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Reduction in CO_2 uptake rates of red tide dinoflagellates due to mixotrophy

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We investigated a possible reduction in CO_2 uptake rate by phototrophic red tide dinoflagellates arising from mixotrophy. We measured the daily ingestion rates of *Prorocentrum minimum* by *Prorocentrum micans* over 5 days in 10 L experimental bottles, and the uptake rates of total dissolved inorganic carbon (C_T) by a mixture of *P. micans* and *P. minimum* (mixotrophic growth), and for the predator *P. micans* (phototrophic growth; control) and prey *P. minimum* (phototrophic growth; control) alone. To account for the effect of pH on the phototrophic growth rates of *P. micans* and *P. minimum*, measurements of C_T and pH in the predator and prey control bottles were continued until the pH reached the same level (pH 9.5) as that in the experimental bottles on the final day of incubation. The measured total C_T uptake rate by the mixture of *P. micans* and *P. minimum* changed from 123 to 161 μmol C_T kg^{-1} d^{-1} over the course of the experiment, and was lower than the C_T uptake rates shown by *P. micans* and *P. minimum* in the predator and prey control bottles, respectively, which changed from 132 to 176 μmol C_T kg^{-1} d^{-1} over the course of the experiment. The reduction in total C_T uptake rate arising from the mixotrophy of *P. micans* was 7-31% of the daily C_T uptake rate seen during photosynthesis. The results suggest that red tide dinoflagellates take up less C_T during mixotrophy.

Key Words: carbon dioxide; dissolved inorganic carbon; marine phytoplankton; mixotrophy; pH; photosynthesis; *Prorocentrum micans*; *Prorocentrum minimum*

INTRODUCTION

Since the industrial revolution, ever increasing quantities of CO_2 have been released into the atmosphere because of the burning of fossil fuels, land use changes, and cement production. During this period approximately half the CO_2 has remained in the atmosphere (Keeling and Whorf 2000, Houghton et al. 2001); the ocean and land biospheres have taken up the remainder. The global oceanic sink of fossil fuel CO_2 has been estimated to be 118 ± 19 petagrams of carbon, accounting for 30% of total emissions during the period 1800-1994 (Sabine et al. 1999, 2002, Lee et al. 2003). However, it is not clear whether the oceanic sink of CO_2 is stable, or will vary in

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response to future ocean changes.

In marine environments phototrophic organisms convert CO_2 to glucose via photosynthesis; part of this fixed carbon is released back into seawater during respiration by the phototrophic organisms and / or their grazers. Through these processes marine organisms influence surface CO_2 concentrations, and thereby contribute to the oceanic sink of CO_2 (e.g., Emerson et al. 1997, Laws et al. 2000, Lee 2001). Accordingly, a mechanistic understanding of the key processes that control CO_2 uptake and release by marine algal groups will increase knowledge of the roles of marine organisms in influencing surface water CO_2 concentrations in time scales ranging from days to seasons. There have been many studies on CO_2 uptake and release by cyanophytes, diatoms, microflagellates, macroalgae, and symbiotic dinoflagellates (e.g., Burkhardt et al. 2001, Miyachi et al. 2003, Rost et al. 2003, Kim et al. 2015*a*). Although phototrophic dinoflagellates are ubiquitous and sometimes dominant in terms of the biomass of phototrophic organisms, few studies have investigated their CO_2 uptake and release (Nimer et al. 1999, Giordano et al. 2005, Rost et al. 2006).

Many phototrophic dinoflagellates originally thought to be exclusively autotrophic are now known to be mixotrophic (i.e., capable of both photosynthesis and ingestion of prey) (Jacobson and Anderson 1996, Stoecker 1999, Berge et al. 2008, Burkholder et al. 2008, Kim et al. 2015*b*, Lee et al. 2016). Furthermore, several newly described phototrophic dinoflagellates have been revealed to be mixotrophic (Lee et al. 2014*a*, 2014*b*, Reñé et al. 2014, Lim et al. 2015). Mixotrophic dinoflagellates are able to feed on diverse prey including heterotrophic bacteria (Nygaard and Tobiesen 1993, Seong et al. 2006, Jeong et al. 2012), cyanobacteria (Jeong et al. 2005*a*, 2012, Glibert et al. 2009), diatoms (Yoo et al. 2009), phytoflagellates (Jeong et al. 2005*b*), other phototrophic dinoflagellates (Legrand et al. 1998), heterotrophic dinoflagellates (Jeong et al. 1997), and ciliates (Bockstahler and Coats 1993, Park et al. 2006). If the heterotrophic activity of mixotrophic dinoflagellates exceeds their autotrophic activity, the release of CO_2 by these organisms may be greater than CO_2 uptake. Therefore, feeding by mixotrophic dinoflagellates on co-occurring prey may play an important role in the CO_2 cycle. There may be complex predator-prey relationships among mixotrophic dinoflagellates, as these organisms commonly co-occur in natural environments (e.g., Jeong et al. 2005*b*, 2010, 2015). Populations of mixotrophic dinoflagellates feeding on each other may affect the CO_2 cycle because predation feeding may reduce photosynthesis or increase respiration.

Two important questions arise concerning predatory interactions amongst mixotrophic dinoflagellates: 1) do they affect seawater CO_2 concentrations and 2) if so, to what degree are the concentrations affected? To answer these questions, we established one set of experimental bottles containing a mixture of the mixotrophic dinoflagellate predator *Prorocentrum micans* and its mixotrophic dinoflagellate prey *Prorocentrum minimum*, and sets of control bottles containing *P. micans* and *P. minimum* alone. Over a 5 d incubation period we measured the daily ingestion rate of *P. minimum* by *P. micans* in the experimental culture, and the rates of uptake of total dissolved inorganic carbon ($C_T = [CO_{2aq}] + [HCO_3^-] + [CO_3^{2-}]$) in both the experimental culture and the control (prey and predator alone) bottles. The results of the study provide insights into the effects of phototrophic red tide dinoflagellate mixotrophy on seawater CO_2 concentrations in marine ecosystems.

