Growth rates and nitrate uptake of co-occurring red-tide dinoflagellates *Alexandrium affine* and *A. fraterculus* as a function of nitrate concentration under light-dark and continuous light conditions

Kyung Ha Lee¹,a, Hae Jin Jeong¹,²,* Hee Chang Kang¹, Jin Hee Ok¹, Ji Hyun You¹ and Sang Ah Park¹

¹School of Earth and Environmental Sciences, College of Natural Sciences, Seoul National University, Seoul 08826, Korea
²Research Institute of Oceanography, Seoul National University, Seoul 08826, Korea

The dinoflagellate genus *Alexandrium* is known to often form harmful algal blooms causing human illness and large-scale mortality of marine organisms. Therefore, the population dynamics of *Alexandrium* species are of primary concern to scientists and aquaculture farmers. The growth rate of the *Alexandrium* species is the most important parameter in prediction models and nutrient conditions are critical parameters affecting the growth of phototrophic species. In Korean coastal waters, *Alexandrium affine* and *Alexandrium fraterculus*, of similar sizes, often form red-tide patches together. Thus, to understand bloom dynamics of *A. affine* and *A. fraterculus*, growth rates and nitrate uptake of each species as a function of nitrate (NO₃) concentration at 100 μmol photons m⁻² s⁻¹ under 14-h light: 10-h dark and continuous light conditions were determined using a nutrient repletion method. With increasing NO₃ concentration, growth rates and NO₃ uptake of *A. affine* or *A. fraterculus* increased, but became saturated. Under light: dark conditions, the maximum growth rates of *A. affine* and *A. fraterculus* were 0.45 and 0.42 d⁻¹, respectively. However, under continuous light conditions, the maximum growth rate of *A. affine* slightly increased to 0.46 d⁻¹, but that of *A. fraterculus* largely decreased. Furthermore, the maximum nitrate uptake of *A. affine* and *A. fraterculus* under light: dark conditions were 12.9 and 30.1 pM cell⁻¹ d⁻¹, respectively. The maximum nitrate uptake of *A. affine* under continuous light conditions was 16.4 pM cell⁻¹ d⁻¹. Thus, *A. affine* and *A. fraterculus* have similar maximum growth rates at the given NO₃ concentration ranges, but they have different maximum nitrate uptake rates. *A. affine* may have a higher conversion rate of NO₃ to body nitrogen than *A. fraterculus*. Moreover, a longer exposure time to the light may confer an advantage to *A. affine* over *A. fraterculus*.

**Key Words:** harmful algal bloom; nitrogen; nutrient; phosphate; protist; red tide

**INTRODUCTION**

The dinoflagellate genus *Alexandrium* often forms red tides or harmful algal blooms, causing human illness and large-scale mortality of marine organisms (Anderson et al. 2012). Therefore, the presence and population dynamics of *Alexandrium* species are primary concerns to scientists and aquaculture farmers (Dias et al. 2015, Eckford-Soper et al. 2016, Hatfield et al. 2019).
most important parameter in prediction models of population dynamics (Jeong et al. 2015). Among 34 officially described *Alexandrium* species, 6-7 species have been revealed to be mixotrophic species (Jacobson and Anderson 1986, Jeong et al. 2005a, 2005b, 2010, Seong et al. 2006, Yoo et al. 2009, Blossom et al. 2012, Lim et al. 2015, Lee et al. 2016). Both exclusively autotrophic and mixotrophic species need nutrients for their growth, and thus nutrient conditions are critical parameters affecting their growth (Yamamoto and Tarutani 1999, Lim et al. 2006, Maguer et al. 2007, Li et al. 2009, Jauzein et al. 2010, Lee et al. 2019). Thus, to understand the population dynamics of an *Alexandrium* species and to predict the outbreak of red tides or harmful algal blooms by them, growth rates of the species under different nutrient conditions should be determined.

In Korean coastal waters, *Alexandrium affine* and *Alexandrium fraterculus* often co-occur (Lee et al. 1998, Kim 2017, our unpublished data). Both species are known to be potentially toxic and also immobilize and / or lyse other protists (Nguyen-Ngoc 2004, Katsu et al. 2007, Anderson et al. 2012, Basti et al. 2015, Lee et al. 2016, Kang et al. 2018). One or both of these two species usually form red-tide patches before the outbreak of red tides by the ichthyotoxic dinoflagellate *Margalefidinium (Cochlodinium) polykrikoides* (Jeong et al. 2017). Thus, the population dynamics of *A. affine* and / or *A. fraterculus* can be used for predicting the outbreak of *M. polykrikoides* red tides. Lee et al. (2016) revealed that *A. affine* and *A. fraterculus* lacked mixotrophic ability, whereas *Alexandrium andersonii* had mixotrophic ability. Therefore, nutrient conditions must be critical parameters affecting their growth. Both *A. affine* and *A. fraterculus* are known to have worldwide distributions; *A. affine* has been observed in European, North American, Asian, and Australian waters, while *A. fraterculus* has been reported to be in North and South American, Australian and New Zealand, and Japanese waters (Fraga et al. 1989, Nakanishi et al. 1996, Moita and Vilarinho 1999, Band-Schmidt et al. 2003, Hansen et al. 2003, Lagos 2003, MacKenzie et al. 2004, Nguyen-Ngoc 2004, Leaw et al. 2005, Omachi et al. 2007, Lee et al. 2009, Nagai et al. 2009, McCarthy 2013, Park et al. 2013). These waters show a wide range of nutrients such as nitrate and phosphate (Fraga et al. 1989, Nogueira et al. 1997, Kang et al. 2019). Furthermore, many phototrophic dinoflagellates can conduct vertical migration (Jeong et al. 2015); they ascend toward well-lit surface waters during the daytime, but descend toward eutrophic deep waters at night. Theoretically, using swimming speed, *A. affine* and *A. fraterculus* could be calculated to travel ca. 15-24 m (Lee et al. 2016, Jeong et al. 2017). Thus, they may experience a wide range of nutrient concentrations every day. To understand the population dynamics of *A. affine* and *A. fraterculus*, the effects of nutrient concentrations on their growth rates should be explored. The duration of day or night varies depending on latitudes and season (Van Haren and Compton 2013). The duration of light is known to affect the growth of dinoflagellates and other phytoplankton groups (e.g., Brand and Guillard 1981). Thus, it is worthwhile to explore effects of the duration of daytime or night on growth of *A. affine* and *A. fraterculus*.

