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Qiu, Xuchun
Laboratory of Marine Environmental Science, Division of Animal & Marine Bioresource Science, Department of Bioresource Sciences, Faculty of Agriculture, Kyushu University

Mukai, Kouki
Laboratory of Marine Environmental Science, Division of Animal & Marine Bioresource Science, Department of Bioresource Sciences, Faculty of Agriculture, Kyushu University

Shimasaki, Yohei
Laboratory of Marine Environmental Science, Division of Animal & Marine Bioresource Science, Department of Bioresource Sciences, Faculty of Agriculture, Kyushu University

Tsuyama, Michito
Department of Agro-environmental Sciences, Faculty of Agriculture, Kyushu University

他

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Potential Maximum Quantum Yield of Photosystem II Reflects the Growth Rate of *Chattonella marina* in Field Bloom Samples

Xuchun QIU, Kouki MUKAI, Yohei SHIMASAKI*, Michito TSUYAMA[1], Tadashi MATSUBARA[2, 3], Yu NAKAJIMA[4], Tsuneo HONJO[5] and Yuji OSHIMA

Laboratory of Marine Environmental Science, Division of Animal & Marine Bioresource Science, Department of Bioresource Sciences, Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan
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Time series variations in the potential maximum quantum yield of photosystem II (F_v/F_m ratio) and growth rate of field *Chattonella marina* cells were investigated during a HAB occurred in the inner part of Ariake Sea, Japan (5–14 September, 2012). This study aimed at investigating the usability of Fv/Fm ratio in evaluating the growth of *C. marina* and its HAB dynamic under natural conditions, by analyzing its correlations with algal growth rate and various environmental variables. Field observation showed that exhaustion of dissolved inorganic nitrogen (DIN) was likely responsible for controlling the growth of *C. marina* cells and finally induced the bloom termination. As the bloom progressed, both the F_v/F_m ratio (0.62 to 0.72) and growth rate (–0.25 to 0.81 div. d^{-1}) of *C. marina* cells tended to decrease, and there was a significant positive correlation between the two parameters. Both the F_v/F_m ratio and growth rate of *C. marina* cells were positively correlated with DIN concentrations, which also supported the inference that DIN was responsible for controlling the dynamics *C. marina* HAB. Thus, our results suggest that the F_v/F_m ratio, combined with information about environmental factors, may be useful in evaluating the nutrient status and growth potential of *C. marina* during field blooms.

Key words: *Chattonella marina*, F_v/F_m ratio, growth rate, environmental factor

INTRODUCTION

So far, the harmful algal blooms (HABs) of *Chattonella* (Raphidophyceae) causing significant economic and ecological damages have been reported in various regions of the world (e.g., Hallegraeff *et al*., 1998; Tiffany *et al*., 2001; Imai and Yamaguchi, 2012). Over the last 20 years, the HABs of *Chattonella marina* (Subrahmanyan) Hara & Chihara var. *marina* and *Chattonella marina* var. *antiqua* (Hada) Demura & Kawachi have caused serious damages to aquaculture and fishery production in the coastal waters of western Japan (Yamatogi *et al*., 2006; Imai and Yamaguchi, 2012; Katano *et al*. 2012). Based on its minimum cell quota for nutrients reported by Nakamura (1985), *C. marina* var. *antiqua* can easily reach the warning level (about 100 cells ml^{-1}) by consuming only small amounts of nitrogen (0.78 μM) and phosphorus (0.062 μM), and subsequently may maintain at high abundance for several weeks, causing considerable mortality in cultured fish (Imai *et al*., 2006; Matsubara *et al*., 2009; Katano *et al*., 2012). At the end of a dense bloom, decomposition of senescent *C. marina* cells is thought to increase dissolved oxygen consumption and thereby contribute to the development of a severely hypoxic water mass, leading to mass mortality of clams (Nakada *et al*., 2010).

