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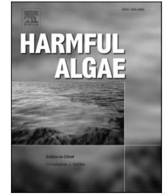

Effects of nutrient depletion duration on growth, photosynthesis and toxins (OA and DTX) in the dinoflagellate Prorocentrum lima

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ABSTRACT

Prorocentrum lima is a marine benthic dinoflagellate known for producing toxins such as okadaic acid (OA) and dinophysistoxin (DTX), which cause diarrheal shellfish poisoning (DSP). *P. lima* is known to increase toxin production under low nutrient concentrations, but there have been few studies examining the effect of prolonged nutrient depletion. This study investigates changes in growth, photosynthetic efficiency, pigments (Chl *a* and carotenoids) and toxin levels (OA and DTX) during the period of nutrient depletion. Nutrient addition was stopped when the cell concentration reached the stationary phase at approximately 200,000 cells ml^{-1}. After stopping nutrient addition, sampling was conducted at 10-day intervals for 30 days. During the exponential growth phase, *P. lima* took up more than 90 % of nitrate and nitrite from the medium within 3 hours. Even after the nutrient supply was stopped, cell density continued to increase, reaching about 340,000 cells ml^{-1}. Chl *a* and carotenoids did not show significant differences, but photosynthetic parameters, such as relative electron transport rate (rETR), ETRmax and Ik decreased. The levels of OA and DTX-1 were also significantly higher on day 30 compared to day 0. These results suggest that, while the cell density can be maintained during nutrient depletion, the toxin content per cell increases significantly, and photosynthetic efficiency decreases.

1. Introduction

Prorocentrum sp. are marine epiphytic and benthic dinoflagellate (Nascimento et al., 2005). Species within *Prorocentrum* genus, including *P. compressum, P. lima* and *P. micans*, are known to form harmful algae blooms (HABs) in eutrophic environments (Hallegraeff, 2003; Turkoglu, 2016; Türkoğlu and Erdoğan, 2010). *Prorocentrum* sp. blooms typically occur in the summer and sometimes in late winter or early spring (Turkoglu, 2016; Turkoglu and Oner, 2010). *Prorocentrum* sp. are known to produce toxins such as okadaic acid (OA) and dinophysistoxin (DTX), which cause diarrheal shellfish poisoning (DSP) (Lawrence et al., 2000; Morton, 1999; Yasutaka Murakami and Takeshi, 1982). DSP has been recognized as a significant public health issue since the late 20th century (Lassus, 1985). The alga grows widely in coastal waters and observed in Asia, Europe, and America (Gayoso et al., 2002; Lawrence et al., 2000; Nascimento et al., 2005). *P. lima* can attach to the surfaces of organisms such as macroalgae and corals as well as to sand and rocks (Faust, 1991; Lawrence, 1998). Despite this, it can also be consumed by filter feeding organisms, such as mussels. (Gayoso et al., 2002).

In general, toxins in shellfish are absorbed from algae through filter feeding, and DSP is transmitted to humans who ingest contaminated shellfish, potentially leading to serious gastrointestinal illness (Anderson, 1998; Foden et al., 2005; Lawrence et al., 2000). These illnesses are primarily caused by shellfish contaminated with dinoflagellate toxins, particularly okadaic acid produced by species such as *Prorocentrum lima* (Lawrence et al., 2000). Numerous outbreaks worldwide have been linked to DSP toxins, this not only poses a threat to human health but also reduces seafood demand (Hallegraeff, 1993).

Nutrient availability and composition significantly affect algal community structure, biomass, phytoplankton biochemistry, and toxin production (Granéli and Flynn, 2006). Nutrients such as nitrate and phosphate can become depleted in the marine environment, and this depletion is more pronounced during microalgal blooms (Bristow et al., 2017; Glibert and Burford, 2017; Paerl et al., 2015). Some studies

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showed that *P. lima* increases toxin production at low nutrient concentrations and low temperature (Granéli et al., 1998; Li and Sun, 2016; McLachlan et al., 1994). *P. lima* can maintain high toxin concentrations even under nutrient limited conditions (Li and Sun, 2016; Zhao et al., 2009). However, there is no research on the growth and toxin production of *Prorocentrum* sp. according to the period of nutrient depletion. In the present study, *P. lima* was cultured under nutrient-enriched conditions until it reached the stationary phase, after which nutrient supply was stopped. The aim of this study is to observe changes in growth, photosynthetic efficiency, and toxin production in *P. lima* during the nutrient depletion period.

