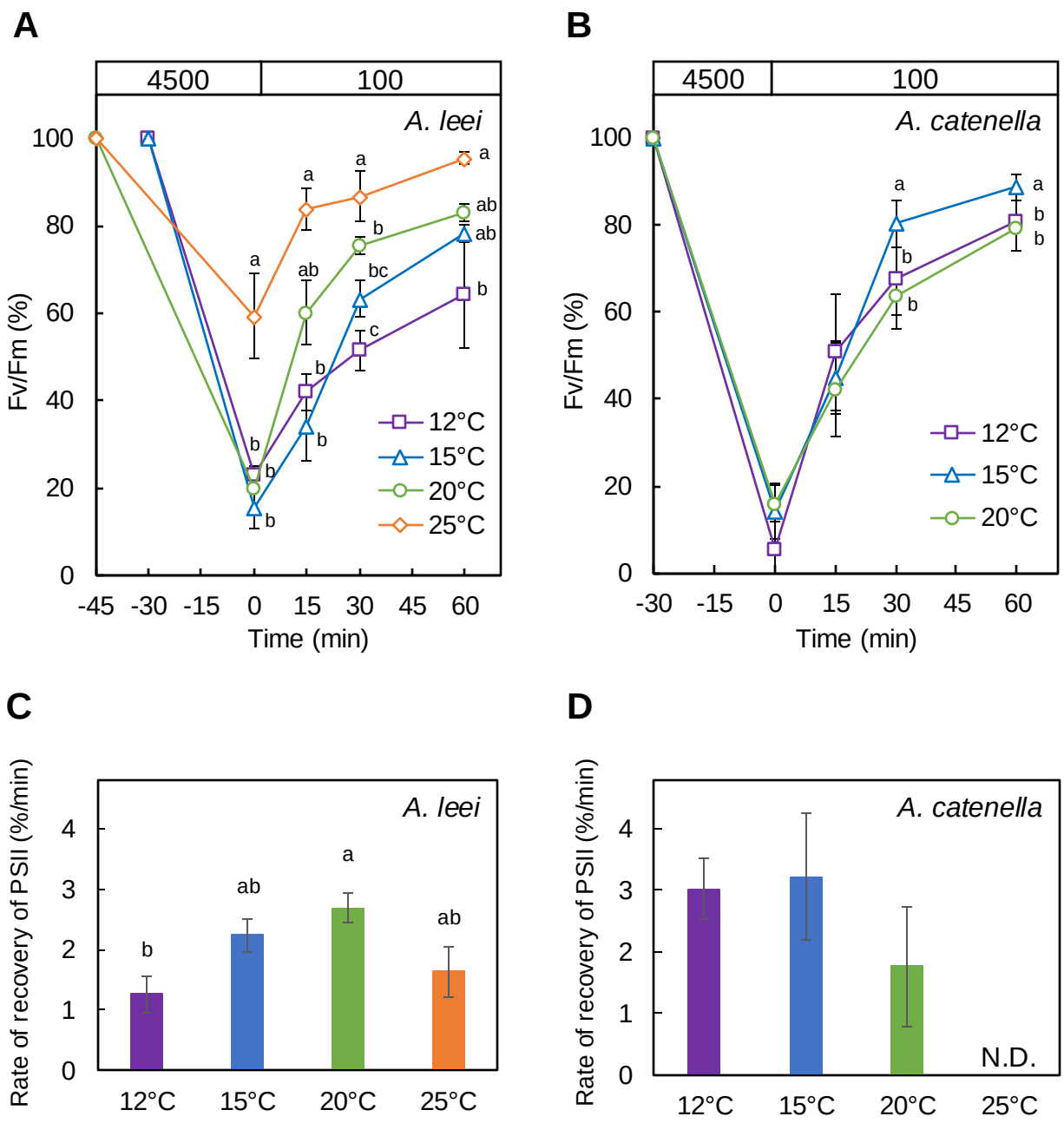
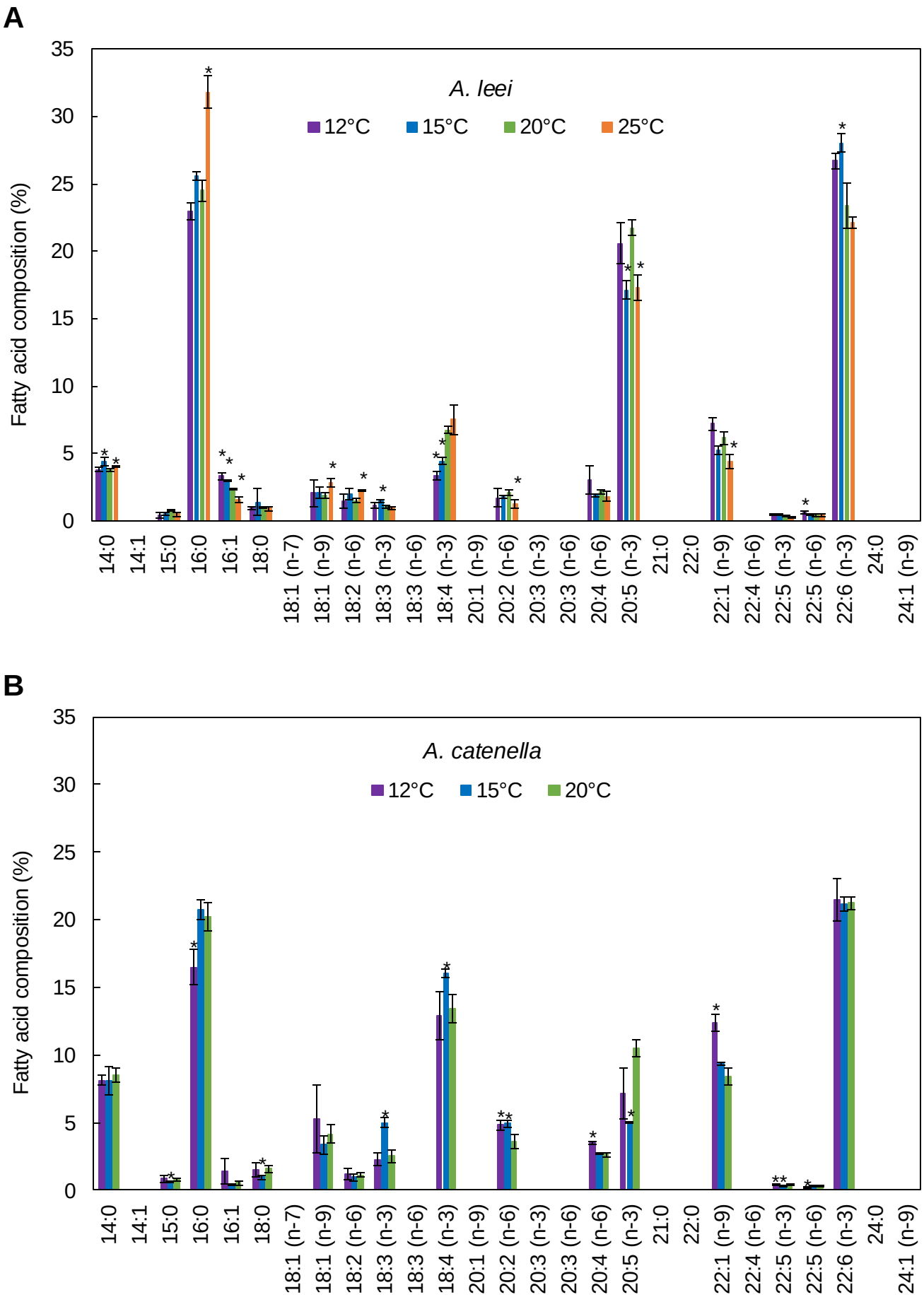
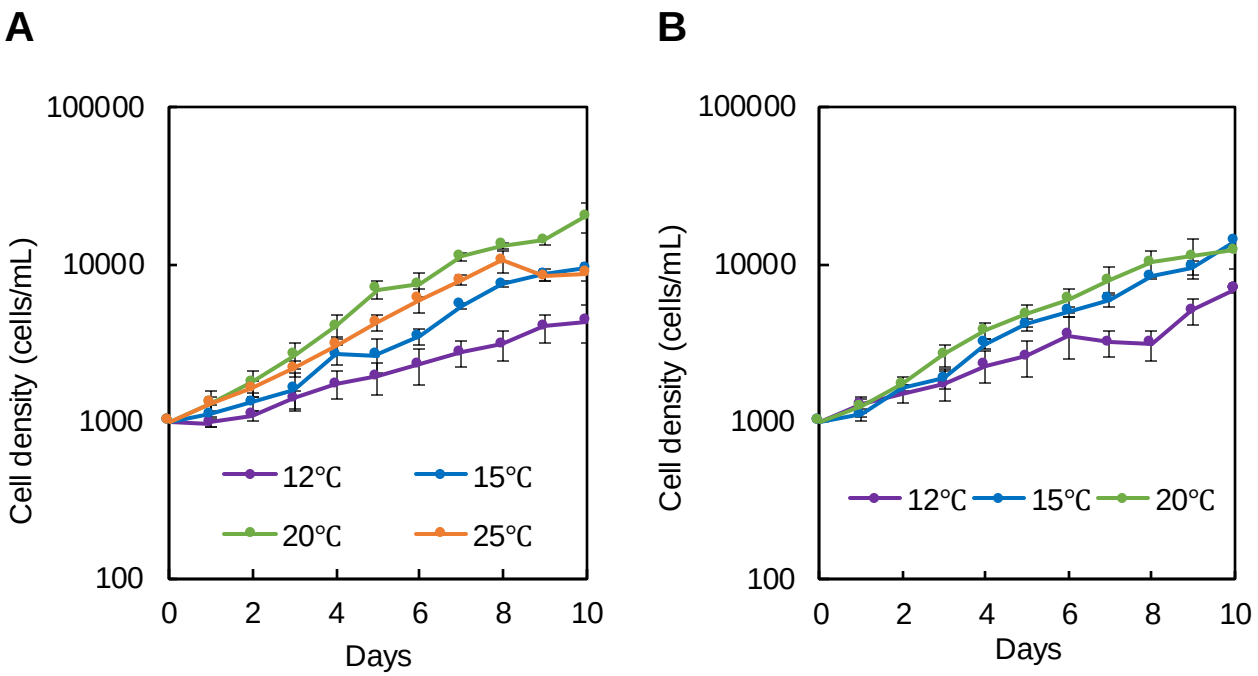

A
4500
100
A. leei
Fv/Fm (%)
Time (min)
12°C
15°C
20°C
25°C
B
4500
100
A. catenella
Fv/Fm (%)
Time (min)
12°C
15°C
20°C
C
A. leei
Rate of recovery of PSII (%/min)
12°C
15°C
20°C
25°C
D
A. catenella
Rate of recovery of PSII (%/min)
N.D.
12°C
15°C
20°C
25°C

Fig. 6


A
A. leei
12°C
15°C
20°C
25°C