Because information on bloom dynamics is necessary for the development of countermeasures to mitigate the fisheries damages caused by *Chattonella* (e.g. stop feeding, moving culture cages), the technology to evaluate cell growth and predict the wax and wane of a bloom is important, and the need to develop such technology is urgent (Imai *et al*., 2006). The HAB of a particular species is the result of complex interactions of environmental variables acting at both population and cellular levels, which also involved the ecophysiological responses of the species (Smayda, 1997; Vargo *et al*., 2009). Thus, a parameter that would reflect both cellular physiological responses to environmental variables and algal population growth seems to be important for successful evaluation of the wax and wane of a bloom (Qiu *et al*., 2013).

The potential maximum quantum yield of photosystem II (F_v/F_m ratio), which reflects the efficiency of photochemical conversion of light energy, is one of the parameters most often used in studies of aquatic photosynthesis (Genty *et al*., 1989; Schreiber *et al*., 1995; Goto *et al*., 2008). Because light energy conversion in photosystem II is directly related to cell productivity and growth, significant correlations between the F_v/F_m ratio and growth rate have been observed in cultures of several phytoplankton species (Kruskopf and Flynn, 2006; Wang *et al*., 2011). In laboratory cultures of *C. marina* var. *antiqua* under

[1] Department of Agro–environmental Sciences, Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan
[2] Saga Prefectural Ariake Fisheries Research and Development Center, Nagata 2753–2, Ashikari–cho, Ogi, Saga 849–0313, Japan
[3] National Research Institute of Fisheries and Enviroment of Inland Sea, Fisheries Research and Education Agency, Hatsukaichi, Hiroshima 739-0452, Japan
[4] The University of Tokyo Graduate School of Frontier Science Dept. of Natural Environmental Studies, Kashiwanoha 5–1–5, Kashiwa–shi, Chiba 277–8564, Japan
[5] Seto Inland Sea Regional Research Center, Kagawa University, Saiwaichou 1–1, Takamatsu, Kagawa 760–8521, Japan
* Corresponding author (E–mail: simasaki@agr.kyushu–u.ac.jp)

various conditions, significant positive correlations between the F_v/F_m ratio and daily growth rate has been observed, and its F_v/F_m ratio was significantly affected by low nutrient levels or elevated irradiance (Qiu *et al*., 2013). Unlike single–species laboratory cultures grown under constant conditions, in nature the F_v/F_m ratio is influenced by the independent or combined action of various factors (Goto *et al*., 2008). Therefore, evaluation of algal growth in the field by using the F_v/F_m ratio should be based on detailed investigation on the target species. However, only a few studies have investigated the impacts of light and nutrients on the F_v/F_m ratio of cultured *Chattonella* spp. (Warner and Madden, 2007; Qiu *et al*., 2013), while investigations of the F_v/F_m ratio during *Chattonella* blooms are absent.

In this study we monitored temporal variations in the F_v/F_m ratio and environmental variables during a HAB caused by *C. marina*, and determined the growth rate of *C. marina* by culturing sea water samples under laboratory conditions. Subsequently, we analyzed the correlations between the F_v/F_m ratio, growth rate, and the environmental factor. This study aimed to investigate the usability of F_v/F_m ratio in evaluating the growth of *C. marina* and its HAB dynamic under natural conditions.

MATERIALS AND METHODS

Field survey

Seawater samples were collected at two routine stations (A and B; sampled on 5, 7, 10, 12, 14, and 18 September 2012) and at six temporary stations (T1–T6; sampled when seawater become discolored from 5 to 14 September 2012) in the inner part of the Ariake Sea, Japan (Fig. 1). About 2 l seawater was collected from depths of 0, 2, and (depth permitting) 5 m with a Niskin water sampler (Model 1080, Rigo, Saitama, Japan), and vertical profiles of water temperature and salinity were measured with a Compact–CTD recorder (Model ASTD687, ALEC Electronics Co., Ltd., Kobe, Japan). Samples were brought to the laboratory within 4 h and used for subsequent experiments. For phytoplankton counting, each bottle was gently turned upside down five times before subsamples were taken. Vegetative cells were counted under a microscope in triplicate 0.1 ml subsamples. For macronutrient analysis, 50 ml subsamples were gravity–filtered through glass microfiber filters (GF/C, Whatman International Ltd., Maidstone, UK). Filtrates were then passed through 0.22–μm syringe filters and frozen at –80°C until analysis. Dissolved inorganic nitrogen (DIN: NO_2^-, NO_3^-, and NH_4^+) and dissolved inorganic phosphorus (DIP: PO_4^{3-}) were measured with an Autoanalyzer (TRACCS 2000; Bran + Luebbe, Hamburg, Germany).