In this present study, growth rates and nitrate uptake of *A. affine* and *A. fraterculus* as a function of nitrate concentration at 100 μmol photons m⁻² s⁻¹ under 14-h light : 10-h dark and continuous light conditions were determined using a nutrient repletion method (Lee et al. 2017). The results of the present study provide a basis for understanding ecophysiology of *A. affine* and *A. fraterculus* and their bloom dynamics.

**MATERIALS AND METHODS**

**Preparation of experimental organisms**

*A. affine* was isolated from coastal waters off Taean (western Korea) in August 2013 when the water temperature and salinity were 21.5°C and 32.2, respectively (Table 1). *A. fraterculus* was isolated from Yeosu (southern Korea) in September 2013 when the water temperature and salinity were 23.4°C and 32.8, respectively. Clonal cultures for both species were established from two serial single isolations.

*A. affine* was grown in enriched f/2-Si seawater medium (Guillard and Ryther 1962), while *A. fraterculus* was grown in enriched L1-Si seawater medium (Guilliard and Hargraves 1993) at 20°C under an illumination of 20 μmol photons m⁻² s⁻¹ of cool white fluorescent light on a 14-h light : 10-h dark cycle.

**Growth and nitrate uptake rates under light and dark conditions**

Experiments 1 and 2 were designed to investigate the growth rates and nitrate (NO₃⁻) uptake of *A. affine* and *A. fraterculus* as a function of NO₃⁻ concentration under light and dark conditions (Table 2). For this measurement, a nutrient repletion method was used (Lee et al. 2017). Dense cultures of *A. affine* and *A. fraterculus*, growing in f/2-Si and L1-Si medium, respectively, were trans-
fered to 800-mL culture flasks. The flasks were placed in a culture room at 20°C under 14-h light : 10-h dark cycle and acclimated at 100 μmol photons m⁻² s⁻¹ for 4 d. Three 1-mL aliquots were subsampled and then cells were enumerated to determine the cell concentration. Ten-milliliter aliquots were filtered through GF/F filters (Whatman Inc., Floreham Park, NJ, USA) and then concentrations of NO₃ (actually nitrate + nitrite in the Cd-coil reduction method) and phosphate (PO₄) were measured using a nutrient analyzer (QuAatro, Seal Analytical, Norderstedt, Germany).

Cells of either species were added to triplicate 800-mL culture flasks by transferring predetermined volumes of cultures (final cell concentration = 100 cells mL⁻¹ for both species). A stock solution of NO₃ made based on the f/2 medium concentration was added for target final concentrations of NO₃ = ca. 110 μM. A sufficient amount of stock solution of PO₄ (final concentration = ca. 20 μM), also prepared based on the f/2 medium concentration, was added so that it would not be limiting before NO₃ was limiting. Trace metals and vitamins were also added plentifully with consideration of the ratio of nitrate to each chemical in an f/2 medium.

The flasks were placed in a temperature-controlled culture room and incubated at 20°C under an illumination of 100 μmol photons m⁻² s⁻¹ of cool white fluorescent light on a 14-h light : 10-h dark cycle. Thirty-milliliter aliquots were subsampled from each flask every day for 2 weeks, and 10-mL aliquots were used for the determination of cell concentration and 20-mL aliquots for the determination of NO₃ and PO₄ concentrations were filtered through GF/F filters. Cell concentrations (abundances) were determined by enumerating cells on three 1-mL Sedgwick-Rafter counting chambers. The concentrations of nutrients were measured using a nutrient analyzer.

The mean NO₃ concentration (N*) at each interval was calculated as:

\[ N^* (\mu M) = \frac{[N_{t2} - N_{t1}]}{\ln(N_{t2} / N_{t1})} \]  

where \( N = NO₃ \) concentration at a single day, \( t_2 - t_1 = 1 \) d.

The specific growth rates of each Alexandrium species (\( \mu, \text{ d}^{-1} \)) at each N* were calculated as:

\[ \mu = \frac{\ln(C_{t2} / C_{t1})}{(t_2 - t_1)} \]

where \( C_{t1} \) and \( C_{t2} = \text{cell concentrations of each Alexandrium species at Day } t_1 \text{ and Day } t_2, \text{ respectively.} \)

The maximum growth rate (\( \mu_{\text{max}}, \text{ d}^{-1} \)) of each Alexandrium species was obtained after data were fitted to a Michaelis-Menten equation:

\[ \mu = \mu_{\text{max}} [N^* / (K_{GR-NO3} + N^*)] \]

where \( K_{GR-NO3} = \text{the NO₃ concentration sustaining } 1/2\mu_{\text{max}} \).

Data were iteratively fitted to the model using DeltaGraph (SPSS Inc., Chicago, IL, USA).

The daily NO₃ uptake of each Alexandrium cell was determined by dividing the reduction in N* by the mean cell concentration (C*) at 1 d intervals.

\[ \frac{(N_{t2} - N_{t1})}{(N^{*t2} - N^{*t1})} \]

\[ C^* (\text{cells mL}^{-1}) = \frac{[C_{t2} - C_{t1}]}{\ln(C_{t2} / C_{t1})} \]

where \( t_2 - t_1 = 1 \) d. Day 0 to 3 for A. affine and Day 0 to 1 for A. fraterculus were treated as the acclimation period, and thus data from these days were not used in calculations. The maxi-

| Table 1. Conditions for the isolation of Alexandrium affine and A. fraterculus used in this study |
|--------|------|--------|--------|--------|--------|
| Species | Strain name | ESD | Location | Time | T | S |
|------|--------|------|--------|------|--------|--------|
| Alexandrium affine | AATA1308 | 31.4 | Yeosu, Korea | Aug 2013 | 21.5 | 32.2 |
| Alexandrium fraterculus | AFVS1309 | 32.3 | Taean, Korea | Sep 2013 | 23.4 | 32.8 |

ESD, equivalent spherical diameter (μM); T, temperature (°C); S, salinity.

| Table 2. Experimental design and the light and nutrient conditions |
|-------|------|--------|--------|--------|--------|
| Experiment | Species | L | L : D cycle | ICC | INO₃ | IPO₄ |
|------|--------|------|--------|------|--------|--------|
| 1   | Alexandrium affine | 100 | 14 : 10 | 115 | 105.1 | 21.2 |
| 2   | Alexandrium fraterculus | 100 | 14 : 10 | 95 | 103.6 | 21.2 |
| 3   | Alexandrium affine | 100 | Continuous light | 105 | 110.7 | 21.2 |
| 4   | Alexandrium fraterculus | 100 | Continuous light | 102 | 104.3 | 21.2 |

L, light intensity (μmol photons m⁻² s⁻¹); L : D cycle, light and dark cycle (hour : hour); ICC, initial cell concentration (cells mL⁻¹); INO₃, initial concentration of NO₃ (μM); IPO₄, initial concentration of PO₄ (μM).
Growth and nitrate uptake rates under continuous light conditions

Experiments 3 and 4 were designed to investigate the growth rates and NO$_3$ uptake of each _Alexandrium_ species as a function of NO$_3$ concentrations under continuous light conditions (Table 2). For this measurement, a nutrient repletion method was used (Lee et al. 2017). The procedure of setting up incubating flasks, subsampling, determining cell abundances and nutrient concentrations, and incubation conditions was the same as in experiments 1 and 2 except for the light conditions.

