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Evaluation of the ETR_{max} in Microalgae Using the PHYTO-PAM Fluorometer

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In this study, the PHYTO-PAM-fluorometric method was used to evaluate the ETR_{max} in terms of sensitivity to DIN/DIP against 14 microalgae: *Prorocentrum micans*, *Heterocapsa triquetra*, *Gymnodinium impudicum*, *Gymnodinium catenatum*, *Amphidinium caterae*, *Chlorella vulgaris*, *Chroococcus minutus*, *Microcystis aeruginosa*, *Chlorella ellipsoidea*, *Nannochloris oculata*, *Oocystis lacustris*, *Chroomonas salina*, *Gloeocystis gigas*, and *Prymnessium parvum*. We found that *P. micans*, *H. triquetra*, and *A. caterae* exposed to the maximum level of DIN/DIP were significantly smaller in the ETR_{max} than that of the minimum and moderate mixture. Unlikely the ETR_{max}, the initial slope alpha was not significantly different at the level of 60 DIN/DIP. In *G. catenatum*, the moderate levels of 15 and 20 in DIN/DIP were found to be significantly different from the ETR_{max} at Ch1-Ch4. *Gymnodinium impudicum* had a higher value than that of the ETR_{max} than that of dinoflagellates used in this study, ranging from 306.1 (Ch4, DIN/DIP: 10) to 520.1 (Ch4, DIN/DIP: 30). The ETR_{max} value obtained from other microalgae was similar to *G. impudicum* at any of the ratios of DIN/DIP and channels. Consequently, the influence of offshore water current assures us of the suppression of photosynthesis and electron transport rate in dinoflagellates. *Gymnodinium impudicum* has not been researched in the area of red tides in Korea, but it will be enough to creat the massive algal blooms in the future because of higher potential photochemical availability.

Key Words : PHYTO-PAM fluorometer, ETR_{max}, Microalgae, Photochemical efficiency, DIN/DIP, Photosynthesis

1. 서 론

The absorption of light by chlorophyll causes its conversion to a highly unstable excited state. Chlorophyll fluorescence can be used as a tool to determine both the maximal and the effective efficiency of photo usage in photochemistry[1~3]. One of features of the PHYTO-PAM phytoplankton analyzer (Heinz Walz GmbH, Effeltrich, Germany) measures the fluorescence emitted from the chlorophylls of PS II, reflecting the efficiency of photochemical energy conversion of a PS II reaction center[4,5]. The PHYTO-PAM provides a powerful tool to discriminate cyanobacteria, green algae and diatoms because of 4 different excitation wavelengths[4,6]. Moreover, the application of the PHYTO-PAM to assessing phytoplankton community structure (i.e., productivity and composition) and chemistry in an aquatic environment was tested[5,7,8]. Consequently, the introduction of the PHYTO-PAM measuring technique with a compact and portable fluorometer became available, which contributed to many practical applications[9~12].

Possibly, much of the ambient nutrients flux, continuous remineralisation, rapid recycling of dissolved and particulate organic matters, and desirable temperature in marine systems may play an important role in the outbreak of dinoflagellates. It has long been known that nitrogen and phosphorus sources play an important role in limiting physiological characteristics and metabolic activities in phytoplankton growth in oceanic and coastal waters[13,14]. It has become increasingly apparent that the flux of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) may be directed to the growth of dinoflagellates in nature. Consequently, it is hypothesized that the

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desirable combination of the ratio of DIN/DIP can be associated with dramatic fluctuations in molecular and biochemical activities.

We carefully investigated the sensitivity of some species of Dinophyceae, Chlorophyceae, Cyanophyceae, and Haptophyceae to the inhibitory effects of DIN/DIP in cultures using the PHYTO-PAM fluorometer.

Materials and Methods

Cultures

A total of 14 microalgae were obtained from Korea Marine Microalgae Culture Center, Pukyong University, Busan, Korea (Table 1). More detail, Dinophyceae (*Prorocentrum micans*, *Heterocapsa triquetra*, *Gymnodinium impudicum*, *Gymnodinium catenatum*, and *Amphidinium caterae*), Chlorophyceae (*Nannochloris oculata*, *Gloeocystis gigas*, *Chlorella vulgaris*, *Chlorella ellipsoidea*, *Oocystis lacustris*, and *Chroomonas salina*), Cyanophyceae (*Chroococcus minutus*, and *Microsystis aeruginosa*), Haptophyceae (*Prymnessium parvum*) were tested. *M. aeruginosa* and *C. ellipsoidea* were isolated from freshwater, whereas *C. minutus* and *O. lacustris* were collected from estuary waters. The organism were grown in f/2-Si medium[15] at 20℃ under 50 μmol m^{-2} s^{-1} light intensity with 12L:12D cycle and maintained in exponential growth phase by serial transfers of a constant volume of inoculum to fresh medium once 25 days until required.