MATERIALS AND METHODS

Experimental organisms

P. micans PMCJH99, isolated from Jinhae Bay in 1999 and *P. minimum* PMJH00, isolated from Jinhae Bay in 2000 were grown at 20°C in enriched f/2 seawater media (Guillard and Ryther 1962) without silicate, under a 14 : 10 h light-dark cycle, using cool white fluorescent light (50 μmol photons m^{-2} s^{-1}).

Transmission electron microscopy

Transmission electron microscopy (TEM) analysis was used to confirm predation by *P. micans* on *P. minimum* (Jeong et al. 2005*b*). We incubated predator and prey cells in a 250-mL polycarbonate (PC) bottle for 24 h. A 50 mL aliquot from the PC bottle was transferred to a 50-mL centrifuge tube, and the cells were fixed for 1.5 to 2 h by the addition of glutaraldehyde (final concentration 2.5%) in culture medium. Cells were centrifuged and the pellet was agarized. After several rinses with culture medium the cells were postfixed in 1% (w/v) osmium tetroxide in deionized water, then dehydrated using a graded ethanol series (50, 60, 70, 80, 90, and 100% [all v/v] ethanol, followed by two washes with 100% ethanol). The cells were embedded in Spurr's low viscosity resin (Spurr 1969), sectioned using a RMC MT-XL ultramicrotome (Boeckeler Instruments Inc., Tucson, AZ, USA), and post-stained with 3% (w/v) aqueous uranyl acetate followed by lead citrate. Stained sections were viewed with a JEOL-1010 electron microscope (Jeol Ltd., Tokyo, Japan).

Ingestion rates

We measured the ingestion and clearance rates of *P. minimum* by *P. micans* as a function of incubation time. Dense photosynthetic cultures of *P. micans* and *P. minimum* were grown for approximately 1 month and transferred to 10 L PC bottles. 1 mL aliquots from the PC bottles were removed at various times, and cell counts were made using a compound microscope. The experimental starting concentrations of *P. micans* and *P. minimum* were established by addition of appropriate volumes of dense cultures. We established triplicate cultures for each of 1) an experimental treatment comprising a mixture of *P. micans* and *P. minimum*, 2) a control comprising the predator (*P. micans*) alone, and 3) a control comprising the prey (*P. minimum*) alone. In addition, we established an additional bottle containing a filtrate of the predator and prey culture (seawater control).

We used mixed filtrates of the two organisms as a basal medium to ensure consistent water conditions across treatment and controls. To achieve this a predator culture was filtered through a 0.7 µm GF/F filter, and volumes of this filtrate (equal to the volume of predator culture added into the predator control and experimental bottles for each predator-prey combination) were added into the prey control and seawater control bottles. A prey culture was also filtered through a 0.7 µm GF/F filter, and volumes of this filtrate (equal to the volume of prey culture added into the prey control and experimental bottles) were added into both the predator control and seawater control bottles. Two liters of f/2 medium were added to all bottles, which were then filled to 6 L with freshly filtered seawater. Each bottle was then fitted with a cap through which three silicon tubes were inserted. To determine the cell densities (cells mL^{-1}) of predator and prey at the beginning of the experiment and after 1-5 d of incubation, a 20 mL aliquot was removed from each bottle through one of the tubes at each sampling time; 10 mL was fixed with 5% (v/v) Lugol's solution and 10 mL was fixed with 4% (v/v) formalin. More than 300 cells in three 1 mL Sedgwick-Rafter counting chambers were enumerated. The treatment and control bottles were placed on a shelf without being refilled after subsampling, and incubated at 20°C as described above. To account for the effect of pH on ingestion rates (see next section), incubation of prey control bottles was continued until pH values attained the same pH found in the experimental bottles on the fifth day of incubation, and the prey cell density was determined daily.

For each sampling interval the predation rates of *P. minimum* by *P. micans* (cells $predator^{-1}$ d^{-1}) in the experimental bottles were calculated as described by Jeong et al. (2005*c*), with the exception of the calculation of growth rate (k). An increase in pH during incubation is known to affect the growth rates of *P. micans* and *P. minimum* (Hansen et al. 2007). Therefore, to obtain unique k values for *P. minimum* and *P. micans* in the experimental bottles at a given pH, we used the measured k values in the prey and predator control bottles at the same pH as in the experimental bottles.

A 30 mL aliquot was removed from each bottle at each sampling time, and the concentrations of chlorophyll *a* (chl-*a*) were measured as described by Arar and Collins (1997).

Determination of C_T and pH

Seawater C_T and A_T for all bottles were measured using coulometric and potentiometric titration in a VINDTA system (Marianda, Kiel, Germany). The accuracy and precision of C_T and A_T measurements were checked daily against seawater reference materials with known C_T and A_T values (certified by A. Dickson, Scripps Institution of Oceanography, San Diego, CA, USA). The measurement precisions were approximately ±1.5 µmol kg^{-1} for both C_T and A_T (a total of 14 measurements for each parameter, one set of measurements per day).

Seawater pH values for all bottles were calculated from C_T and A_T measurements using the carbonic acid dissociation constants of Mehrbach et al. (1973) as refitted in different functional forms by Dickson and Millero (1987). This set of thermodynamic constants has proved to be the most consistent for laboratory (Lee et al. 1996, Lueker et al. 2000, Millero et al. 2006) and field (Wanninkhof et al. 1999, Lee et al. 2000, Millero et al. 2002) measurements of carbon parameters. Given the uncertainty (±1.5 µmol kg^{-1}) in C_T and A_T measurements, the predicted pH values based on C_T and A_T measurements were accurate to ±0.005 units. To monitor changes in seawater pH in experiments such as these it is recommended that measurements of C_T and A_T be performed daily, because conventional pH measurement using glass electrodes does not provide stable signals in high pH solutions (pH > 8.5).