Fig. 1. (A-C) Change in the concentration (abundance, AB) of _Alexandrium affine_ (cells mL$^{-1}$) as a function of elapsed time (d) under an illumination of 100 μmol photons m$^{-2}$ s$^{-1}$ on a 14-h light : 10-h dark (LD) cycle. The concentration of _A. affine_ provided in the normal scale (A) and natural log (Ln) scale at Day 0 to 14 (B) and at Day 1 to 9 (C). (D-F) Change in the NO$_3$ (D) and PO$_4$ (E) concentrations and ratio of NO$_3$ relative to PO$_4$ (F) concentration as a function of elapsed time (d). Symbols represent each treatment. The curves in (B) and (C) are fitted to a linear regression using all the treatments obtained from Day 0 to 14 (B) and from Day 1 to 9 (C). (B) Ln (AB) = 0.332 (d) + 4.89, $r^2 = 0.957$; (C) Ln (AB) = 0.425 (d) + 4.50, $r^2 = 0.993$.

The maximum NO$_3$ uptake of each _Alexandrium_ species ($V$, pM cell$^{-1}$ d$^{-1}$) was obtained after data were fitted to a Michaelis-Menten equation:

$$V = V_{\text{max}} \frac{N^*}{(K_{UT-NO3} + N^*)}$$  \hspace{1cm} (6)

where $V_{\text{max}}$ = maximum uptake rate (pM cell$^{-1}$ d$^{-1}$), $N^*$ = mean NO$_3$ concentration (μM), and $K_{UT-NO3}$ = half saturation constant for NO$_3$ uptake (μM).

The mean PO$_4$ concentration ($P^*$), specific growth rates of each _Alexandrium_ species at each $P^*$, and daily PO$_4$ uptake of each cell were calculated in the same manner.
**RESULTS**

**Growth and nitrate uptake rates of* Alexandrium affine* under light and dark conditions**

In experiment 1, with increasing incubation time, the concentration of *A. affime* rapidly increased from 115 cells mL\(^{-1}\) at Day 0 to 5,272 cells mL\(^{-1}\) at Day 10 and then continued to slightly increase (Fig. 1A). The maximum cell concentration (mean ± standard error [SE]) of *A. affime* concentrations was 7,250 ± 468 cells mL\(^{-1}\) which was achieved at Day 14, the last day of the experiment. The growth rate of *A. affime*, calculated from linear regression equation, using data between Day 1 and 9, was 0.425 d\(^{-1}\) (Fig. 1B & C). Furthermore, with increasing incubation time, the NO\(_3\) concentration (mean ± SE) rapidly decreased from 105.1 ± 0.4 μM at Day 0 to 11.0 ± 0.6 μM at Day 10 and then became 0.9-1.2 μM at Day 11 to 14 (Fig. 1D). Moreover, with increasing incubation time, the PO\(_4\) concentration (mean ± SE) rapidly decreased from 21.2 ± 0.3 μM at Day 0 to 4.5 ± 0.2 μM at Day 14 (Fig. 1E). With increasing elapsed incubation time, the ratio of NO\(_3\) relative to PO\(_4\) decreased from 5.0-5.3 at Day 0-4 to 2.5 at Day 9 and 0.1-0.2 at Day 11-14 (Fig. 1F).

With increasing NO\(_3\) concentration, the growth rate of *A. affime* rapidly increased and then became saturated (Fig. 2A). When the data were fit to Eq. (3), the maximum growth rate (μ\(_{max}\)) of *A. affime* was 0.447 d\(^{-1}\) and the half saturation constant for growth rate (K\(_{GR-NO3}\)) was 8.7 μM. However, at the given range of PO\(_4\) concentrations, the growth rate of *A. affime* linearly increased with increasing PO\(_4\) concentration (Fig. 2B).

With increasing NO\(_3\) concentration, the NO\(_3\) uptake of *A. affime* rapidly increased initially, but then increased...
more slowly (Fig. 3A). When the data were fit to Eq. (4-6), the maximum NO₃ uptake of *A. affine* was 12.9 pM cell⁻¹ d⁻¹ and the half saturation constant for uptake (K_{UT-NO3}) was 37.5 μM. However, at the given range of PO₄ concentrations, the PO₄ uptake of *A. affine* linearly increased with increasing PO₄ concentration (Fig. 3B).

**Growth and nitrate uptake rates of *Alexandrium fraterculus* under light and dark conditions**

In experiment 2, with increasing incubation time, the concentration of *A. fraterculus* increased from 95 cells mL⁻¹ at Day 0 to ca. 4,350 cells mL⁻¹ at Day 13 and then slightly decreased (Fig. 4A). The growth rate of *A. fraterculus* calculated from linear regression equation using data between Day 1 and 9 was 0.389 d⁻¹ (Fig. 4B & C). Furthermore, with increasing incubation time, the NO₃ concentration rapidly decreased from 103.6 ± 0.5 μM at Day 0 to 4.2 ± 2.7 μM at Day 9 and then became 0.8-1.3 μM at Day 10 to 14 (Fig. 4D). Moreover, with increasing incubation time, the PO₄ concentration decreased from 21.2 ± 0.2 μM at Day 0 to 11.0 ± 0.2 μM at Day 14 (Fig. 4E). With increasing elapsed incubation time, the ratio of NO₃ relative to PO₄ concentration decreased from 4.9-5.0 at Day 0-2 to 1.5 at Day 8 and 0.1-0.3 at Day 9-14 (Fig. 4F).

With increasing NO₃ concentration, the growth rate of *A. fraterculus* rapidly increased and then became saturated (Fig. 5A). When the data were fit to Eq. (3), the maximum growth rate (μ_{max}) of *A. fraterculus* was 0.422 d⁻¹ and half saturation constant for growth rate (K_{GR-NO3}) was 5.4...
concentration of \(A. \text{fraterculus}\) linearly increased with increasing \(\text{PO}_4\) concentration (Fig. 5B).