DIN/DIP

The N/P ratio was carried out in seven different levels (5, 10, 15, 20, 30, 40, 60) for the N ($NaNO_3$) and P sources ($NaH_2PO_4 \cdot H_2O$) with the supplement of f/2-Si medium[15]. Microalgae were exposed to 50 ml container (Nalgen) for 10 days at each combination of DIN and DIP under above-mentioned culture conditions. These containers were cultured in triplicate. Final concentration of Channel (Ch)l was adjusted to approximately 50 μg l^{-1} based on Chl fluorescence analysis

Analysis

Chl fluorescence was measured with the PHYTO-PAM (Walz, Germany) equipped with a standard 10 × 10 mm quartz cuvette. For excitation of Chl fluorescence the PHYTO-PAM applies an array of four different types of light emitting diodes (LED) peaking at 470, 535, 620, and 650 nm. The PHYTO-PAM was operated in conjunction with a notebook personal computer and PhytoWin software provided with the instrument. Control determined the filtrate of a sample that passed through a 0.45 μm filter retaining all microalgae. The parameters of the maximum electron transport rate (ETR_{max}) and initial slope alpha were provided for opening a window listing the Curve Fit Parameters for Ch1-Ch4. The ETR is given in relative units as calculated by the fluorometer software. The relative units are proportional to μmol electrons m^{-2}

Table 1. Microalgae used in this study

Species	Strain No.	Location	Time
Prorocentrum micans	D-008	Busan	none[1]
Heterocapsa triquetra	D-009	Yeosu	December, 1998
Gymnodinium impudicum	D-085	Narodo	none[1]
Gymnodinium catenatum	D-099	Yellow Sea	none[1]
Amphidinium caterae	D-019	Yoido	March, 1999
Chlorella vulgaris	EC-003	Hwajinpo	July, 1995
Chroococcus minutus	CY-042	Busan	August, 1995
Microcystis aeruginosa	FC-070	Nakdong river	August, 1995
Chlorella ellipsoidea	FC-006	none[2]	none[2]
Nannochloris oculata	C-031	none[2]	none[2]
Oocystis lacustris	EC-016	Busan	September, 1999
Chroomonas salina	CR-002	Haeundae	May, 1997
Gloeocystis gigas	C-133	Yeosu	April, 1998
Prymnessium parvum	H-20	Jejudo	March, 1996

Note: [1] means no registration of sampling time in KMCC (Korea Marine Microalgae Culture Center), [2] represents to provide the sample from UTEX

s^{-1}. ETR_{max} was calculated as:

$$ETR\ (\mu mol\ electrons\ g^{-1}\ Chl\ a \cdot S^{-1}) = \frac{PAR}{a^{*}\ PAR^{2} + b^{*}\ PAR + c}$$

where, a, b, c is the least square deviation and PAR (μmol quanta m^{-2} s^{-1}) is a scalar irradiance. Multiple Duncan test was performed with SPSS ver 10.0 software at a significance level of $p<0.05$.

Results

The ETR_{max}, initial slope alpha, and r^2 of rapid light curves of microalgae using the PHYTO-PAM exposed to different ratio of DIN/DIP in cultures (Table 2). In dinoflagellates, the cells of *P. micans*, *H. triquetra*, and *A. caterae* exposed to the maximum level of DIN/DIP in cultures were found to have the ETR_{max} which was significantly (Duncan Multiple test, $p<0.05$) smaller than that of the minimum and moderate mixture. Subsequently, the values of r^2 were shown to be somewhat lower. In contrast to the ETR_{max}, the initial slope alpha was not significantly different, although the cells were cultured to the value of 60 with DIN/DIP. Interestingly, *G. impudicum* had a higher value of the ETR_{max} than that of dinoflagellates used in this study. The ETR_{max} in *G. impudicum* ranged from 306.1 (Ch4, DIN/DIP: 10) to 520.1 (Ch4, DIN/DIP: 30). In particular, the cell showed higher levels of the ETR_{max} and r^2 than that of Ch and the mixture of DIN/DIP. *Gymnodinium catenatum* had a somewhat different value of the ETR_{max} against *P. micans*, *H. triquetra*, and *A. caterae*. Those cells had significantly different signs of the ETR_{max} exposed to 60 of DIN/DIP, whereas *G. catenatum* did not show anything significant in this regard. The moderate levels of 15 and 20 in DIN/DIP were shown to be significantly different from the ETR_{max} at Ch1-Ch4, compared with any combination. The ETR_{max} from *C. vulgaris*, *C. minutus*, *M. aeruginosa*, *C. ellipsoidea*, *N. oculata*, *O. lacustris*, *C. salina*, *G. gigas*, and *P. parvum* was relatively similar to *G. impudicum* at any of the ratios of DIN/DIP and channels. On the basis of statistical analysis, a comparison of those cells and *G. impudicum* among dinoflagellates did not reveal any significant differences, but other dinoflagellates were extremely separated from those cells. The contribution to variance of the ETR_{max} from DIN/DIP was remarkably higher depending on microalgae than that of initial slope alpha, which was not significantly different.