The measurements of C_T and pH were performed in parallel with measurements of the growth, ingestion, and clearance rates of *P. minimum* by *P. micans*, as described in the preceding section.

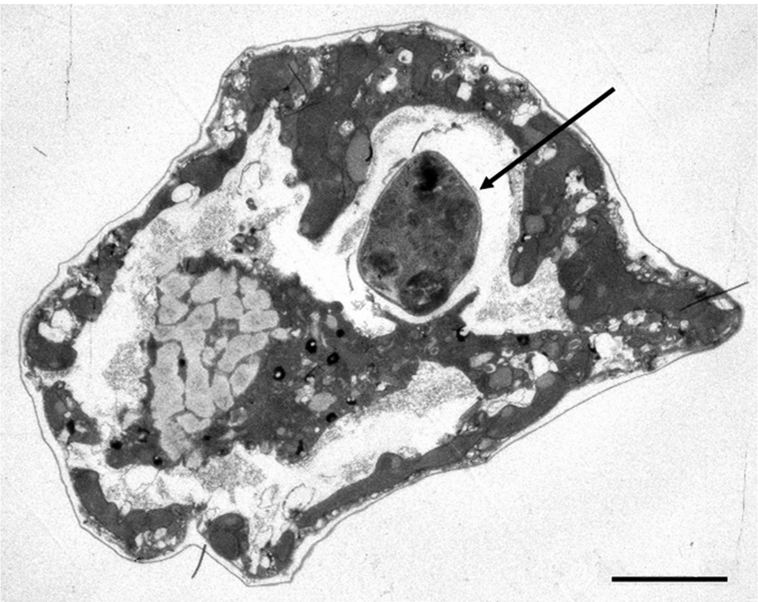

Fig. 1. *Prorocentrum micans* containing an ingested *P. minimum* cell (arrow). Scale bar represents: 5 µm.

Calculation of C_T uptake rate

As mixotrophic dinoflagellates can perform multiple activities (including photosynthesis, feeding, digestion, and / or respiration), we choose the C_T assay over the ^{14}C-based short-term incubation method, as the former provides a measure of the net result of multiple activities. We calculated the C_T uptake rate by *P. micans* cells (nmol C_T dinoflagellate^{-1} d^{-1}) in predator control bottles at each sampling interval (1 day) by dividing the reduction in C_T (nmol C_T g^{-1} d^{-1} or µmol C_T kg^{-1} d^{-1}) by the mean predator concentration (cells mL^{-1}). The C_T uptake rate by *P. minimum* cells in prey control bottles was also calculated by dividing the reduction in C_T by the mean prey concentration at each time interval. The mean predator (and prey) concentration at each interval was calculated following the method described by Jeong and Latz (1994).

At each sampling time the expected total C_T uptake rate by the populations of *P. micans* and *P. minimum* (µmol C_T kg^{-1} d^{-1}) in the experimental bottles was determined by summing the calculated uptake rates of their equivalent individual populations in the predator and prey control bottles, respectively. In this calculation the C_T uptake rate by the population of *P. micans* (nmol C_T g^{-1} d^{-1}, or µmol C_T kg^{-1} d^{-1}) in the experimental bottles at each time was obtained by multiplying the mean C_T uptake rate for *P. micans* cells (nmol C_T dinoflagellate^{-1} d^{-1}) measured in the predator control bottles by the mean *P. micans* concentration (cells mL^{-1}) in the experimental bottles. Similarly, the C_T uptake rate by *P. minimum* was calculated by multiplying the mean C_T uptake rate of *P. minimum* cells measured in the prey bottles by the mean *P. minimum* concentration in the experimental bottles. To minimize the effect of pH on the C_T uptake rate, the mean C_T uptake rates (nmol C_T dinoflagellate^{-1} d^{-1}) obtained from the prey and predator control bottles at the same pH as in the experimental bottles were used.

Statistical treatment of results

Experimental data were treated with linear regression analysis of variance. If the p-value is smaller than 0.05, the ANOVA test implies that a relation does exist between variables. Using this result along with the scatter plot of the figure consisting of a pair of variables (one for x-axis, and one for y-axis), it can be concluded that the relationship between two variables is linear.

RESULTS

Cell abundance

A TEM image (Fig. 1) shows a *P. minimum* cell within a cell of the *P. micans* predator. Using an inverted microscope we previously captured an image of *P. micans* engulfing a *P. minimum* cell through the sutures (Jeong et al. 2005*b*).

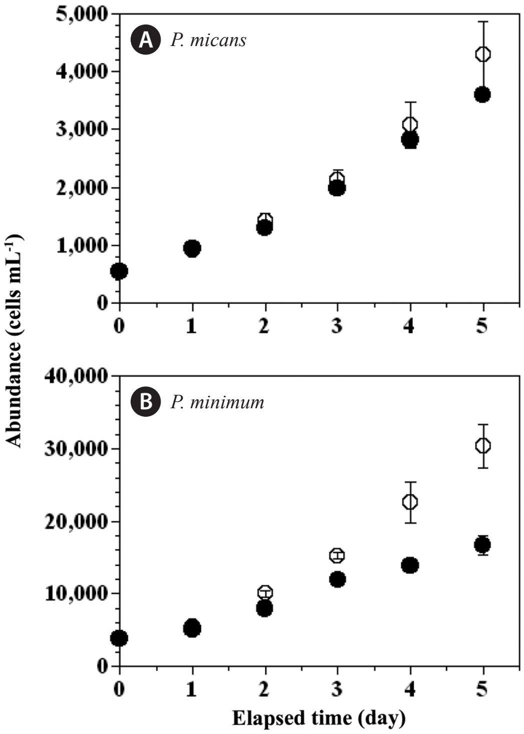

Fig. 2. Cell abundances (cells mL^{-1}) of the predator *Prorocentrum micans* (A) and the prey *P. minimum* (B) as a function of incubation time in the predator and prey control bottles (open circles), and the experimental treatment bottles (closed circles). Symbols and error bars represent means ± 1 standard error (n = 3).