With increasing \(\text{NO}_3\) concentration, the \(\text{NO}_3\) uptake of \(A. \text{fraterculus}\) rapidly increased initially, but then increased more slowly (Fig. 6A). When the data were fit to Eq. (4-6), the maximum \(\text{NO}_3\) uptake by \(A. \text{fraterculus}\) was 30.1 pM cell\(^{-1}\) d\(^{-1}\) and the half saturation constant for uptake \(K_{\text{UT-NO}_3}\) was 44.4 μM. However, at the given range of \(\text{PO}_4\) concentrations, the \(\text{PO}_4\) uptake of \(A. \text{fraterculus}\) linearly increased with increasing \(\text{PO}_4\) concentration (Fig. 6B).

**Growth and nitrate uptake rates of *Alexandrium affine* under the continuous light conditions**

In experiment 3, with increasing incubation time, the concentration of \(A. \text{affine}\) rapidly increased from 105 cells mL\(^{-1}\) at Day 0 to 5,779 cells mL\(^{-1}\) at Day 12 and then became saturated (Fig. 7A). The growth rate of \(A. \text{affine}\), calculated from a linear regression equation using data between Day 1 and 9, was 0.405 d\(^{-1}\) (Fig. 7B & C). Furthermore, with increasing incubation time, the \(\text{NO}_3\) concentration rapidly decreased from 110.7 ± 6.3 μM at Day 0 to 15.7 ± 4.2 μM at Day 11 and then became 1.1-2.6 μM at Day 12 to 14 (Fig. 7D). Moreover, with increasing elapsed incubation time, the \(\text{PO}_4\) concentration decreased from 21.2 ± 0.3 μM at Day 0 to 8.4 ± 0.5 μM at Day 14 (Fig. 7E). With increasing incubation time, the ratio of \(\text{NO}_3\) relative to \(\text{PO}_4\) decreased from 5.0-5.2 at Day 0-5 to 1.3 at Day 11 and 0.1-0.3 at Day 12-14 (Fig. 7F).

With increasing \(\text{NO}_3\) concentration, the growth rate of \(A. \text{affine}\) rapidly increased and then became saturated (Fig. 8A). When the data were fit to Eq. (3), the maximum...
Growth and nitrate uptake rates of *Alexandrium fraterculus* under the continuous light conditions

In experiment 4, with increasing incubation time, the concentration of *A. fraterculus* increased from 102 cells mL\(^{-1}\) at Day 0 to 1,587 cells mL\(^{-1}\) at Day 14 (Fig. 10A). The growth rate of *A. fraterculus*, calculated from linear regression equation using data between Day 2 and 9, was 0.295 d\(^{-1}\) (Fig. 10B & C). Furthermore, with increasing incubation time, the NO\(_3\) concentration decreased from 104.3 ± 0.3 μM at Day 0 to 49.9 ± 4.1 μM at Day 14 (Fig. 10D). Moreover, with increasing incubation time, the PO\(_4\) concentration increased from 87.7 ± 1.2 μM at Day 0 to 49.9 ± 4.1 μM at Day 14 (Fig. 10E). Conversely, the ratio of NO\(_3\) to PO\(_4\) concentration decreased from 2.1 ± 0.1 at Day 0 to 1.0 ± 0.1 at Day 14 (Fig. 10F).

**Fig. 7.** (A-C) Change in the concentration of *Alexandrium affine* (cells mL\(^{-1}\)) as a function of elapsed time (d) at 100 μmol photons m\(^{-2}\) s\(^{-1}\) under continuous light conditions (CL). The concentration of *A. affine* provided in the normal scale (A) and natural log (Ln) scale at Day 0 to 14 (B) and at Day 1 to 9 (C). (D-F) Change in the NO\(_3\) (D) and PO\(_4\) (E) concentrations and ratio of NO\(_3\) relative to PO\(_4\) (F) concentration as a function of elapsed time (d). Symbols represent each treatment. The curves in (B) and (C) are fitted to a linear regression using all the treatments obtained from Day 0 to 14 (B) and from Day 1 to 9 (C). (B) Ln (AB) = 0.324 (d) + 4.79, \(r^2 = 0.961\); (C) Ln (AB) = 0.405 (d) + 4.46, \(r^2 = 0.986\).
DISCUSSION

The growth rate of *A. affine* at 64 μM, which was the mean NO₃ concentration between Day 1 and 9, calculated using the Michaelis-Menten type equation in Fig. 2A (0.394 d⁻¹), is only 8% different from that calculated using the linear regression equation in Fig. 1C (0.425 d⁻¹). Similarly, the growth rate of *A. fraterculus* at 31 μM, which was the mean NO₃ concentration between Day 0 to 9, calculated using the Michaelis-Menten type equation in Fig. 5A (0.360 d⁻¹), is also only 8% different from that calculated using the linear regression equation in Fig. 4C (0.389 d⁻¹). Thus, the nutrient repletion method is a reasonable tool for determining growth rates of *A. affine* and *A. fraterculus* as a function of NO₃ concentration.

The maximum growth rate of *A. affine* obtained in the present study is comparable to that reported in Nguyen-
from Taean (West Sea of Korea) and Yeosu (South Sea of Korea) from which *A. affine* and *A. fraterculus* were isolated, were 1.6-10.7 and 0.8-26.4 μM, respectively (Kang et al. 2019). Furthermore, the range of NO$_3$ concentrations in Junk Bay, Hong Kong and Ria de Vigo, northwest Spain, from which *A. affine* was found were 0-15.0 and 0-30.0 μM, respectively (Fraga et al. 1989, Nogueira et al. 1997, Hodgkiss and Lu 2004, Lee et al. 2009). The K$\text{GR-NO}_3$ of *A. affine* (8.7 μM) and *A. fraterculus* (5.4 μM) fall in the ranges of the NO$_3$ concentrations in the waters off Taean and Yeosu, Korea, Junk Bay, Hong Kong, and Ria de Vigo, Spain. Therefore, a small change in NO$_3$ concentrations in these waters may sometimes cause a large change in growth rates of *A. affine* and *A. fraterculus*.