Fatty acid composition (%)
0
5
10
15
20
25
30
35
14:0
14:1
15:0
16:0
16:1
18:0
18:1 (n-7)
18:1 (n-9)
18:2 (n-6)
18:3 (n-3)
18:3 (n-6)
18:4 (n-3)
20:1 (n-9)
20:2 (n-6)
20:3 (n-3)
20:3 (n-6)
20:4 (n-6)
20:5 (n-3)
21:0
22:0
22:1 (n-9)
22:4 (n-6)
22:5 (n-3)
22:5 (n-6)
22:6 (n-3)
24:0
24:1 (n-9)
B
A. catenella
12°C
15°C
20°C
Fatty acid composition (%)
0
5
10
15
20
25
30
35
14:0
14:1
15:0
16:0
16:1
18:0
18:1 (n-7)
18:1 (n-9)
18:2 (n-6)
18:3 (n-3)
18:3 (n-6)
18:4 (n-3)
20:1 (n-9)
20:2 (n-6)
20:3 (n-3)
20:3 (n-6)
20:4 (n-6)
20:5 (n-3)
21:0
22:0
22:1 (n-9)
22:4 (n-6)
22:5 (n-3)
22:5 (n-6)
22:6 (n-3)
24:0
24:1 (n-9)



Supplemental Fig. S1. Effects of various temperatures on growth. Cells of *A. leei* (A) and *A. catenella* (B) were grown at various temperatures and cell number was counted. Values are means ± S.D. (bars) of results from three independent experiments.