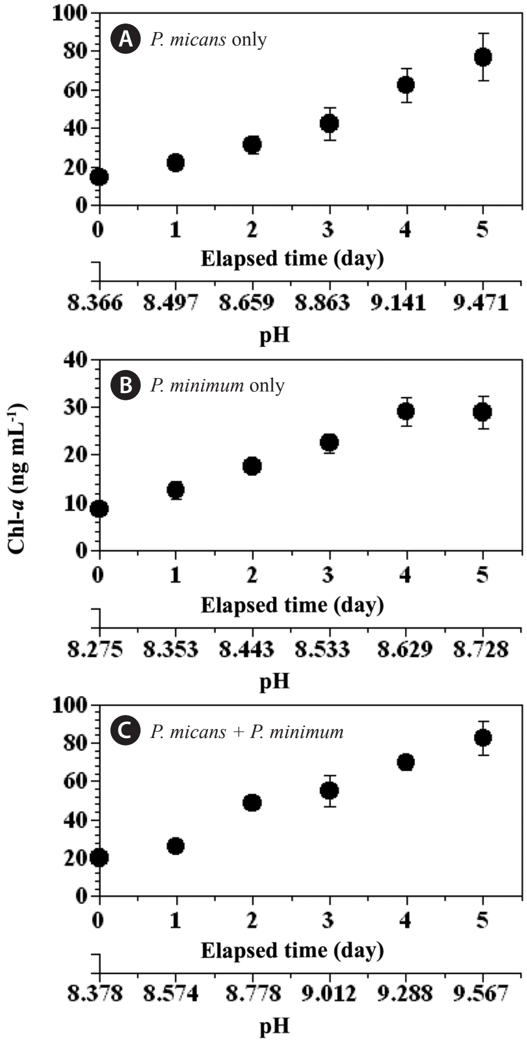

Fig. 3. Concentrations of chlorophyll-*a* (chl-*a*, ng mL^{-1}) in the predator *Prorocentrum micans* (A) and prey *P. minimum* (B) control bottles, and the experimental treatment bottles (C) as a function of incubation time and seawater pH. Symbols and error bars represent means ± 1 standard error (n = 3).

With increasing incubation time, the abundance of *P. micans* in the experimental treatment bottles increased from 550 cells mL^{-1} (day 0) to 3,596 cells mL^{-1} (day 5), whereas abundance in the predator control bottles increased from 562 to 4,290 cells mL^{-1} (Fig. 2A). Over the same period the abundance of *P. minimum* in the experimental treatment bottles increased from 3,820 to 16,639 cells mL^{-1}, whereas their abundance in the prey control bottles increased from 3,878 to 30,333 cells mL^{-1} (Fig. 2B).

Changes in chl-*a* and pH

The chl-*a* concentration in the predator and prey control bottles increased (day 0-5) from 14.5 to 76.9 ng mL^{-1}, and 8.7 to 28.9 ng mL^{-1}, respectively (Fig. 3A & B), whereas the chl-*a* concentration in the experimental treatment bottles increased from 20.2 to 82.4 ng mL^{-1} over the same period (Fig. 3C). Over the same period (day 0-5) the pH increased considerably in the predator control (from 8.36 to 9.47), the prey control (from 8.27 to 8.73), and the experimental treatment (from 8.38 to 9.57) bottles (Fig. 4A-C), whereas the pH in the seawater control bottle remained approximately unchanged (from 8.10 to 8.12).

Changes in C_T concentration

During the incubation period (day 0-5) the concentration of C_T in the predator control and prey control bottles decreased from 1,800 to 1,100 µmol C_T kg^{-1} and from 1,850 to 1,550 µmol C_T kg^{-1}, respectively (Fig. 5A & B). The C_T value for the experimental treatment bottles decreased from 1,790 to 1,060 µmol C_T kg^{-1} (Fig. 5C), whereas The concentration of C_T in the seawater control bottle remained between 2,092 and 2,099 µmol C_T kg^{-1}.

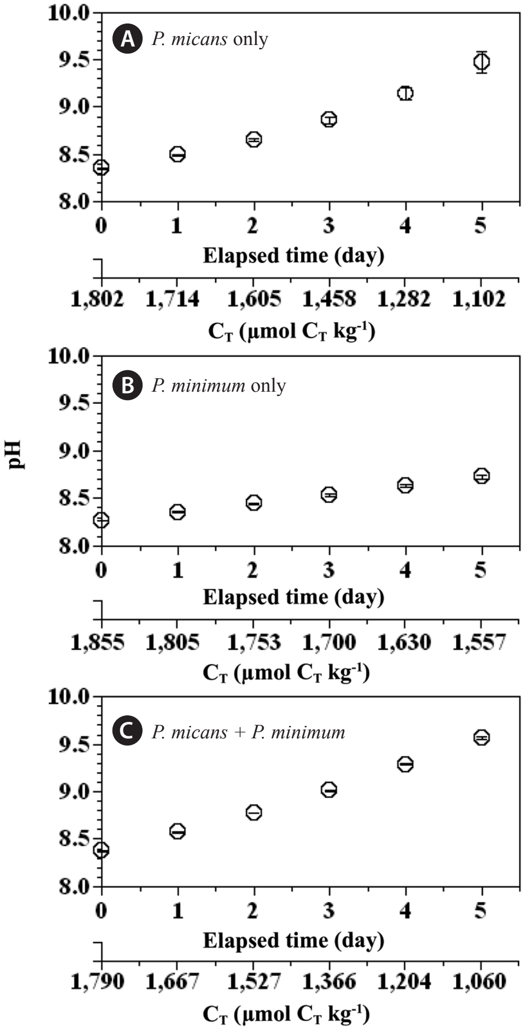

Fig. 4. Seawater pH (seawater scale, kg per seawater unit) in the predator *Prorocentrum micans* (A) and prey *P. minimum* (B) control bottles, and the experimental treatment bottles (C) as a function of incubation time and total dissolved inorganic carbon concentration (C_T). Symbols and error bars represent means ± 1 standard error (n = 3).