Ngoc (2004) and Lim et al. (2019), while the maximum growth rate of *A. fraterculus* obtained in the present study is slightly higher than Lim et al. (2007) (Table 3). The present study reports for the first time the half saturation constant for growth rate (K$\text{GR-NO}_3$) and for NO$_3$ uptake (K$\text{UT-NO}_3$) of *A. affine* and *A. fraterculus* (Table 3). The values of K$\text{GR-NO}_3$ and K$\text{UT-NO}_3$ are important in understanding competition among red-tide or harmful algal bloom species, because those with lower K$\text{GR-NO}_3$ and K$\text{UT-NO}_3$ can grow and uptake NO$_3$ rapidly at lower NO$_3$ concentrations. Furthermore, growth rates and NO$_3$ uptake of the species largely change when NO$_3$ concentrations change near K$\text{GR-NO}_3$ and K$\text{UT-NO}_3$. The range of NO$_3$ concentrations in the waters, collected from April 2015 to October 2018, from Taean (West Sea of Korea) and Yeosu (South Sea of Korea) from which *A. affine* and *A. fraterculus* were isolated, were 1.6-10.7 and 0.8-26.4 μM, respectively (Kang et al. 2019). Furthermore, the range of NO$_3$ concentrations in Junk Bay, Hong Kong and Ria de Vigo, northwest Spain, from which *A. affine* was found were 0-15.0 and 0-30.0 μM, respectively (Fraga et al. 1989, Nogueira et al. 1997, Hodgkiss and Lu 2004, Lee et al. 2009). The K$\text{GR-NO}_3$ of *A. affine* (8.7 μM) and *A. fraterculus* (5.4 μM) fall in the ranges of the NO$_3$ concentrations in the waters off Taean and Yeosu, Korea, Junk Bay, Hong Kong, and Ria de Vigo, Spain. Therefore, a small change in NO$_3$ concentrations in these waters may sometimes cause a large change in growth rates of *A. affine* and *A. fraterculus*.

Fig. 10. (A-C) Change in the concentration of *Alexandrium fraterculus* (cells mL$^{-1}$) as a function of elapsed time (d) at 100 μmol photons m$^{-2}$ s$^{-1}$ under continuous light conditions (CL). The concentration of *A. fraterculus* provided in the normal scale (A) and natural log (Ln) scale at Day 0 to 14 (B) and at Day 2 to 9 (C). (D-F) Change in the NO$_3$ (D) and PO$_4$ (E) concentrations and ratio of NO$_3$ relative to PO$_4$ (F) concentration as a function of elapsed time (d). Symbols represent each treatment. The curves in (B) and (C) are fitted to a linear regression using all the treatments obtained from Day 0 to 14 (B) and from Day 2 to 9 (C). (B) Ln (AB) = 0.217 (d) + 4.75, $r^2$ = 0.936; (C) Ln (AB) = 0.295 (d) + 4.43, $r^2$ = 0.951.
Table 3. Maximum growth rates ($\mu_{max}$, d$^{-1}$) of *Alexandrium affine* and *A. fraterculus*, half saturation constant for growth rate as a function of nitrate concentration ($K_{GR-NO_3}$, μM), nitrate maximum uptake ($V_{max-NO_3}$, pM cell$^{-1}$ d$^{-1}$), half saturation constant for the nitrate uptake as a function of nitrate concentration ($K_{UT-NO_3}$, μM) and conditions for growth

| Species / ESD | $\mu_{max}$ | $K_{GR-NO_3}$ | $V_{max-NO_3}$ | $K_{UT-NO_3}$ | T     | S     | L : D cycle | NO$_3$ | Reference                  |
|---------------|-------------|---------------|----------------|----------------|-------|-------|-------------|--------|---------------------------|
| *A. affine*   |             |               |                |                |       |       |             |        |                           |
| 29.7          | 0.60        | -             | -              | 25             | -     | -     | -           | -      | Jeong et al. (2010)       |
| 31.4          | 0.34        | -             | -              | 5-30           | 230   | 10 : 14| -           | -      | Band-Schmidt et al. (2003)|
| 31.4          | 0.37        | -             | -              | 15-25          | 150   | 15 : 9 | -           | -      | Lim et al. (2007)         |
| 31.4          | 0.43        | -             | -              | 15-30          | 100   | 12 : 12| -           | -      | Lim et al. (2019)         |
| 31.4          | 0.45        | 8.7           | 12.9           | 37.5           | 20    | -     | 100         | 14 : 10| This study                |
| 31.4          | 0.46        | 21.5          | 16.4           | 83.3           | 20    | -     | 100         | CL     | 111                       |
| 31.4          | 0.90        | -             | -              | 22             | 120   | 16 : 8| 5-400       |        | Lee et al. (2009)         |
| 35.0          | 0.49        | -             | -              | 21-27          | 25    | 12 : 12| -           | -      | Nguyen-Ngoc (2004)        |
| *A. fraterculus* |         |               |                |                |       |       |             |        |                           |
| 32.3          | 0.35        | -             | -              | 15-25          | 150   | 15 : 9 | -           | -      | Lim et al. (2007)         |
| 32.3          | 0.42        | 5.4           | 30.1           | 44.4           | 20    | 100   | 14 : 10     | 104    | This study                |

ESD, equivalent spherical diameter (μm); T, temperature (°C); S, salinity; L, light intensity (μmol photons m$^{-2}$ s$^{-1}$); L : D cycle, light and dark cycle (hour : hour); NO$_3$, range of concentration of nitrate used in experiment (μM); CL, continuous light.

*Data from Kang et al. (2018).*
The range of NO$_3$ concentrations in the waters, collected from April 2015 to October 2018, from 9 stations located in South Sea of Korea in which _A. affine_ and _A. fraterculus_ have caused red tides or harmful algal blooms, was 0.4-102.9 μM (Kang et al. 2019). Moreover, the range of NO$_3$ concentrations in Seto Inland Sea, Japan in which _A. affine_ and _A. fraterculus_ were found was 5.4-70.0 μM (Nakanishi et al. 1996, Montani et al. 1998, Nagai et al. 2009). The $K_{\text{UT-NO3}}$ of _A. affine_ (37.5 μM) and _A. fraterculus_ (44.4 μM) fall in the ranges of the NO$_3$ concentrations in South Sea of Korea and Seto Inland Sea. Therefore, a small change in NO$_3$ concentrations in South Sea of Korea and Seto Inland Sea may sometimes cause a large change in the NO$_3$ uptake of _A. affine_ and _A. fraterculus_. In addition, the $K_{\text{UT-NO3}}$ of _A. affine_ was lower than that of _A. fraterculus_, while the $K_{\text{GR-NO3}}$ of _A. fraterculus_ was lower than that of _A. affine_. Therefore, _A. affine_ rapidly uptakes NO$_3$ at lower NO$_3$ concentrations than _A. fraterculus_, but _A. fraterculus_ rapidly grows at lower NO$_3$ concentrations than _A. affine_.