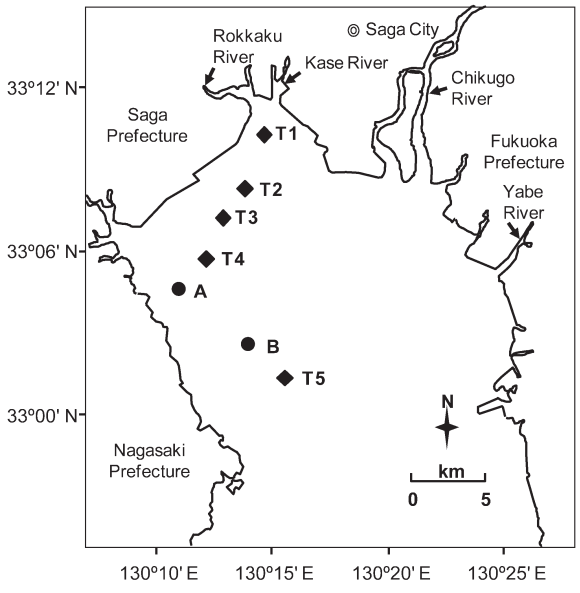

Fig. 1. The sampling stations in the Ariake Sea, Japan. Circles indicate the two routine stations: A (33°04.715'N, 130° 10.885'E) and B (33°02.700'N, 130°14.261'E); Diamonds indicate the five temporary sampling stations: T1 (33° 10.571'N, 130°14.218'E), T2 (33°07.963'N, 130°13.436'E), T3 (33°07.157'N, 130°12.886'E), T4 (33°04.813'N, 130°11.737'E), and T5 (33°01.165'N, 130°15.971'E).

Determination of the potential maximum quantum yield of photosystem II (F_v/F_m ratio)

The F_v/F_m ratio of seawater samples were used as the photosynthetic activity indicator and determined on the sampling day. To avoid the potential impact of elevated irradiance on F_v/F_m ratio during sampling, we kept samples under weak natural light (<10 μmol photons m^{-2} s^{-1}) during transport (3 to 4 h) and placed them under 110 ± 10 μmol photons m^{-2} s^{-1} for 1 h in the laboratory before taking subsamples. Subsequently, 1.5 ml subsamples were kept in darkness for at least 30 min and then assayed with a Xe–PAM fluorometer (H. Walz, Effeltrich, Germany), as described by Qiu *et al*. (2013).

Determination of growth rate of *C. marina*

To determine the growth rate of *C. marina* cells, triplicate 25 ml subsamples were transferred into 70–ml sterile flasks (Nunc, Thermo Fisher Scientific Inc., Suwanee, GA, USA) on the sampling day (defined as day 1), and algae were grown in an incubator at 27.5°C under cool–white fluorescent illumination (110 ± 10 µmol photons m^{-2} s^{-1}) at a 14:10 h light:dark cycle for 3 days. Phytoplankton cells were counted under a microscope in triplicate 0.1 ml subsamples on the day 3, and the growth rate (GR, div. d^{-1}) was determined as $GR = \ln(N_3/N_1) / 2\ln(2)$, where N_1 and N_3 are cell densities on days 1 and 3 (Guillard, 1973).

Statistical analysis

Simple linear regressions were used to fit the relationship between the F_v/F_m ratio and the growth rate of *C. marina* cells. Spearman's rank correlation coefficient (r_s) was used to examine (1) temporal variations in F_v/F_m and growth rate, (2) correlations of each environmental factor (temperature, salinity, DIN, and DIP) with F_v/F_m ratio and with growth rate of *C. marina*. All statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS 11.0; SPSS, Inc., Chicago, IL, USA).

