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Photosynthetic activity of benthic diatoms in response to different temperatures

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Abstract The photosynthetic activities of benthic diatoms in response to temperature changes were assessed by measuring chlorophyll fluorescence kinetics. Small benthic diatom species with large surface area to volume (SA/*V*) ratios responded to increasing temperature differently from large diatoms, since larger ratios caused lower photosynthetic activity under high-temperature conditions. The small SA/*V* ratios of large cells may be advantageous in benthic environments under adverse conditions such as high temperature and/or strong light. A size-dependent differential response of benthic diatoms to changes in environmental factors such as temperature may result in an altered distribution of the different diatom communities.

Keywords Benthic diatoms · Temperature · Photosynthesis · SA/*V* ratio · Chlorophyll fluorescence

Introduction

Benthic microalgae are important primary producers in coastal and estuarine ecosystems and contribute up to 50% of total primary production (Underwood and Kromkamp 1999). Most benthic microalgae are composed of motile pennate diatoms (Serôdio et al. 1997), which are able to migrate into the sediments to avoid damage from excess light and strong tides when diatom cells are exposed to them (Kromkamp et al. 1998; Underwood and Kromkamp 1999). Among benthic photosynthetic factors, temperature varies more rapidly within a short time period. The surface (upper 200 µm) temperature in muddy sediments in temperate regions can easily change by 10°C during emersion periods, with rates of temperature change as high as 4°C h^{-1} (Harrison 1985). However, there are only a few recent studies on the photosynthetic response of benthic diatoms to changes in temperature (Morris and Kromkamp 2003; Hancke et al. 2008).

A broader awareness of global warming has underlined the importance of understanding the effects of increasing temperature on marine primary producers. In this study, we determined the ratio of surface area to volume (SA/*V*) for five diatom species and applied this information to an examination of temperature effects on the photosynthetic activity of benthic diatoms. Because the SA/*V* ratio of unicellular algae is a highly relevant factor, both ecologically and physiologically, is closely related to their growth rates, nutrient uptakes, and sinking rates (Smith and Kalff 1982; Harris 1986; Sumich 1984).

Materials and methods

Unialgal cultures of *Amphora coffeaeformis*, *Navicula* sp., *Nitzschia* sp., *Cylindrotheca closterium*, and *Pleurosigma elongatum* (isolated from the Nakdong River Estuary in Korea) were grown in f/2 medium (Guillard and Ryther 1962) with pre-filtered seawater (0.2-µm membrane filter;

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salinity, 34 psu). These species are pennate diatoms and are the dominant species in the area (Du and Chung 2007). All cultures were incubated under constant conditions at 15°C with a light (100 μmol photons m^{-2} s^{-1}):dark photoperiod of 12 h:12 h. To minimize effects of cell growth, we chose the samples from the beginning of the stationary phase and sub-sampled for measurements of cell size by scanning electron microscopy (SEM; TOPCON ABT-32). The sub-samples were fixed in 2% glutaraldehyde for 3 h at 4°C, post-fixed in 1% osmium tetroxide, and after the fixations rinsed with sodium cacodylate buffer (pH 7.5). The fixed specimens were then dehydrated through an ethanol series and dried in hexamethyldisilazane. Finally, they were coated with gold ions using an IB-3 (Eiko, Japan), and the sizes of ten cells were measured. From the largest to the smallest cells, the sizes (mean ± s.d.) were as follows: *P. elongatum* (170±10.9 μm), *C. closterium* (100±6.3 μm), *A. coffeaeformis* (25±3.9 μm), *Navicula* sp. (22±2.7 μm), and *Nitzschia* sp. (18±2.1 μm). The surface areas and volumes of the cells were determined based on geometric shapes and equations, using the method of Hillebrand et al. (1999), to obtain the SA/*V* ratio, a parameter that reflects both the size and shape of a cell.

The photosynthetic response of each species was measured by chlorophyll fluorescence using Diving-PAM (Walz, Germany). Photosynthetic activity can be assessed by measuring the effective quantum yield of PSII (Φ_{PSII}) which measures the proportion of the light absorbed by chlorophyll associated with PSII used in photochemistry (Genty et al. 1989). The chlorophyll fluorescence technique allows not only the quick and non-intrusive measurement of photosynthetic activity but also the examination of processes such as quantum efficiency, photoprotection, and photoinhibition, which provide important information on the physiological states of benthic microalgae (Kromkamp et al. 1998). For the fluorescence measurements, six sub-samples for each species were filtered on different glass microfiber filters (Whatman GF/F 47 mm) (Consalvey et al. 2004) and the filters were placed on a tissue culture plate filled with agar medium. Subsequently, six plates for each species were put at six different temperatures (10, 15, 20, 25, 30, and 35°C) on the temperature gradient table with a water bath at the same time and measured every 2 h for 24 h. The samples were acclimated to the same irradiance (100 μmol photons m^{-2} s^{-1}) as the culture conditions before the start of the curve. Photosynthetic activities were assessed using rapid-light curves (RLCs), where samples were exposed to eight incremental steps of irradiance (10 s per step) ranging from 0 to 988 μmol photons m^{-2} s^{-1}. A 10-s light step is most commonly used for aquatic photo-autotrophs (e.g., Ralph et al. 1998; Serôdio et al. 2005).