Discussion

With the fluctuation of the ratio of DIN/DIP in culture, tested dinoflagellates showed a suppression of photochemical efficiency and oxygen availability. Our study clearly suggested that N-deficient and P-deficient nutrient mediums should be attained to significantly decrease the relative ETR_{max} for *P. micans*, *H. triquetra*, *G. catenatum*, and *A. caterae*. Rapid light curves which were used to provide information on the inhibition of the photosynthesis were also remarkably decreased for testing dinoflagellates. The reduction of the ETR_{max} is associated with the inhibition of PSII activity rather than the dark reactions of photosynthesis[15]. Consequently, the effect of the lack of nitrogen and phosphorus sources on photosynthetic activity is caused indirectly so that uptake efficiency is lower. Therefore, it becomes obvious that photochemical yield from electron transport gives an indication of the DIN/DIP effect. *Prorocentrum micans*, *H. triquetra*, *G. catenatum*, and *A. caterae* are highly dependent and sensitive to inhibitory photochemical availability according to the effect of DIN/DIP.

In a comparison of the ETR_{max} of different species of dinoflagellates, *G. impudicum* under the influence of DIN/DIP showed a considerable difference. In this study, the fluctuations of the ETR_{max} in *G. impudicum* with the effect of DIN/DIP were not found at any of channels (Table 2). *Gymnodinium impudicum* was found at the similar ETR_{max} with cyanobacterial species. In particular, the correlations with rapid light curves and corresponding photo intensity were sharply and lineally increased. It is assumed that *G. impudicum* has a physiological efficiency to adapt and use higher irradiance without any photo inhibition response. It is suggested that *G. impudicum* should be regarded as one of the most photochemically active dinoflagellates. Lee et al[16]. reported that the growth response of *G. impudicum* to different combinations of temperature, salinity, irradiance and nutrients showed euryhalic and higher characteristics. This indicates that the results from growth experiments in cultures somewhat agreed with our present study using the PHYTO-PAM flurometer.

Although *G. impudicum* has a remarkable capability

Table 2. Multiple comparison of the ETR_{max}, initial slope alpha, and r^2 of rapid light curves of microalgae using PHYTO-PAM for 10 days after inoculation. The PHYTO-PAM uses 4 different excitation wavelengths, which peak at 470 nm (Ch1), 535 nm (Ch2), 620 nm (Ch3), and 650 nm (Ch4). 14 species of microalgae were grown at different ratio of DIN/DIP under the light intensity of 50 μmol m^{-2} s^{-1} at 20℃ in L:D cycle of 12:12. Chl concentrations of the culture was adjusted to approximately 50 μg l^{-1} obtained from the calculation by Chl fluorescence analysis. Values are mean ± SE (n=3). Means sharing the same letter are not significantly different (p<0.05, Duncan test). Ch, channel