Interestingly, the $K_{\text{GR-NO3}}$ of _A. affine_ and _A. fraterculus_ were much lower than their $K_{\text{UT-NO3}}$. That is, the growth rates of _A. affine_ and _A. fraterculus_ became saturated at the NO$_3$ concentrations at which NO$_3$ uptakes increased. For a cell to divide, the cell should uptake a certain amount of NO$_3$. Thus, NO$_3$ acquired by the cell may not have been high enough to be used for cell division. Similar patterns are often observed in feeding by mixotrophic and heterotrophic protists; growth rate of a mixotrophic or heterotrophic protist on algal prey become saturated at prey concentrations at which its ingestion rates increase (Jeong et al. 2007, 2014, 2018a, 2018b, Lim et al. 2014). In conclusion, the values of $K_{\text{GR-NO3}}$ and $K_{\text{UT-NO3}}$ of a species should be determined separately because they can be different from each other. The nutrient repletion method can be a useful tool for determining $K_{\text{GR-NO3}}$ and $K_{\text{UT-NO3}}$ of the species as a function of NO$_3$ concentration simultaneously.

The maximum NO$_3$ uptake of _A. affine_ (ca. 13-16 pM cell$^{-1}$ d$^{-1}$) is comparable to that of _Alexandrium minutum_ (4.3-16.8 pM cell$^{-1}$ d$^{-1}$) and _Alexandrium tamiyavanichii_ (ca. 10-20 pM cell$^{-1}$ d$^{-1}$), but the maximum NO$_3$ uptake of _A. fraterculus_ (ca. 30 pM cell$^{-1}$ d$^{-1}$) is greater than that of these _Alexandrium_ species (Lim et al. 2006, Maguer et al. 2007). The size of _A. fraterculus_ (32.3 μm) is larger than that of _A. minimum_ (20.5 μm), comparable to that of _A. affine_ (31.4 μm), but smaller than that of _A. tamiyavanichii_ (38.6 μm). The maximum NO$_3$ uptake of these _Alexandrium_ species was not significantly correlated with their size (p > 0.1, linear regression ANOVA). Thus, the maximum NO$_3$ uptake of an _Alexandrium_ species should be measured because it may not be calculated using the equation of a regression between the maximum NO$_3$ uptake and size of _Alexandrium_ species.

The maximum growth rate of _A. fraterculus_ under continuous light conditions was much lower than that under light and dark conditions, whereas the maximum growth rate of _A. affine_ grown under continuous light conditions was slightly higher than that under light and dark conditions. This evidence suggests that growth rate of _A. fraterculus_ may be inhibited by continuous light. One scenario is that _A. fraterculus_ has a strong circadian rhythm and a continuous light condition disturbs this rhythm. There have been many dinoflagellates species showing circadian rhythms (Prézelin et al. 1977, Knaust et al. 1998, Van Dolah et al. 2007, Dapena et al. 2015); _A. minutum_ showed nuclear and morphological changes during cell cycle and growth, indicating a strong circadian rhythm (Dapena et al. 2015). In contrast, to our best knowledge, there has only been one dinoflagellate species identified as showing inhibition of growth under a continuous light condition; the growth rate of _Tripodiscus (Ceratium) ranipes_ under a continuous light of 320 μmol photons m$^{-2}$ s$^{-1}$ was much lower than that under light and dark cycle (Brand and Guillard 1981). Thus, the present study adds _A. fraterculus_ to a few dinoflagellate species showing inhibition of growth under a continuous light condition. Another scenario is that an excessive amount of photons inhibits the operation of photosystems of _A. fraterculus_ (Richardson et al. 1983, Krause 1988). The amount of photons produced by a continuous light is ca. 40% greater than that by 14-h illumination. Thus, the amount of photons produced by a continuous light at 100 μmol photons m$^{-2}$ s$^{-1}$ is equivalent to that at 140 μmol photons m$^{-2}$ s$^{-1}$ under 14-h light : 10-h dark cycle. The autotrophic growth of the mixotrophic dinoflagellate _Takayama helix_ is known to be inhibited at 115 μmol photons m$^{-2}$ s$^{-1}$ under 14-h light : 10-h dark cycle (Ok et al. 2019). Therefore, it is worthwhile to explore possible inhibition of growth of _A. fraterculus_ at high light intensities under 14-h light : 10-h dark cycle to test this hypothesis.

**ACKNOWLEDGEMENTS**

We thank An Suk Lim, Sung Yeon Lee, and Se Hyun Jang for technical support. This research was supported by the Useful Dinoflagellate program of Korea Institute of Marine Science and Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (MOF) and...
the National Research Foundation (NRF) funded by the Ministry of Science and ICT (NRF-2015M1A5A1041806; NRF-2017R1E1A01074419) award to HJ and (NRF-2019R1C1C1008546) to KHL.

REFERENCES

Anderson, D. M., Alpermann, T. J., Cembella, A. D., Collos, Y., Masseret, E. & Montgomery, M. 2012. The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health. Harmful Algae 14:10-35.

Band-Schmidt, C. J., Lechuga-Devêze, C. H., Kulis, D. M. & Anderson, D. M. 2003. Culture studies of *Alexandrium affine* (Dinophyceae), a non-toxic cyst forming dinoflagellate from Bahía Concepción, Gulf of California. Bot. Mar. 46:44-54.

Basti, L., Nagai, S., Go, J., Okano, S., Nagai, K., Watanabe, R., Suzuki, T. & Tanaka, Y. 2015. Differential inimical effects of *Alexandrium* spp. and *Karenia* spp. on cleavage, hatching, and two larval stages of Japanese pearl oyster *Pinctada fucata martensii*. Harmful Algae 43:1-12.

Blossom, H. E., Daugbjerg, N. & Hansen, P. J. 2012. Toxic mucus traps: a novel mechanism that mediates prey uptake in the mixotrophic dinoflagellate *Alexandrium pseuderogonyaulax*. Harmful Algae 17:40-53.

Brand, L. E. & Guillard, R. R. L. 1981. The effects of continuous light and light intensity on the reproduction rates of twenty-two species of marine phytoplankton. J. Exp. Mar. Biol. Ecol. 50:119-132.

Dapena, C., Bravo, I., Cuadrado, A. & Figueroa, R. I. 2015. Nuclear and cell morphological changes during the cell cycle and growth of the toxic dinoflagellate *Alexandrium minutum*. Protist 166:146-160.

Dias, J., Muñoz, J., Huisman, J. & McDonald, J. 2015. Biosecurity monitoring of Harmful Algal Bloom (HAB) species in Western Australian waters: first confirmed record of *Alexandrium catenella* (Dinophyceae). Bioinvasions Rec. 4:233-241.

Eckford-Soper, L. K., Bresnan, E., Lacaze, J. -P., Green, D. H. & Davidson, K. 2016. The competitive dynamics of toxic *Alexandrium fundyense* and non-toxic *Alexandrium tamarense*: the role of temperature. Harmful Algae 53:135-144.