From fluorescence measurements, Φ_{PSII} multiplied by PAR gives a relative indication of the photosynthetic electron transport rate (rETR), which together with Φ_{PSII} indicates the actual state of photosynthesis (Ralph and Gademann 2005). The maximum relative electron transfer rate ($rETR_{max}$) was derived from the relative electron transport rate (rETR) fitted to the model of Platt et al. (1980). The $rETR_{max}$ has been emphasized in many recent photosynthetic studies because it is closely related to the maximum photosynthetic capacity which is obtained when the rate of photosynthesis is limited by the activity of the electron transport chain or Calvin cycle enzymes (Behrenfeld et al. 2004; Ralph and Gademann 2005), although $rETR_{max}$ values obtained from RLCs have some discrepancies with the values measured from steady-state light curves, which depend on the ambient irradiance (Serôdio et al. 2006).

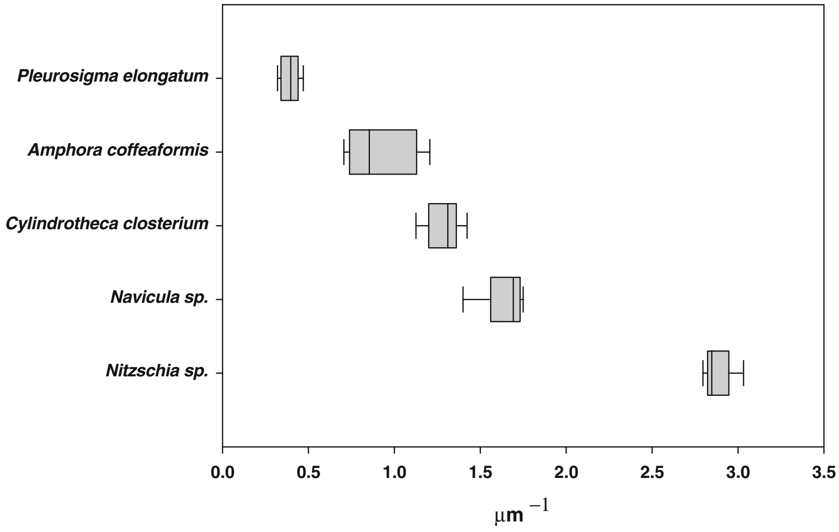

Fig. 1 Box plots showing the distribution of SA/*V* ratios for each species. *Boxes* encompass the 25% and 75% quartiles of all data for each algal type. The *central line* represents the median and bars (*whiskers*) are the 10% and 90% quartiles

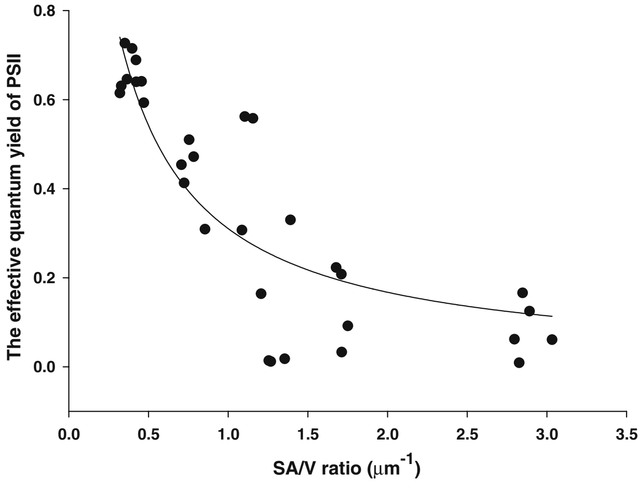

Fig. 2 Relationship between the SA/*V* ratio and the effective quantum yield of PSII at the same irradiance as the growth irradiance (100 μmol photons m^{-2} s^{-1}) in samples exposed to a temperature of 35°C. The equation of the curve is $y = a\ (1+b\times x)$, (R^2=0.76, $p<0.01$)

Results

The mean SA/*V* ratio ranged from 0.39±0.05 to 2.88±0.08 μm^{-1} and depended mainly on cell size (Fig. 1). Among the five different species, the smallest and thinnest *Nitzschia* sp. had the largest SA/*V* ratio, whereas the largest *P. elongatum* had the smallest ratio. *C. closterium* had a relatively large SA/*V* ratio because surface area was large, but volume was small due to their long, thin shapes. In contrast, *A. coffeaeformis* had a relatively small SA/*V* ratio, although the range was broad. Depending on the SA/*V* ratio, their photosynthetic activities differed (Fig. 2). In samples exposed to 35°C, the highest of the investigated temperatures, the SA/*V* ratio and Φ_{PSII} were negatively correlated (Fig. 2), indicating that smaller species (larger SA/*V* ratios) are more negatively affected by higher temperature than larger species.