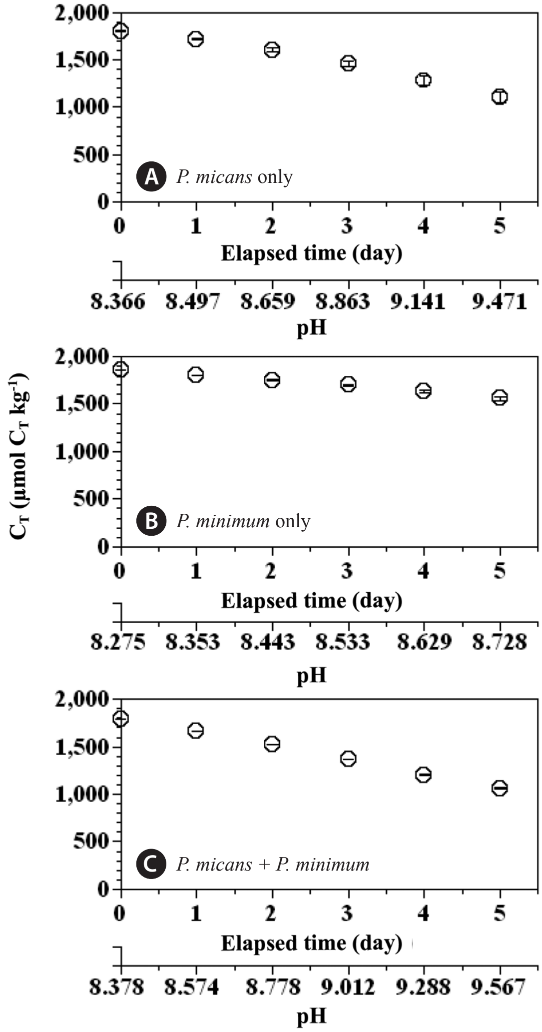

Fig. 5. Concentrations of total dissolved inorganic carbon (C_T; μmol C_T kg^{-1}) in the predator *Prorocentrum micans* (A) and prey *P. minimum* (B) control bottles, and the experimental treatment bottles (C) as a function of incubation time and seawater pH. Symbols and error bars represent means ± 1 standard error (n = 3).

The effect of pH on growth rates of *Prorocentrum micans* and *Prorocentrum minimum*

Seawater pH is known to affect the growth rates of *P. micans* and *P. minimum*; in general, the higher the pH the lower the growth rate. The pH in the predator control and experimental treatment bottles increased more rapidly than in the prey control bottles. Therefore, our measurements of abundance of *P. minimum*, pH, and C_T in the prey control bottles were extended up to day 12 (Fig. 6), as opposed to day 5 in the predator control and experimental bottles. The abundance of *P. minimum* in the prey control bottles increased from 35,708 cells mL^{-1} at day 6 to 68,583 cells mL^{-1} at day 12 (Fig. 6A). The pH increased in the prey control bottles from 8.85 to 9.48 (Fig. 6B) between day 6 and day 12, and the C_T concentrations decreased from 1,480 to 1,100 μmol C_T kg^{-1} (Fig. 6C).

Rate of carbon (C_T) uptake

The C_T uptake rates by *P. micans* in the predator control bottles (0.049-0.118 nmol C_T dinoflagellate^{-1} d^{-1}) (Fig. 7A) were an order of magnitude higher than those for *P. minimum* in the prey control bottles (0.004-0.011 nmol C_T dinoflagellate^{-1} d^{-1}) (Fig. 7B). However, the chl-*a* specific C_T uptake rates of *P. micans* in the predator control bottles (112-220 μmol C_T [mg chl-*a*]$^{-1}$ h^{-1}) were similar in magnitude to those of *P. minimum* in the prey control bottles

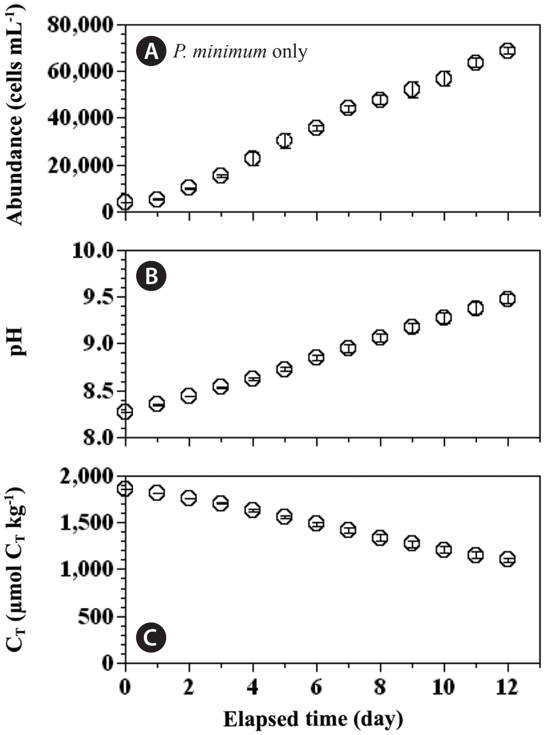

Fig. 6. Cell abundances (cells mL^{-1}) of *Prorocentrum minimum* (A), seawater pH (seawater scale, kg per seawater unit) (B), and concentrations of total dissolved inorganic carbon (C_T; µmol C_T kg^{-1}) in the *P. minimum* control bottles (C). Symbols and error bars represent means ± 1 standard error (n = 3).