2. Materials and methods

2.1. Prorocentrum lima culture

Prorocentrum lima was provided by NEB (Seoul, Korea) and the algae were collected at JeJu Island, Korea in 2021. The algae were cultivated in 500 ml flasks, containing 300 ml of f/2 medium (Fritz aquatics, Texas, USA; Guillard., 1975) at the optimal growth conditions, 20°C of temperature, 100 μmol m^{-2} s^{-1} of irradiance and 12L:12D of photoperiod. The initial cell concentration was approximately 3000 cells ml^{-1}, and the medium was completely changed every 6 days. Before replacing the medium, the algae attached to the flask were removed using a scraper. The culture medium containing the algae were completely mixed and divided into several 50 ml conical tubes. The tubes were centrifuged at 1000 g for 10 minutes, and the supernatant was discarded. The cells were transferred to flasks and new f/2 medium was added. Ambient seawater collected from Yeongheung Island, Incheon was used after filtration and autoclave. Once the cell concentration of this alga reached the stationary phase at approximately 200,000 cells ml^{-1} in 40 days, additional nutrient supply was stopped. Sampling for cell counting, pigments and toxins was then conducted at 10-day intervals for 30 days.

2.2. Cell counting

Cell counting was performed every 4 days. Prior to counting, the culture medium was mixed thoroughly. The medium containing *Prorocentrum lima* (3 ml) was transferred into a well plate, and 1 % Lugol's solution was added to fix and stain the cells. The sample was allowed to sit for approximately 5 minutes to ensure proper fixation and staining. The number of cells per ml of medium was determined using a Sedgwick Rafter counting chamber (SR chamber). Twenty cells out of a total of 1000 cells observed in the SR chamber were counted and the cell concentrations was calculated based on this count.

For additional analyses, approximately 15 ml of culture medium were collected and centrifuged. The pellet was stored at -80°C for toxin analysis, while the supernatant was filtered through a syringe filter for nutrient analyses. Only intracellular toxins were extracted from the cell pellet, and extracellular toxin fractions in the culture medium were not analyzed in this study. Previous studies have shown that DSTs in *P. lima* have been mainly analyzed in their intracellular forms, rather than as extracellular toxins in the surrounding medium (Vanucci et al., 2010; Varkitzi et al., 2010; Wu et al., 2020). The filtered medium was stored at -20°C until analysis. Nitrate, nitrite and phosphorus concentrations in the medium were analyzed using a QuAAtro39 Auto Analyzer (SEAL Analytical, Wisconsin, USA).

2.3. Toxin analysis

For toxin (OA and DTX-1) analysis, the cells were thawed, and 3 ml of methanol was added to the pellet. The mixture was sonicated in an ice bath for 5 minutes, and the supernatant was collected by centrifugation at 3500 rpm for 5 minutes. A Strata-X cartridge was preconditioned with 3 ml of 100 % methanol and 3 ml of deionized water. The extract was then loaded onto the cartridge, ensuring that the methanol concentration was less than 10 %. After sample loading, the cartridge was washed with 3 ml of 15 % methanol to remove interferences, and then dried using a vacuum pump. The samples were eluted with 3 ml of 100 % methanol containing 1 % NH_4OH and concentrated to 1 ml under N_2 gas. It should be noted that this procedure quantified only the free forms of OA and DTX-1 without alkaline hydrolysis, and therefore esterified forms were not included in the final measurements. Since *Prorocentrum* and *Dinophysis* are known to produce fatty acid esters of DSP, the total toxin content might be underestimated in the absence of hydrolysis (Wu et al., 2020). However, the analysis of free intracellular toxins without hydrolysis has been widely applied in previous studies on *P. lima*, and thus remains a commonly accepted approach for comparative purposes (Vanucci et al., 2010; Varkitzi et al., 2010).

The samples were quantitatively and qualitatively analyzed using UPLC-MS/MS (Kim et al., 2022; Kim et al., 2023). Certified reference materials for OA and DTX-1 were obtained from the National Research Council Canada (Ottawa, ON, Canada). Analyses were performed using an Agilent 1290 Infinity II liquid chromatograph coupled with an Agilent 6470 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was achieved using an X-Bridge C18 column (3.0 mm × 150 mm, 5.0 μm particle size), with a binary mobile phase system consisting of (A) 0.05 % ammonium hydroxide in water and (B) 0.05 % ammonium hydroxide in 90:10 (v/v) acetonitrile/water. The LC operating conditions and MRM transitions were adopted from previously validated protocols with no further modification (Kim et al., 2022; Kim et al., 2023).