Microalgae	N/P	ETR_{max} (μmol electrons g^{-1} Chl a s^{-1})				Alpha				r^2
		Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4	
Prorocentrum micans	5	137.6±15.7^{a}	71.3±10.9^{a}	130.2±22.1^{a}	64.9±9.7^{a}	0.23±0.04^{d}	0.37±0.07^{d}	0.50±0.02^{d}	0.39±0.02^{d}	0.911
	10	66.2±11.9^{a}	83.3±13.1^{a}	133.3±10.9^{a}	147.5±20.4^{a}	0.28±0.01^{d}	0.56±0.02^{d}	0.21±0.02^{d}	0.53±0.04^{d}	0.745
	15	46.8±10.9^{b}	70.1±12.3^{a}	72.4±10.3^{a}	161.5±19.3^{a}	0.55±0.04^{d}	0.43±0.03^{d}	0.69±0.05^{d}	0.67±0.05^{d}	0.803
	20	203.6±15.3^{a}	155.4±19.3^{a}	235.1±10.1^{a}	222.0±11.9^{a}	0.46±0.03^{d}	0.43±0.01^{d}	0.36±0.06^{d}	0.40±0.05^{d}	0.982
	30	105.4±10.7^{a}	197.3±19.1^{a}	170.0±11.0^{a}	154.6±12.1^{a}	0.35±0.03^{d}	0.38±0.06^{d}	0.30±0.04^{d}	0.36±0.02^{d}	0.760
	40	196.2±11.4^{a}	225.4±11.8^{a}	216.4±12.9^{a}	202.0±15.7^{a}	0.46±0.01^{d}	0.40±0.08^{d}	0.29±0.03^{d}	0.30±0.03^{d}	0.895
	60	79.1±10.8^{b}	64.2±15.4^{b}	67.3±15.2^{b}	93.3±13.3^{b}	0.58±0.05^{d}	0.62±0.01^{d}	0.59±0.05^{d}	0.51±0.05^{d}	0.435
Heterocapsa triquetra	5	66.0±9.5^{b}	55.6±10.4^{b}	51.9±10.0^{b}	180.7±21.4^{a}	0.21±0.02^{d}	0.28±0.03^{d}	0.26±0.03^{d}	0.27±0.01^{d}	0.105
	10	226.6±19.2^{a}	327.4±21.4^{c}	187.5±15.2^{a}	396.1±17.0^{c}	0.62±0.08^{d}	0.26±0.04^{d}	0.48±0.02^{d}	0.20±0.06^{d}	0.950
	15	126.1±9.7^{a}	151.8±10.7^{a}	137.3±15.1^{a}	131.9±10.3^{a}	0.57±0.02^{d}	0.48±0.05^{d}	0.76±0.08^{d}	0.55±0.02^{d}	0.639
	20	150.1±10.4^{a}	215.6±15.4^{a}	147.8±14.0^{a}	151.2±13.2^{a}	0.48±0.08^{d}	0.53±0.03^{d}	0.20±0.01^{d}	0.28±0.04^{d}	0.999
	30	163.4±12.1^{a}	127.4±11.4^{a}	162.5±15.6^{a}	273.1±14.9^{c}	0.35±0.05^{d}	0.39±0.05^{d}	0.28±0.02^{d}	0.20±0.03^{d}	0.814
	40	115.2±14.7^{a}	116.5±17.7^{a}	114.3±10.7^{a}	213.4±13.7^{a}	0.27±0.04^{d}	0.54±0.09^{d}	0.67±0.06^{d}	0.49±0.05^{d}	0.887
	60	24.3±15.5^{b}	72.5±10.9^{b}	14.9±12.4^{b}	38.9±10.7^{b}	0.21±0.01^{d}	0.20±0.02^{d}	0.37±0.07^{d}	0.54±0.06^{d}	0.661
Gymnodinium impudicum	5	331.7±10.7^{c}	392.2±19.3^{c}	357.1±15.9^{c}	412.1±12.7^{c}	0.41±0.07^{d}	0.46±0.04^{d}	0.50±0.04^{d}	0.52±0.07^{d}	0.929
	10	357.6±15.4^{c}	375.0±10.9^{c}	377.7±17.9^{c}	306.1±10.4^{c}	0.39±0.05^{d}	0.22±0.01^{d}	0.27±0.03^{d}	0.31±0.02^{d}	0.979
	15	387.8±10.1^{c}	308.8±13.9^{c}	436.4±15.7^{c}	459.6±10.7^{c}	0.71±0.04^{d}	0.37±0.06^{d}	0.35±0.02^{d}	0.71±0.05^{d}	0.980
	20	453.7±13.4^{c}	498.8±10.8^{c}	498.7±12.9^{c}	486.3±19.7^{c}	0.23±0.07^{d}	0.35±0.05^{d}	0.35±0.02^{d}	0.41±0.03^{d}	0.947
	30	437.0±11.9^{c}	444.0±15.5^{c}	479.9±12.0^{c}	520.1±10.9^{c}	0.22±0.04^{d}	0.25±0.07^{d}	0.27±0.08^{d}	0.30±0.03^{d}	0.924
	40	414.7±11.3^{c}	435.7±12.9^{c}	479.4±10.2^{c}	457.2±14.2^{c}	0.35±0.01^{d}	0.39±0.04^{d}	0.41±0.04^{d}	0.49±0.04^{d}	0.931
	60	420.4±15.4^{c}	457.4±10.7^{c}	492.1±18.7^{c}	453.4±13.9^{c}	0.43±0.02^{d}	0.44±0.02^{d}	0.43±0.07^{d}	0.55±0.01^{d}	0.862