RESULTS

Field observation

A total of 51 seawater samples were collected. *Chattonella marina* cells were dominant (≥300 cells ml^{-1}) in 29 samples collected from 5 to 14 September 2012 and then disappeared on 18 September, 2012 (Fig. 2A). The peak density was observed at 9.4×10^3 cells ml^{-1} in station T4 on 7 September, 2012. *Skeletonema* spp. were the most frequent accompanying species; they gradually increased from 10 September and became dominant on 18 September (Fig. 2B). There were small variations in water temperature (26.2–30.7°C) and salinity (19.0–28.9); the water temperature gradually decreased and salinity gradually increased from 7 September (Fig. 2C and D). The DIN concentrations recorded from 5 to 14 September were generally <2.8 μM (except for one sample on 10 September), and those on 12 and 14 September were <1.0 μM (Fig. 2E). The DIP concentrations ranged from 0.20 to 2.45 μM and gradually increased after 10 September (Fig. 2F). In addition, the abundance of *Gyrodinium dominans*, which is known as predator of *Chattonella* (Nakamura *et al*., 1992), was ≤66 cells ml^{-1} (data not shown).

Variations in the F_v/F_m ratio and growth rate of *C. marina* during the HAB

The F_v/F_m ratio of seawater samples were considered to be contributed by *C. marina* cells only when they dominated the phytoplankton community (i.e. ≥300 cells ml^{-1}; N = 29), which were used for the further correlation analysis. The F_v/F_m ratio and growth rate of *C. marina* cells ranged from 0.62 to 0.72 and ranged from –0.25 to 0.81 div. d^{-1}, respectively (Fig. 3A and B). As the bloom progressed, there were significant decreases in both the F_v/F_m ratio (Spearman rank correlation, r_s = –0.67, N = 29, $P < 0.01$) and growth rate (Spearman rank correlation, r_s = –0.77, N = 29, $P < 0.01$). There was a significant positive correlation between F_v/F_m ratio and growth rate (Linear regression, $r^2 = 0.32$, $F_{1,28} = 12.62$, $P < 0.01$; Fig. 3C). The DIN concentration was significantly correlated with both F_v/F_m ratio (Spearman rank correlation, r_s = 0.57, N = 29, $P < 0.01$) and growth rate (Spearman rank correlation, r_s = 0.43, N = 29, $P < 0.05$) of *C. marina* cells (Table 1). Growth rate was also significantly but weakly correlated with water temperature (Spearman rank correlation, r_s = 0.39, N = 29, $P < 0.05$) and salinity (Spearman rank correlation, r_s = –0.44, N = 29, $P < 0.05$).

DISCUSSION

Observation on environmental variables suggested that the macronutrient concentrations play critical roles in promoting the bloom dynamics of *C. marina* during the current field survey (Fig. 2). Yamatogi *et al*. (2006) reported that *C. marina* isolated from the Ariake sea has potential to rapidly grow (>0.7 div. d^{-1}) at the temperatures of 25–32°C and salinities of 20–36, suggesting that the water temperature and salinity during the current field survey (Fig. 2 C and D) are suitable for the growth of *C. marina*. In contrast, Nakamura *et al*. (1988)