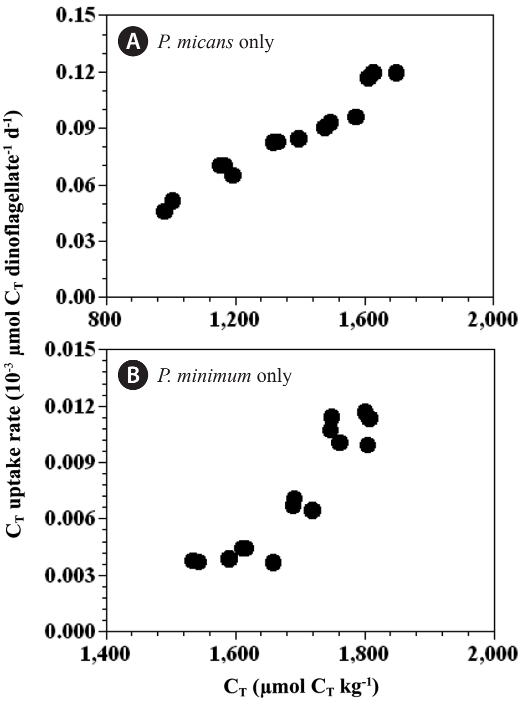

Fig. 7. Rate of C_T uptake (10^{-3} µmol C_T dinoflagellates^{-1} d^{-1}) by *Prorocentrum micans* (A) and *P. minimum* (B) as a function of C_T. Symbols and error bars represent means ± 1 standard error (n = 3).

(98-254 µmol C_T [mg chl-*a*]$^{-1}$ h^{-1}). The C_T uptake rates (per cell) of *P. micans* and *P. minimum* were significantly positively correlated with the C_T concentration ($p < 0.05$, linear regression ANOVA) (Fig. 7).

The total C_T uptake rate of the combined populations of *P. micans* and *P. minimum* in the experimental bottles was 123-161 µmol C_T kg^{-1} d^{-1}, which was lower than the expected total C_T uptake rate (136-212 µmol C_T kg^{-1} d^{-1}) solely by a phototrophic growth of *P. micans* and *P. minimum* (Fig. 8A). With increasing incubation time the difference in the total C_T uptake rate (expected total C_T uptake rate - measured total C_T uptake rate) increased from 8 to 56 µmol C_T kg^{-1} d^{-1} (Fig. 8B). Even for C_T uptake corrected for the effect of pH, the measured total C_T uptake rate was lower than the expected rate (132-176 µmol C_T kg^{-1} d^{-1}) (Fig. 8C), and the reduction in the total C_T uptake rate arising from the mixotrophy of *P. micans* increased from 6 to 25 µmol C_T kg^{-1} d^{-1} (Fig. 8D).

Ingestion rates

The higher pH (or lower C_T) observed in the prey control bottles between day 6 and day 12 could have lowered the growth rate of *P. minimum*. Thus, we calculated two ingestion rates of *P. minimum* by *P. micans*; one rate corrected for pH effects and the other not so corrected. To account for the effect of pH on ingestion rate, we calculated the ingestion rate of *P. minimum* by *P. micans* by replacing the growth rate of *P. minimum* in the experimental bottles by that obtained for *P. minimum* in the prey control bottles at similar pH and C_T levels.

Without correcting for pH effects the apparent predation rate of *P. minimum* by *P. micans* was 236-2,784 cells d^{-1} at day 5 (Fig. 9A). In contrast, the pH-corrected predation rate of *P. minimum* by *P. micans* was 630-1,946 cells d^{-1} at day 5 (Fig. 9B).

Correlations

Our results indicate that the differences between the expected and measured rates of C_T uptake in the experimental treatment bottles were significantly positively correlated with the rate of predation of *P. minimum* by *P. micans* ($p < 0.05$ for both, linear regression ANOVA) (Fig. 10A & B). These relationships suggest that feeding by *P. micans* on *P. minimum* may be an important factor in controlling respective rates of carbon uptake.