Table 2. continued

Microalgae	N/P	ETR_{max} (μmol electrons g^{-1} Chl *a* s^{-1})				Alpha				r^2
		Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4	
Gymnodinium catenatum	5	155.2±18.4^a	30.4±9.7^b	18.3±12.2^b	154.9±16.7^a	0.95±0.01^d	0.89±0.05^d	0.55±0.02^d	0.20±0.05^d	0.180
	10	113.2±13.4^a	127.1±10.4^a	125.0±16.4^a	141.1±12.2^a	0.24±0.03^d	0.71±0.01^d	0.79±0.03^d	0.42±0.03^d	0.953
	15	330.8±19.4^c	16.1±6.4^b	23.1±12.1^b	16.2±10.3^b	0.67±0.06^d	0.67±0.04^d	0.39±0.02^d	0.55±0.02^d	0.926
	20	16.0±10.1^b	126.3±15.7^a	56.1±13.9^b	23.1±12.1^b	0.69±0.04^d	0.84±0.02^d	0.54±0.06^d	0.75±0.02^d	0.762
	30	105.2±15.9^a	20.0±10.7^b	25.4±10.4^b	74.6±9.7^a	0.49±0.07^d	0.21±0.03^d	0.83±0.05^d	0.89±0.03^d	0.911
	40	173.2±11.4^a	59.5±13.7^a	71.0±9.7^a	160.4±15.7^a	0.29±0.05^d	0.36±0.04^d	0.47±0.05^d	0.38±0.04^d	0.850
	60	154.8±15.7^a	91.7±12.9^a	41.3±10.6^b	182.1±20.4^a	0.42±0.04^d	0.34±0.05^d	0.65±0.04^d	0.23±0.06^d	0.904
Amphidinium caterae	5	42.8±14.3^b	152.8±19.9a	154.1±12.6^a	174.5±16.7^a	0.68±0.01^d	0.81±0.03^d	0.20±0.08^d	0.25±0.09^d	0.915
	10	254.2±15.5^a	46.9±15.9^b	42.6±10.3^b	38.7±12.2^b	0.56±0.03^d	0.88±0.01^d	0.30±0.04^d	0.49±0.05^d	0.849
	15	104.8±15.9^a	48.8±16.0^b	298.5±26.7^c	39.8±10.8^b	0.27±0.02^d	0.38±0.03^d	0.44±0.04^d	0.36±0.05^d	0.534
	20	288.0±12.4^c	227.9±15.7^a	157.8±13.2^a	206.8±12.4^a	0.32±0.06^d	0.28±0.02^d	0.33±0.06^d	0.32±0.04^d	0.764
	30	66.9±10.7^a	74.5±13.6^a	111.5±10.5^a	69.1±15.7^a	0.22±0.02^d	0.35±0.03^d	0.87±0.02^d	0.36±0.06^d	0.970
	40	121.1±19.7^a	108.2±19.4^a	139.8±13.7^a	129.6±10.4^a	0.84±0.05^d	0.84±0.05^d	0.74±0.05^d	0.82±0.02^d	0.595
	60	41.5±10.4^b	40.3±15.4^b	48.4±10.2^b	46.4±11.1^b	0.93±0.06^d	0.22±0.01^d	0.46±0.01^d	0.50±0.03^d	0.992
Chlorella vulgaris	5	361.1±12.4^c	260.4±10.4^c	343.7±15.9^c	494.6±14.9^d	0.88±0.05^d	0.35±0.02^d	0.28±0.06^d	0.47±0.04^d	0.808
	10	495.6±12.8^c	396.7±12.1^c	485.5±15.4^c	246.6±16.4^c	0.23±0.04^d	0.40±0.05^d	0.40±0.03^d	0.4210.04^d	0.917
	15	308.5±15.9^c	464.1±13.2^c	403.9±10.1^c	345.8±10.5^c	0.35±0.02^d	0.50±0.02^d	0.50±0.02^d	0.41±0.01^d	0.884
	20	401.2±11.4^c	455.8±16.7^c	416.6±10.9^c	243.3±10.7^c	0.34±0.05^d	0.39±0.03^d	0.48±0.03^d	0.43±0.05^d	0.705
	30	415.8±10.8^c	255.2±11.6^c	316.0±16.6^c	358.5±12.4^c	0.52±0.06^d	0.61±0.01^d	0.62±0.06^d	0.77±0.06^d	0.692
	40	222.6±10.4^c	312.7±15.1^c	346.2±10.7^c	313.3±14.2^d	0.63±0.02^d	0.59±0.04^d	0.65±0.08^d	0.71±0.03^d	0.892
	60	290.0±12.4^c	378.4±10.9^c	418.3±16.9^c	361.1±19.7^c	0.61±0.05^d	0.67±0.05^d	0.63±0.02^d	0.78±0.05^d	0.794
Chroococcus minutus	5	485.5±19.7^c	486.2±16.7^c	467.1±17.8^c	461.8±13.5^c	0.92±0.01^d	0.99±0.07^d	057±0.04^d	0.89±0.01^d	0.965
	10	488.1±15.9^c	349.9±15.1^c	264.9±12.1^c	361.5±16.4^c	0.55±0.06^d	0.49±0.08^d	0.52±0.06^d	0.53±0.04^d	0.435
	15	409.0±13.1^c	399.4±13.9^c	425.8±19.7^c	411.9±15.1^c	0.27±0.02^d	0.24±0.02^d	0.28±0.02^d	0.28±0.06^d	0.935
	20	360.8±10.7^c	242.9±15.7^c	325.9±15.8^c	241.7±19.1^c	0.28±0.01^d	0.23±0.03^d	0.29±0.05^d	0.31±0.04^d	0.855
	30	480.1±19.9^c	345.7±12.7^c	278.3±13.1^c	388.6±12.7^c	0.26±0.03^d	0.31±0.04^d	0.31±0.04^d	0.39±0.02^d	0.842
	40	399.0±10.7^c	451.1±19.9^c	467.5±13.6^c	417.5±16.7^c	0.58±0.01^d	0.57±0.01^d	0.56±0.04^d	0.62±0.05^d	0.919
	60	477.8±15.7^c	424.7±10.1^c	375.0±13.1^c	429.3±15.1^c	0.45±0.05^d	0.26±0.03^d	0.73±0.06^d	0.30±0.07^d	0.897