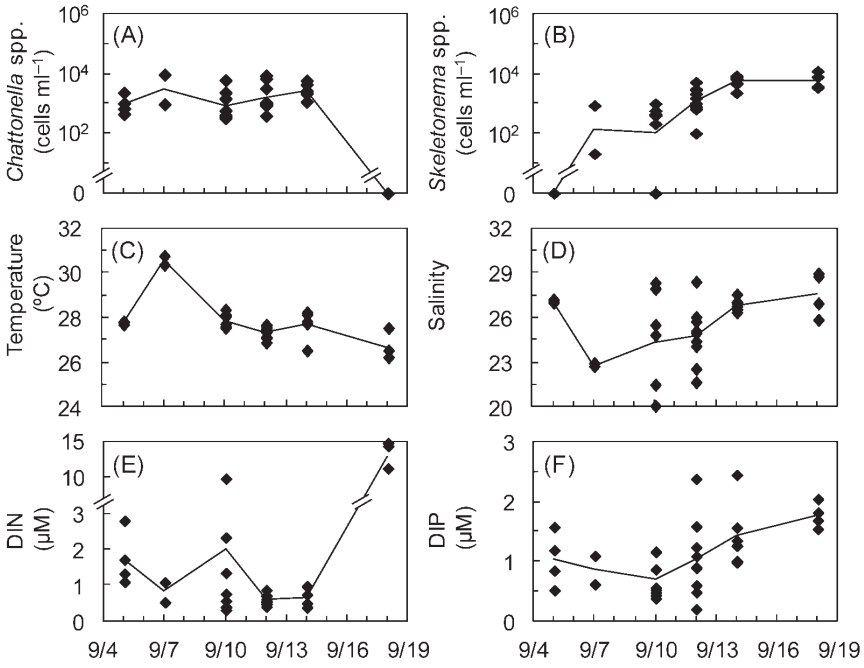

Fig. 2. Temporal variations in the (A) abundance of *Chattonella marina*, (B) abundance of *Skeletonema* spp., (C) water temperature, (D) water salinity, (E) dissolved inorganic nitrogen (DIN), and (F) dissolved inorganic phosphorus (DIP) during the field survey in the Ariake Sea, Japan, from 5 to 18 September 2012. Data are shown only when *C. marina* was dominant. Symbols (◆) indicate values for each station, and lines indicate the average values of all stations on the same sampling day.

reported that the half–saturation constants for the growth of *C. marina* var. *antiqua* for nitrate and phosphate are 1.0 μM and 0.11 μM, respectively. From the aspect of the half–saturation constants, exhaustion of DIN (declined to <1.0 μM on 12 and 14 September, Fig. 2E), compared with the DIP concentrations (0.20–2.45 μM from 5 to 18 September, Fig. 2F), were likely responsible for controlling the growth of *C. marina* cells during the bloom. Indeed, the growth rate of *Chattonella* cells decreased to <0 div. d^{-1} on 14 September, and thus the bloom had reached its terminal stage, because a field population with a low or negative growth rate will be rapidly dissipated by various processes (Nakamura *et al*. 1992; Imai *et al*. 2001; Vargo *et al*. 2009).

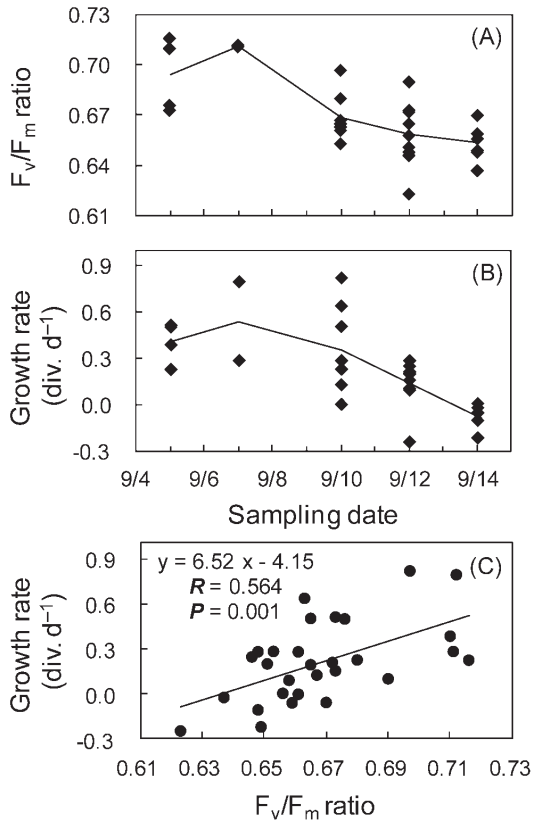

Fig. 3. Temporal variations in (A) F_v/F_m ratio and (B) growth of *Chattonella marina* cells in seawater samples collected when they dominated the phytoplankton community, as well as (C) the relationship between the above two parameters. In (A) and (B), symbols (◆)indicate values for each station, and lines indicate the average values of all stations on the same sampling day. In (C), *R* indicates Pearson's correlation coefficient (N = 29) and *P* indicates the significance of the correlation.