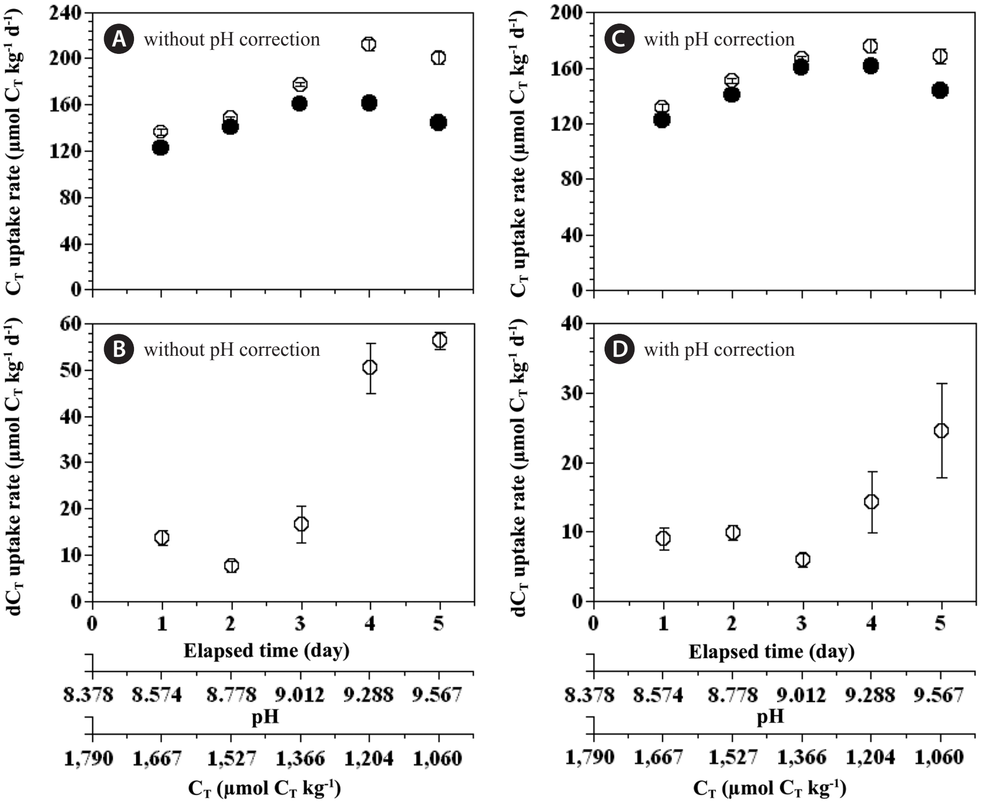

Fig. 8. Expected (open circles) and measured (closed circles) total C_T uptake rates (A & C) by the red tide dinoflagellates and their anomalies (B & D) (dC_T uptake rate = expected total C_T uptake rate - measured total C_T uptake rate) as a function of the incubation time, seawater pH, and total dissolved inorganic carbon concentration (C_T). In A and B the total C_T uptake rates and corresponding anomalies were not corrected for the effect of pH, whereas in C and D the total C_T uptake rates and corresponding anomalies were corrected for pH. Symbols and error bars represent means ± 1 standard error (n = 3).

DISCUSSION

Few studies have reported CO_2 uptake rates (and / or maximum chl-*a* specific CO_2 uptake rates) for phototrophic dinoflagellates (Rost et al. 2006). In this study the C_T uptake rate of *P. micans* (0.049-0.118 nmol C_T dinoflagellate^{-1} d^{-1}) was approximately 10 times higher than that of *P. minimum* (0.004-0.011 nmol C_T dinoflagellate^{-1} d^{-1}). Both the cell volume and chl-*a* content of *P. micans* are approximately 10-fold those of *P. minimum*. Therefore, the chl-*a* specific C_T uptake rates of *P. micans* were comparable in magnitude to those of *P. minimum*, indicating that the concentration of chl-*a* is an important factor in determining C_T uptake rate. The measured maximum chl-*a* specific C_T uptake rates of *P. micans* (220 μmol C_T [mg chl-*a*]$^{-1}$ h^{-1}) and *P. minimum* (254 μmol C_T [mg chl-*a*]$^{-1}$ h^{-1}) in the present study are similar in magnitude to those of the diatoms *Phaeodactylum tricornutum*, *Thalassiosira weissflogii* and *Skeletonema costatum*, the prymnesiophyte *Phaeocystis globosa* (ca. 200-300 μmol [mg chl-*a*]$^{-1}$ h^{-1}) (Burkhardt et al. 2001, Rost et al. 2003), and the coccolithophorid *Emiliania huxleyi* (ca. 200-300 μmol [mg chl-*a*]$^{-1}$ h^{-1}) (Rost et al. 2003). The C_T uptake rates, normalized to the chl-*a* content, are similar in magnitude across planktonic groups. Therefore, the chl-*a* concentrations in marine algae may be useful in estimating C_T uptake rates.

The maximum chl-*a* specific C_T uptake rate of *P. minimum* calculated in the present study is much lower than the maximum chl-*a* specific HCO_3^- uptake rate (ca. 700 μmol [mg chl-*a*]$^{-1}$ h^{-1}) determined by Rost et al. (2006). The latter uptake rate for *P. minimum* was measured using the ^{14}C method, in which HCO_3^- is taken up over a short period. This evidence suggests that the maximum chl-*a* specific HCO_3^- uptake rate obtained using the ^{14}C method is higher than that measured using relatively long-term incubation involving a light-dark cycle.

The mean C_T uptake rates per cell (*P. micans* or *P. minimum*) were positively correlated with C_T concentrations,

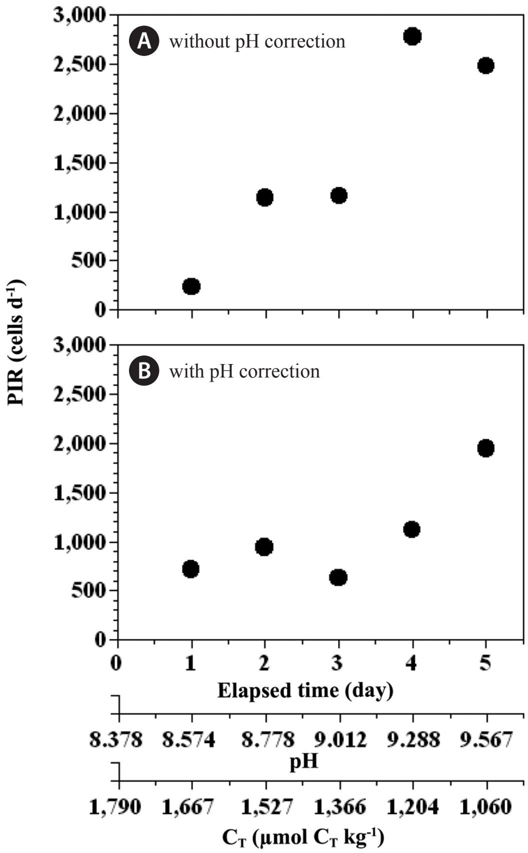

Fig. 9. Total *Prorocentrum minimum* cells ingested by *P. micans* per day (PIR, cells d^{-1}) as a function of incubation time, seawater pH, and total dissolved inorganic carbon concentration (C_T) without correction for the effect of pH (A), and with correction for the effect of pH (B). Symbols and error bars represent means ± 1 standard error (n = 3).