Table 2. continued

Microalgae	N/P	ETR_{max} (μmol electrons g^{-1} Chl *a* s^{-1})				Alpha				r^2
		Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4	
Microcystis aeruginosa	5	298.5±15.7^{c}	318.4±14.7^{c}	399.4±6.7^{d}	278.1±13.5^{c}	0.45±0.02^{d}	0.67±0.05^{d}	0.64±0.05^{d}	0.55±0.04^{d}	0.914
	10	311.7±12.1^{c}	422.7±13.4^{c}	380.7±13.1^{c}	526.2±10.1^{c}	0.41±0.03^{d}	0.48±0.04^{d}	0.47±0.04^{d}	0.42±0.06^{d}	0.620
	15	347.6±14.1^{c}	361.9±13.7^{c}	365.7±10.6^{c}	331.3±14.4^{c}	0.73±0.01^{d}	0.90±0.06^{d}	0.72±0.06^{d}	0.21±0.04^{d}	0.832
	20	442.6±13.4^{c}	422.7±15.4^{c}	417.8±15.4^{c}	377.3±13.7^{c}	0.59±0.08^{d}	0.55±0.04^{d}	0.77±0.03^{d}	0.48±0.07^{d}	0.952
	30	301.8±15.1^{c}	363.1±10.5^{c}	319.6±16.9^{c}	313.0±12.4^{c}	0.70±0.04^{d}	0.64±0.01^{d}	0.79±0.02^{d}	0.75±0.04^{d}	0.792
	40	373.9±18.4^{c}	393.5±11.9^{c}	442.4±10.1^{c}	415.1±10.4^{c}	0.57±0.05^{d}	0.62±0.03^{d}	0.59±0.08^{d}	0.65±0.05^{d}	0.882
	60	443.8±14.6^{c}	455.1±19.7^{c}	476.1±13.1^{c}	381.0±15.7^{c}	0.54±0.02^{d}	0.50±0.08^{d}	0.53±0.06^{d}	0.68±0.06^{d}	0.692
Chlorella ellipsoidea	5	323.5±13.1^{c}	307.6±15.9^{c}	335.2±11.4^{c}	298.6±12.4^{c}	0.50±0.05^{d}	0.56±0.07^{d}	0.53±0.09^{d}	0.59±0.04^{d}	0.785
	10	373.0±12.4^{c}	299.6±12.1^{c}	523.6±10.8^{c}	280.4±10.4^{c}	0.56±0.01^{d}	0.23±0.06^{d}	0.49±0.05^{d}	0.53±0.05^{d}	0.637
	15	334.6±13.4^{c}	377.4±19.4^{c}	367.8±15.9^{d}	342.5±10.5^{c}	0.44±0.03^{d}	0.43±0.04^{d}	0.33±0.04^{d}	0.53±0.04^{d}	0.843
	20	327.0±15.4^{c}	343.7±10.4^{c}	325.6±15.4^{c}	306.6±12.1^{c}	0.36±0.02^{d}	0.61±0.08^{d}	0.49±0.07^{d}	0.18±0.06^{d}	0.692
	30	320.4±12.1^{c}	327.7±16.4^{c}	305.3±10.5^{c}	308.4±14.8^{c}	0.48±0.06^{d}	0.49±0.04^{d}	0.37±0.04^{d}	0.57±0.02^{d}	0.831
	40	408.0±16.7^{c}	415.9±12.2^{c}	339.7±12.0^{c}	442.2±15.9^{c}	0.65±0.01^{d}	0.62±0.02^{d}	0.72±0.06^{d}	0.57±0.04^{d}	0.970