The maximum relative electron transfer rate ($rETR_{max}$) also showed a substantial inter-specific variability in the responses to temperature (Fig. 3). The $rETR_{max}$ of species with large SA/*V* ratios, such as *Navicula* sp., *Nitzschia* sp., and *C. closterium*, decreased significantly (t test, $p<0.01$) at the highest temperature (35°C). By contrast, *P. elongatum* with the smallest SA/*V* ratio had a high photosynthetic capacity under increased temperature conditions. *A. coffeaeformis* with a relatively small SA/*V* ratio was also less affected than other species although decreased at the highest temperature. The $rETR_{max}$ values of *P. elongatum* and *A. coffeaeformis* were significantly (t test, $p<0.01$) higher than those of other small species. The results from this study show that the impact of temperature on the photosynthetic response of benthic diatoms differs depending on the SA/*V* ratio of the species.

The photosynthetic activity of *P. elongatum*, which has the largest cells and thus the smallest SA/*V* ratio, was not significantly (t test, $p>0.05$) affected by an increase in temperature from 30 to 35°C . By contrast, species characterized by small cells, such as *Nitzschia* sp. and *Navicula* sp., were more vulnerable to temperature increases because of their large SA/*V* ratios.

Discussion

In marine primary producers, a large SA/*V* ratio would appear to be advantageous for acclimation to different environments. The small cell size of algae produces a relatively large SA/*V* ratio to increase uptake efficiency of diluted inorganic nutrients (Smith and Kalff 1982; Harris 1986). Similarly, a small cell more efficiently harvests light as a result of the smaller packaging effect (Raven 1998). In addition, a large SA/*V* ratio slows a cell's sinking rate and thus increases their residence time in the upper, sunlit waters of the ocean (Sumich 1984). However, our results show that large SA/*V* ratios can be disadvantageous under adverse conditions such as high temperature and/or strong light. Montagnes and Franklin (2001) found that diatoms with an exceptionally large cell size (>300 μm) increased their size even further with increasing temperature, whereas the size of small diatoms decreased. They interpreted the enlargement of large diatoms as being mainly due to a vegetative increase. Our results show that a larger cell size corresponds to a smaller SA/*V* ratio and a decreased vulnerability to higher temperatures. This could be one reason for why the findings of Montagnes and Franklin

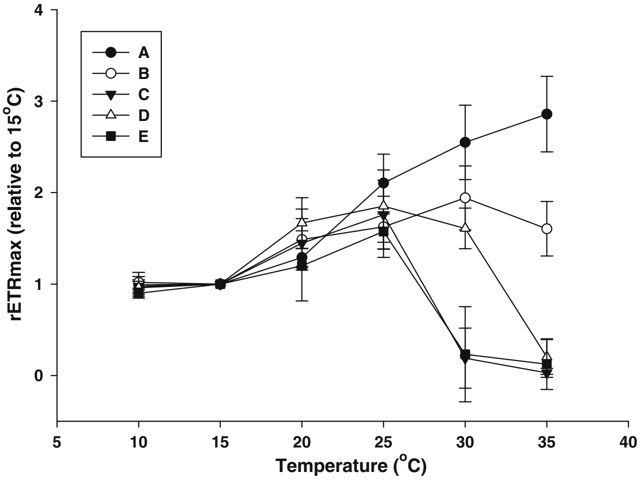

Fig. 3 Relative effect of temperature on $rETR_{max}$: *P. elongatum* (**A**), *A. coffeaeformis* (**B**), *C. closterium* (**C**), *Navicula* sp. (**D**), and *Nitzschia* sp. (**E**). All parameters were normalized to the values at 15°C where they were used to be grown at. Data represent the means and standard deviations

(2001) observed an enlargement in the size of the diatom population with increasing temperatures.

Based on the results from this study, the vertical distribution patterns of benthic diatoms at high temperature can be predicted. When the surface temperature increases, large species with small SA/*V* ratios, such as *P. elongatum*, can exist at the top surface of sediment. In contrast, small species with large SA/*V* ratios, such as *Nitzschia* sp., will be forced to migrate downward and will eventually be found in the sub-surface, beneath the layer formed by species of large cell size. The other potential consequence of the SA/*V* ratio is that when the vertical migration is too short because of excessive vertical warming, the ratio may be an important determinant in species succession. However, this scenario intrinsically assumes that surface warming is very local, and its confirmation requires a high-resolution vertical temperature profile or a model thereof. In fact, Grant (1986) and Barranguet et al. (1998) showed that temperature can exert tight control on benthic photosynthetic rates and lead to changes in microphyte community composition. In addition, based on results that coincide well with those of our study, Du et al. (2009) found that *A. coffeaeformis* was positively correlated, but *Navicula* sp. was negatively correlated, to sediment temperature, as determined by principal component analysis of diatoms obtained from the same sampling site as ours.

Recently, global environmental conditions have been rapidly changing, with severe potential effects on the natural habitats of marine primary producers such as phytoplankton, benthic algae, and macroalgae. Therefore, it is essential to investigate different physiological responses of photoautotrophs to environmental factors such as temperature, especially given that size-dependent differences in physiological characteristics may alter diatom assemblages.

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