Fraga, S., Gallager, S. M. & Anderson, D. M. 1989. Chain-forming dinoflagellates: an adaptation to red tides. *In Okaichi, T., Anderson, D. M. & Nemoto, T. (Eds.) Red Tides: Biology, Environmental Science and Toxicology*. Elsevier, New York, pp. 281-284.

Guillard, R. R. L. & Hargraves, P. E. 1993. *Stichocorysis immobils* is a diatom, not a chrysophyte. Phycologia 32:234-236.

Guillard, R. R. L. & Ryther, J. H. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol. 8:229-239.

Hansen, G., Daugbjerg, N. & Franco, I. M. 2003. Morphology, toxin composition and LSU rDNA phylogeny of *Alexandrium minutum* (Dinophyceae) from Denmark, with some morphological observations on other European strains. Harmful Algae 2:317-335.

Hatfield, R. G., Bean, T., Turner, A. D., Lees, D. N., Lowther, J., Lewis, A. & Baker-Austin, C. 2019. Development of a TaqMan qPCR assay for detection of *Alexandrium* spp. and application to harmful algal bloom monitoring. Toxicon X 2:100011.

Hodgkiss, I. J. & Lu, S. 2004. The effects of nutrients and their ratios on phytoplankton abundance in Junk Bay, Hong Kong. *In Ang, P. O. (Ed.) Asian Pacific Phycology in the 21st Century: Prospects and Challenges*. Springer, Dordrecht, pp. 215-229.

Jacobson, D. M. & Anderson, D. M. 1986. Thecate heterotrophic dinoflagellates: feeding behavior and mechanisms. J. Phycol. 22:249-258.

Jauzein, C., Labry, C., Youenou, A., Quéré, J., Delmas, D. & Collos, Y. 2010. Growth and phosphorus uptake by the toxic dinoflagellate *Alexandrium catenella* (Dinophyceae) in response to phosphate limitation. J. Phycol. 46:926-936.

Jeong, H. J., Kang, H. C., You, J. H. & Jang, S. H. 2018a. Interactions between the newly described small- and fast-swimming mixotrophic dinoflagellate *Yihiella yeosuensis* and common heterotrophic protists. J. Eukaryot. Microbiol. 65:612-626.

Jeong, H. J., Kim, J. S., Song, J. Y., Kim, J. H., Kim, T. H., Kim, S. K. & Kang, N. S. 2007. Feeding by protists and copepods on the heterotrophic dinoflagellates *Pfiesteria piscicida*, *Stoeckeria algicida*, and *Luciella masanensis*. Mar. Ecol. Prog. Ser. 349:199-211.

Jeong, H. J., Lim, A. S., Franks, P. J. S., Lee, K. H., Kim, J. H., Kang, N. S., Lee, M. J., Jang, S. H., Lee, S. Y., Yoon, E. Y., Park, J. Y., Yoo, Y. D., Seong, K. A., Kwon, J. E. & Jang, T. Y. 2015. A hierarchy of conceptual models of red-tide generation: nutrition, behavior, and biological interactions. Harmful Algae 47:97-115.

Jeong, H. J., Lim, A. S., Lee, K., Lee, M. J., Seong, K. A., Kang, N. S., Jang, S. H., Lee, K. H., Lee, S. Y., Kim, M. O., Kim, J. H., Kwon, J. E., Kang, H. C., Kim, J. S., Yih, W., Shin, K., Jang, P. K., Ryu, J. -H., Kim, S. Y., Park, J. Y. & Kim, K. Y. 2017. Ichthyotoxic *Cochlodinium polykrikoides* red
tides offshore in the South Sea, Korea in 2014: I. temporal variations in three-dimensional distributions of red-tide organisms and environmental factors. Algae 32:101-130.

Jeong, H. J., Lim, A. S., Yoo, Y. D., Lee, M. J., Lee, K. H., Jang, T. Y. & Lee, K. 2014. Feeding by heterotrophic dinoflagellates and ciliates on the free-living dinoflagellate *Symblo-dinium* sp. (Clade E). J. Eukaryot. Microbiol. 61:27-41.

Jeong, H. J., Park, J. Y., Nho, J. H., Park, M. O., Ha, J. H., Seong, K. A., Jeng, C., Seong, C. N., Lee, K. Y. & Yih, W. H. 2005a. Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. Aquat. Microb. Ecol. 41:131-143.

Jeong, H. J., Yoo, Y. D., Kim, J. S., Seong, K. A., Kang, N. S. & Kim, T. H. 2010. Growth, feeding, and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic foods. Ocean Sci. J. 45:65-91.

Jeong, H. J., Yoo, Y. D., Park, J. Y., Song, J. Y., Kim, S. T., Lee, S. H., Kim, K. Y. & Yih, W. H. 2005b. Feeding by the phototrophic red-tide dinoflagellates: five species newly revealed and six species previously known to be mixotrophic. Aquat. Microb. Ecol. 40:133-150.

Jeong, H. J., You, J. H., Lee, K. H., Kim, S. J. & Lee, S. Y. 2018b. Feeding by common heterotrophic protists on the mixotrophic alga *Gymnodinium smithiae* (Dinophyceae), one of the fastest growing dinoflagellates. J. Phycol. 54:734-743.

Kang, H. C., Jeong, H. J., Kim, S. J., You, J. H. & Ok, J. H. 2018. Differential feeding by common heterotrophic protists on 12 different *Alexandrium* species. Harmful Algae 78:106-117.

Kang, H. C., Jeong, H. J., Ok, J. H., You, J. H., Jang, S. H., Lee, S. Y., Lee, K. H., Park, J. Y. & Rho, J. -R. 2019. Spatial and seasonal distributions of the phototrophic dinoflagellate *Biecheleirosis adriatica* (Suessiaceae) in Korea: quantification using qPCR. Algae 34:111-126.

Katsuo, D., Kim, D., Yamaguchi, K., Matsuyama, Y. & Oda, T. 2007. A new simple screening method for the detection of cytotoxic substances produced by harmful red tide phytoplankton. Harmful Algae 6:790-798.

Kim, J. H. 2017. Spatiotemporal distribution and a survival strategy of harmful dinoflagellates *Alexanderium* spp. in Korean coastal waters and application to controlling scuticociliates. M.S. thesis. Seoul National University, Seoul, Korea. 136 pp.

Knaust, R., Urbig, T., Li, L., Taylor, W. & Hastings, J. W. 1998. The circadian rhythm of bioluminescence in *Pyrocystis* is not due to differences in the amount of luciferase: a comparative study of three bioluminescent marine dinoflagellates. J. Phycol. 34:167-172.

Krause, G. H. 1988. Photoinhibition of photosynthesis: an evaluation of damaging and protective mechanisms. Physiol. Plant. 74:566-574.