	60	342.9±15.6^{c}	273.1±10.9^{a}	285.8±12.7^{c}	249.2±14.8^{c}	0.57±0.05^{d}	0.69±0.05^{d}	0.67±0.01^{d}	0.78±0.08^{d}	0.841
Nannochloris oculata	5	322.4±11.4^{c}	337.6±10.4^{c}	388.4±15.7^{c}	352.4±15.4^{c}	0.42±0.09^{d}	0.57±0.07^{d}	0.39±0.04^{d}	0.41±0.04^{d}	0.842
	10	290.0±12.4^{c}	335.0±12.4^{c}	352.5±13.0^{c}	292.9±15.0^{c}	0.72±0.02^{d}	0.71±0.09^{d}	0.65±0.06^{d}	0.75±0.06^{d}	0.766
	15	297.1±13.4^{c}	332.4±9.7^{c}	302.5±10.6^{c}	403.4±16.7^{c}	0.56±0.05^{d}	0.27±0.05^{d}	0.71±0.04^{d}	0.61±0.02^{d}	0.897
	20	348.1±16.7^{c}	353.7±13.2^{c}	379.3±15.4^{c}	302.0±15.7^{c}	0.57±0.02^{d}	0.72±0.04^{d}	0.78±0.08^{d}	0.79±0.03^{d}	0.849
	30	344.3±10.4^{c}	384.6±10.6^{c}	355.9±16.1^{c}	315.7±14.0^{c}	0.27±0.06^{d}	0.54±0.06^{d}	0.66±0.07^{d}	0.49±0.04^{d}	0.948
	40	402.3±11.8^{c}	451.4±12.4^{c}	380.7±12.1^{c}	388.0±13.9^{c}	0.96±0.01^{d}	0.89±0.04^{d}	0.56±0.06^{d}	0.58±0.05^{d}	0.736
	60	356.7±13.8^{c}	299.4±12.7^{c}	352.1±12.4^{c}	412.9±14.7^{c}	0.57±0.04^{d}	0.75±0.05^{d}	0.33±0.04^{d}	0.41±0.08^{d}	0.824
Oocystis lacustris	5	540.8±14.9^{c}	449.5±16.7^{c}	375.2±15.1^{c}	243.4±10.4^{a}	0.41±0.06^{d}	0.98±0.03^{d}	0.57±0.05^{d}	0.90±0.05^{d}	0.755
	10	330.8±19.7^{c}	233.7±10.6^{a}	224.6±18.7^{a}	254.0±10.2^{a}	0.80±0.04^{d}	0.71±0.01^{d}	0.76±0.03^{d}	0.83±0.09^{d}	0.784
	15	467.5±16.4^{c}	355.1±10.7^{c}	403.6±19.4^{c}	304.6±15.7^{c}	0.34±0.05^{d}	0.87±0.05^{d}	0.75±0.07^{d}	0.66±0.04^{d}	0.820
	20	350.5±13.9^{c}	372.7±16.4^{c}	308.6±11.6^{c}	380.0±12.5^{c}	0.50±0.03^{d}	0.53±0.06^{d}	0.82±0.05^{d}	0.58±0.01^{d}	0.636
	30	357.8±15.4^{c}	364.2±16.1^{c}	443.9±14.9^{c}	359.3±16.4^{c}	0.87±0.04^{d}	0.24±0.07^{d}	0.75±0.06^{d}	0.23±0.02^{d}	0.789
	40	404.4±12.7^{c}	283.7±15.7^{c}	383.1±14.0^{c}	488.9±11.6^{c}	0.82±0.05^{d}	0.66±0.09^{d}	0.97±0.09^{d}	0.71±0.05^{d}	0.845
	60	372.2±19.7^{c}	239.2±13.2^{a}	210.3±12.1^{a}	104.1±11.4^{a}	0.74±0.08^{d}	0.99±0.04^{d}	0.45±0.04^{d}	0.26±0.07^{d}	0.634