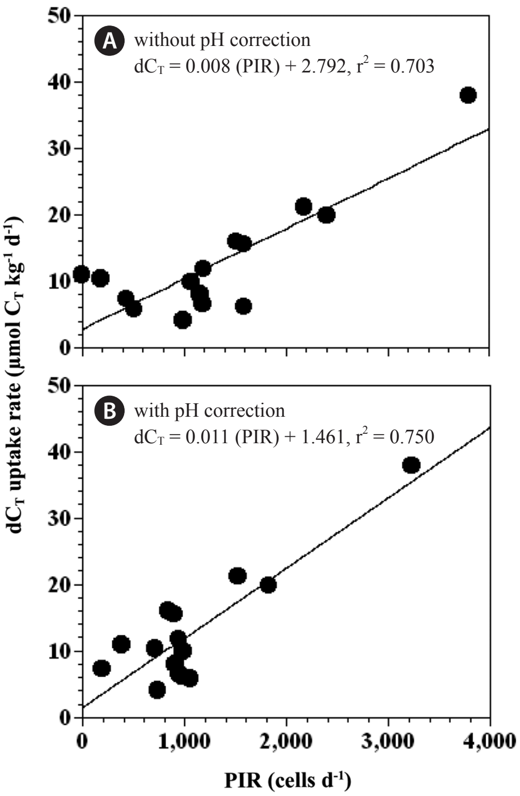

Fig. 10. Anomalies in C_T uptake (dC_T uptake rate) as a function of total *P. minimum* cells ingested by *P. micans* per day (PIR, cells d^{-1}) without correction for the effect of pH (A), and with correction for the effect of pH (B). The equations for the linear regressions are as follows: dC_T uptake rate (μmol C_T kg^{-1} d^{-1}) = 0.008 × (PIR) + 2.792, r^2 = 0.703 ($p < 0.01$) (A) and dC_T uptake rate (μmol C_T kg^{-1} d^{-1}) = 0.011 × (PIR) + 1.461, r^2 = 0.750 ($p < 0.05$) (B).

indicating that this factor also affects the C uptake rate, as previously reported for diatoms and the mixotrophic dinoflagellate *Heterocapsa triquetra* (Burkhardt et al. 2001, Rost et al. 2003, 2006).

The results of the present study show that feeding by a mixotrophic dinoflagellate predator on a phototrophic dinoflagellate prey can lead to a substantial reduction in total C_T uptake rates. The degree of reduction in C_T uptake rates because of the mixotrophy of *P. micans* was 7-31% of the daily C_T uptake rates during photosynthesis. The reductions in C_T uptake rate from mixotrophy were positively correlated with the ingestion rates of *P. minimum* by *P. micans* (Fig. 10). Two possibilities could account for this trend. The first is that *P. micans* may photosynthesize less (i.e., the photosynthetic rate may be considerably reduced) during feeding on *P. minimum* and associated digestion. Second, the respiration rate of *P. micans* in the mixotrophic mode was greater than in the autotrophic mode, suggesting that more organic carbon may be converted to CO_2 in the mixotrophic mode. The present study thus indicates that reduction in C_T uptake by mixotrophic dinoflagellates feeding on co-occurring prey should be taken into account in ecosystem models describing CO_2 dynamics.

When considering ingestion rates among different dinoflagellate predators feeding on the same prey species (e.g., Jeong et al. 2005*b*, 2010, 2015), it should be noted that the degree of reduction in the rate of C_T uptake arising from mixotrophy may be different from that of *P. micans*. To better understand the dynamics of the CO_2 cycle in a given ecosystem, predator-prey relationships among mixotrophic dinoflagellates and co-occurring plankton should be investigated, as should the ingestion rates of mixotrophic dinoflagellates on co-occurring plankton prey. The carbon dynamics within a given ecosystem can be reasonably described by measuring *in situ* ingestion rates of dominant mixotrophic dinoflagellates on co-occurring algal prey. Many mixotrophic dinoflagellates are known to feed on diverse phytoplankton including cyanobacteria, haptophytes, cryptophytes, raphidophytes, and other mixotrophic dinoflagellates (Skovgaard et al. 2000, Jeong et al. 2005*a*, Lee et al. 2015). Therefore, feeding by mixotrophic dinoflagellates on prey is likely to occur frequently because predator and prey usually coexist in natural environments. The CO_2 uptake by mixotrophic dinoflagellates and co-occurring microalgae is likely to be lower when dinoflagellate phagotrophy is occurring than when phagotrophy is absent or rare.

Despite the low availability of CO_2 in the external environment and the low affinity of algal RUBISCO (ribulose-1,5-bisphosphate carboxylase / oxygenase) for CO_2 (Badger et al. 1998), most algae (including coccoliths and diatoms), and cyanobacteria can actively perform photosynthesis by utilizing either CO_2 or HCO_3^- (or both) as external sources of inorganic carbon via a CO_2 concentrating mechanism (CCM) (Reinfelder 2011). Little is known about the mechanisms of C_T uptake in dinoflagellates; however, several species have a CCM in that they are known to have the capability to accumulate inorganic carbon during photosynthesis (Dason et al. 2004). *P. micans* is reported to have a 10-fold higher C_T concentration than seen in the external environment (Nimer et al. 1999). *P. micans* may not need a CCM when feeding on prey, instead reserving C_T for later use when the population of prey is low. Acquiring and reserving C_T via phagotrophy may provide dinoflagellates with a competitive advantage over strictly photosynthetic diatoms. Mixotrophy in dinoflagellates is a unique survival strategy at low CO_2 levels in modern oceans. Dinoflagellates are known to have occurred in the oceans ~400 million years ago (the early Devonian), when CO_2 levels (approximately 3,040 μatm) were eight times higher than at present (~402 μatm) (Falkowski and Raven 1997). As the CO_2 concentration decreased from the early Devonian to the present, dinoflagellates may have risen in importance because of their unique mixotrophic survival strategy. It is worth exploring the relative importance of autotrophy and mixotrophy in dinoflagellates in response to long-term changes in CO_2 concentration.

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