Both the F_v/F_m ratio and growth rate of *C. marina* cells tended to decrease as the bloom progressed (Fig. 3A and B), and there was a significant positive correlation between the two parameters (Fig. 3C). This relationship is consistent with observations of batch cultures of *C. marina* var. *antiqua* under a variety of culture conditions (Qiu *et al*., 2013). The F_v/F_m ratio has been widely used to indicate the degree of potential photosynthetic competence and adverse effects of photoinhibition and nutrient stress on phytoplankton in single–species laboratory cultures (e.g., Bergmann *et al*., 2002; Parkhill *et al*., 2002; Warner and Madden, 2007; Qi *et al*., 2013). Because there are distinct interspecies differences in the responses of the F_v/F_m ratio to nutrient limitation and its correlation with phytoplankton growth rates, several recent studies have questioned the reliability of this ratio as a robust diagnostic of the nutritional status and growth rate of mixed or uncharacterized field populations (e.g., Kruskopf and Flynn, 2006; Wang *et al*., 2011). However, those studies also suggested that the F_v/F_m ratio may be used to determine either nutrient status or relative growth rates of phytoplankton when studying single species, or blooms dominated by one species (Kruskopf and Flynn, 2006). Therefore, the F_v/F_m ratio may serve as an effective parameter for evaluating the physiological status and growth potential of field *C. marina* population, because this species generally has a competitive advantage over other algae and form monospecific blooms in the field (e.g., Mikhail, 2007; Katano *et al*., 2012; Qiu *et al*., 2014). Furthermore, both F_v/F_m ratio and the growth rate and F_v/F_m ratio of *C. marina* cells were positively correlated with DIN concentrations (Table 1). This result also supported the inference that DIN was responsible for controlling the dynamics *C. marina* HAB, and suggested that the F_v/F_m ratio may also be used to indicate their nutritional status during a monospecific bloom.

To our knowledge, this is the first report that simultaneously determined the F_v/F_m ratio and growth rate of phytoplankton in field samples. Our results indicated that the F_v/F_m ratio is potentially useful for reflecting growth rate of *C. marina* during its blooms and may provide information useful for predicting bloom dynamics. As both the F_v/F_m ratio and algal growth are influenced in nature by the independent or combined action

Table 1. Correlations of F_v/F_m ratio and growth rate of *Chattonella marina* cells in seawater samples collected during the field bloom (5–14 September 2012) with environmental variables

		Dissolved inorganic nitrogen	Dissolved inorganic phosphorus	Temperature	Salinity
F_v/F_m ratio	Coefficient	**0.568**	–0.225	0.164	–0.074
	P–value	0.001	0.241	0.397	0.701
Growth rate	Coefficient	**0.433**	–0.252	**0.386**	**–0.443**
	P–value	0.019	0.188	0.039	0.016

Correlations were analyzed by Spearman's rank correlation (N = 29). Values in bold are significant ($P < 0.05$)

of various environmental factors, the evaluation of the growth potential of *C. marina* based on the F_v/F_m ratio should be combined with those factors. On the other hand, little is known about the molecular mechanisms involved in the regulation of the F_v/F_m ratio in *Chattonella*. The decreased expression levels of two proteins, oxygen–evolving enhancer 1 (part of the oxygen–evolving complex of photosystem Ⅱ) and 2–cysteine peroxiredoxin (component of a H_2O_2–scavenging system), in batch cultures of *C. marina* var. *antiqua* have been suggested to contribute to the reduction in the F_v/F_m ratio and cell tolerance to unfavorable growth conditions (Qiu *et al*., 2013). Clarification of the molecular mechanisms that regulate the photosynthetic activity may improve the reliability of the F_v/F_m ratio as an indicator of *C. marina* growth potential.

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