Table 2. continued

Microalgae	N/P	ETR_{max} (μmol electrons g^{-1} Chl *a* s^{-1})				Alpha				r^2
		Ch1	Ch2	Ch3	Ch4	Ch1	Ch2	Ch3	Ch4	
Chroomonas salina	5	247.6±12.4[a]	322.4±12.3[c]	412.7±13.4[c]	389.4±10.4[c]	0.54±0.05[d]	0.44±0.04[d]	0.21±0.05[d]	0.57±0.05[d]	0.745
	10	355.7±10.4[c]	456.7±10.5[c]	402.9±12.4[c]	356.1±13.4[c]	0.77±0.03[d]	0.81±0.03[d]	0.61±0.01[d]	0.22±0.03[d]	0.859
	15	399.1±12.4[c]	254.7±14.7[a]	356.7±10.6[c]	334.2±12.4[c]	0.41±0.04[d]	0.55±0.05[d]	0.75±0.05[d]	0.71±0.01[d]	0.945
	20	388.9±15.4[c]	365.7±15.9[c]	424.9±10.7[c]	400.3±15.8[c]	0.45±0.01[d]	0.58±0.02[d]	0.51±0.09[d]	0.45±0.05[d]	0.863
	30	289.4±13.7[c]	422.4±12.4[c]	397.1±10.9[c]	422.6±12.4[c]	0.37±0.03[d]	0.64±0.06[d]	0.55±0.07[d]	0.41±0.08[d]	0.854
	40	402.7±15.7[c]	364.2±10.6[c]	398.4±19.4[c]	355.7±10.6[c]	0.22±0.02[d]	0.47±0.04[d]	0.74±0.08[d]	0.76±0.06[d]	0.712
	60	227.1±14.7[a]	324.8±12.4[c]	304.7±12.4[c]	420.3±11.9[c]	0.57±0.04[d]	0.57±0.05[d]	0.81±0.04[d]	0.67±0.07[d]	0.730
Gloeocystis gigas	5	394.9±10.5[c]	334.4±13.1[c]	368.3±10.6[c]	298.2±15.8[c]	0.36±0.06[d]	0.29±0.02[d]	0.21±0.06[d]	0.28±0.06[d]	0.821
	10	296.4±16.7[c]	318.9±15.0[c]	326.3±10.7[c]	270.8±16.8[c]	0.36±0.02[d]	0.36±0.01[d]	0.50±0.04[d]	0.48±0.04[d]	0.851
	15	368.4±10.4[c]	262.4±13.6[a]	388.8±15.7[c]	389.0±14.9[c]	0.29±0.01[d]	0.25±0.05[d]	0.28±0.07[d]	0.33±0.07[d]	0.886
	20	294.4±15.4[c]	404.4±15.9[c]	400.1±12.4[c]	293.9±10.5[c]	0.50±0.03[d]	0.44±0.08[d]	0.54±0.05[d]	0.56±0.09[d]	0.852
	30	313.8±12.4[c]	393.2±14.3[c]	418.8±13.9[c]	266.4±13.4[a]	0.54±0.05[d]	0.59±0.05[d]	0.29±0.05[d]	0.47±0.05[d]	0.828
	40	269.8±10.6[a]	282.4±10.8[c]	307.4±12.4[c]	279.9±12.8[c]	0.25±0.06[d]	0.24±0.06[d]	0.97±0.06[d]	0.65±0.04[d]	0.822
	60	399.8±12.5[c]	303.5±10.9[c]	389.0±10.4[c]	240.8±16.7[a]	0.24±0.04[d]	0.58±0.04[d]	0.57±0.05[d]	0.76±0.06[d]	0.343
Prymnessium parvum	5	422.4±13.4[c]	402.1±15.4[c]	405.9±12.6[c]	322.4±15.4[c]	0.41±0.04[d]	0.37±0.05[d]	0.85±0.06[d]	0.37±0.05[d]	0.549
	10	377.1±12.1[c]	399.1±12.7[c]	422.1±10.9[c]	299.4±15.1[c]	0.37±0.02[d]	0.64±0.04[d]	0.71±0.07[d]	0.65±0.05[d]	0.657
	15	304.7±10.9[c]	411.5±10.6[c]	351.7±12.4[c]	367.4±13.9[c]	0.67±0.01[d]	0.34±0.03[d]	0.44±0.08[d]	0.51±0.08[d]	0.678
	20	299.3±18.7[c]	318.7±10.5[c]	452.3±11.4[c]	407.7±12.9[c]	0.53±0.01[d]	0.56±0.02[d]	0.50±0.04[d]	0.59±0.01[d]	0.723
	30	402.1±10.4[c]	355.2±10.4[c]	297.1±12.5[c]	356.7±14.8[c]	0.47±0.05[d]	0.34±0.02[d]	0.32±0.02[d]	0.69±0.06[d]	0.645
	40	255.7±15.4[a]	301.4±19.7[c]	412.7±13.4[c]	399.4±17.6[c]	0.57±0.08[d]	0.54±0.05[d]	0.47±0.05[d]	0.76±0.08[d]	0.633
	60	389.4±12.4[c]	214.7±15.2[a]	255.7±14.9[a]	312.4±16.7[c]	0.64±0.03[d]	0.52±0.06[d]	0.70±0.04[d]	0.74±0.04[d]	0.621

of photosynthesis, there have been no reports on blooming in Korean coastal waters except for the first outbreak in the waters of Tongyong in 1997[17]. *Prorocentrum micans*, *H. triquetra*, *G. catenatum*, and *A. caterae* had annual occurrences of algal blooms in Korean waters[17]. A study of the reasons why blooms caused by *G. impudicum* are not occurring is underway. The present data has been used to understand why the massive blooms of *G. impudicum* in inshore enriched nutrients and offshore waters occur with the depletion of nutrients.

We may conclude that the sensitivity of algae to DIN/DIP evaluated by the PHYTO-PAM fluorometry parameters is significantly heterogenous among species; in particular, some dinoflagellates (*P. micans*, *H. triquetra*, *G. catenatum*, and *A. caterae*) had a decreased the ETR_{max} with the effect of P and N-limited medium in cultures. However, *G. impudicum* and cyanobacterial species were not found to have a considerable fluctuation of the ETR_{max} exposed to DIN/DIP. It is thought that *G. impudicum* may differ in terms of photo-physiological characteristics (i.e. the ways in which this species adapts to the extreme environment) from dinoflagellates. Consequently, the coastal regions of the South Sea of Korea are more vulnerable to the influence of offshore water currents than any other waters in Korea, based on geographical characteristics, possibly resulting in the outbreak of massive algal blooms of *G. impudicum*. In future, the PHYTO-PAM method will be used to biotoxin for monitoring and other prediction programs in Korea.

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