Lagos, N. 2003. Paralytic shellfish poisoning phycotoxins: occurrence in South America. Comments Toxicol. 9:175-193.

Leaw, C. P., Lim, P. T., Ng, B. K., Cheah, M. Y., Ahmad, A. & Usup, G. 2005. Phylogenetic analysis of *Alexandrium* species and *Pyrodinium bahamense* (Dinophyceae) based on theca morphology and nuclear ribosomal gene sequence. Phycologia 44:550-565.

Lee, F. W. -F., Morse, D. & Lo, S. C. -L. 2009. Identification of two plastid proteins in the dinoflagellate *Alexandrium affine* that are substantially down-regulated by nitrogen-depletion. J. Proteome Res. 8:5080-5092.

Lee, J. -B., Kim, D. Y. & Lee, J. 1998. Community dynamics and distribution of dinoflagellates and their cysts in Masan-Chinhae Bay, Korea. Fish. Aquat. Sci. 1:283-292.

Lee, K. H., Jeong, H. J. & Lim, A. S. 2017. Nitrate uptake of the red tide dinoflagellate *Prorocentrum micans* measured using a nutrient repletion method: effect of light intensity. Algae 32:139-153.

Lee, K. H., Jeong, H. J., Kwon, J. E., Kang, H. C., Kim, J. H., Jang, S. H., Park, J. Y., Yoon, E. Y. & Kim, J. S. 2016. Mixotrophic ability of the phototrophic dinoflagellates *Alexandrium andersonii*, *A. affine*, and *A. fraterculus*. Harmful Algae 59:67-81.

Lee, K. H., Jeong, H. J., Lee, K., Franks, P. J. S., Seong, K. A., Lee, S. Y., Lee, M. J., Jang, S. H., Potvin, E., Lim, A. S., Yoon, E. Y., Yoo, Y. D., Kang, N. S. & Kim, K. Y. 2019. Effects of warming and eutrophication on coastal phytoplankton production. Harmful Algae 81:106-118.

Li, T. -S., Yu, R. -C. & Zhou, M. -J. 2009. Demand and adsorption strategies of phosphorus of *Alexandrium catenella* isolated from East China Sea. Mar. Environ. Sci. 28:355-359.

Lim, A. S., Jeong, H. J., Jang, T. Y., Yoo, Y. D., Kang, N. S., Yoon, E. Y. & Kim, G. H. 2014. Feeding by the newly described heterotrophic dinoflagellate *Stoeckeria changwoensis*: a comparison with other species in the family Pfiesteriaceae. Harmful Algae 36:11-21.

Lim, A. S., Jeong, H. J., Kim, J. H., Jang, S. H., Lee, M. J. & Lee, K. 2015. Mixotrophy in the newly described dinoflagellate *Alexandrium pohangense*: a specialist for feeding on the fast-swimming ichthyotoxic dinoflagellate *Cochlodinium polykrikoides*. Harmful Algae 49:10-18.

Lim, P. -T., Leaw, C. -P., Kaga, S., Sekiguchi, K. & Ogata, T. 2007. Growth responses of five non toxic *Alexandrium* species (dinophyceae) to temperature and salinity. Mar. Res. Indonesia 32:189-195.

Lim, P. -T., Leaw, C. -P., Usup, G., Kobiyama, A., Koike, K. 2005. Identification of two plastid proteins in the dinoflagellate *Alexandrium affine* that are substantially down-regulated by nitrogen deprivation. J. Proteome Res. 8:5080-5092.
Lee et al. Growth of *Alexandrium affine* and *A. fraterculus*

Nogueira, E., Pérez, F. E. & Ríos, A. F. 1997. Seasonal patterns and long-term trends in an estuarine upwelling ecosystem (Ría de Vigo, NW Spain). *Estuar. Coast. Shelf Sci.* 44:285-300.

Ok, J. H., Jeong, H. J., Lim, A. S., You, J. H., Kang, H. C., Kim, S. J. & Lee, S. Y. 2019. Effects of light and temperature on the growth of *Takayama helix* (Dinophyceae): mixotrophy as a survival strategy against photoinhibition. *J. Phycol.* Advanced online publication. https://doi.org/10.1111/jpy.12907.

Omachi, C. Y., Tamanaha, M. D. S. & Proença, L. A. D. O. 2007. Bloom of *Alexandrium fraterculus* in coastal waters off Itajaí, SC, Southern Brazil. *Braz. J. Oceanogr.* 55:57-61.

Park, J., Jeong, H. J., Yoo, Y. D. & Yoon, E. Y. 2013. Mixotrophic dinoflagellate red tides in Korean waters: distribution and ecophysiology. *Harmful Algae* 30(Suppl. 1):S28-S40.

Prézelin, B. B., Meeson, B. W. & Sweeney, B. M. 1977. Characterization of photosynthetic rhythms in marine dinoflagellates: I. Pigmentation, photosynthetic capacity and respiration. *Plant Physiol.* 60:384-387.

Richardson, K., Beardall, J. & Raven, J. A. 1983. Adaptation of unicellular algae to irradiance: an analysis of strategies. *New Phytol.* 93:157-191.

Seong, K. A., Jeong, H. J., Kim, S., Kim, G. H. & Kang, J. H. 2006. Bacterivory by co-occurring red-tide algae, heterotrophic nanoflagellates, and ciliates. *Mar. Ecol. Prog. Ser.* 322:85-97.

Van Dolah, F. M., Lidie, K. B., Morey, J. S., Brunelle, S. A., Ryan, J. C., Monroe, E. A. & Haynes, B. L. 2007. Microarray analysis of diurnal- and circadian-regulated genes in the Florida red-tide dinoflagellate *Karenia brevis* (Dinophyceae) 1. *J. Phycol.* 43:741-752.

Van Haren, H. & Compton, T. J. 2013. Diel vertical migration in deep sea plankton is finely tuned to latitudinal and seasonal day length. *PLoS One* 8:e64435.

Yamamoto, T. & Tarutani, K. 1999. Growth and phosphate uptake kinetics of the toxic dinoflagellate *Alexandrium tamarense* from Hiroshima Bay in the Seto Inland Sea, Japan. *Phycologia* 48:177-185.

Nguyen-Ngoc, L. 2004. An autecological study of the potentially toxic dinoflagellate *Alexandrium affine* isolated from Vietnamese waters. *Harmful Algae* 3:117-129.

Nguyen-Ngoc, L. 2004. An autecological study of the potentially toxic dinoflagellate *Alexandrium affine* isolated from Vietnamese waters. *Harmful Algae* 3:117-129.