2.4. Photosynthetic pigments (Chl a and carotenoids) and efficiency (Fv/Fm, ETRmax, Ik and rETR)

For photosynthetic pigments and efficiency, sampling was conducted every 10 days after the medium supply was stopped. After mixing the medium, 5 ml samples were collected and filtered using Whatman GF/F filters (0.7 μm). *Prorocentrum lima* remaining on the filter was placed in a 15 ml tube and stored in dark at -80°C until analysis. The filter paper with algae was treated with 10 ml of 80 % acetone and stored at 4°C for 24 hours. After treatment, the filter paper was removed and the solution was centrifuged at 2000 g for 5 minutes. The supernatant was then measured for absorbance at 470, 646 and 663 nm using a SYNERGY HTX microplate reader (BioTek, Winooski, Vermont, USA). Chl *a* and carotenoids were quantified using these formula (Lichtenthaler and Wellburn, 1983).

$$\text{Chlorophyll } a = \frac{(12.21A_{663} - 2.81A_{646}) \times Volume\ of\ extract}{Sample\ volume\ (ml) \times Number\ of\ cell\ \left(cells\ ml^{-1}\right)}$$

$$\text{Carotenoids} = \frac{(1000A_{470} - 3.27C_a - 104C_b)}{Sample\ volume\ (ml) \times Number\ of\ cell\ \left(cells\ ml^{-1}\right)/229}$$

For photosynthetic efficiency analysis, 5 ml of medium was collected, centrifuged and removed all supernatant. The tube with algae was placed in dark for 10 minutes. Photosynthetic efficiency was measured using Diving-PAM (Walz, Effeltrich, Germany). All samples were irradiated with light at 0, 172, 281, 423, 580, 860, 1158, 1735 and 2505 μmol photons m^{-2} s^{-1}. The light was sequentially irradiated for 10 seconds to express as a light curve, and the saturated light was irradiated to measure the quantum yield. The photosynthetic ability by potential chlorophyll was observed as relative electron transfer rate (rETR). rETR and ETRmax in photosystem II was calculated using this quantum yield value following the equations below (Beer et al., 1998; Genty et al., 1989; Ralph et al., 1998).

$$\text{rETR} = \left(\frac{[\text{F'm} - \text{F}]}{\text{F'm}}\right) \times \text{PAR} \times 0.5 \times 0.84$$

$$\text{ETRmax} = \text{ETRs}[\alpha / (\alpha + \beta)][\beta / (\alpha + \beta)]\beta/\alpha$$

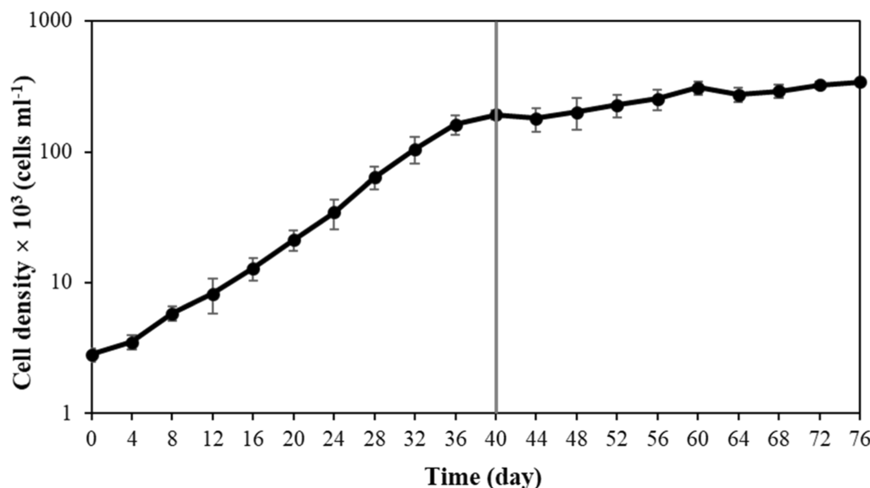

Fig. 1. Cell density (cells ml^{-1}) of *Prorocentrum lima* measured every 4 days. The red line indicates 40 days of culture. Medium supply was stopped after the red line for 30 days. Each coordinate is the overall mean with standard deviation of 5 replicates.

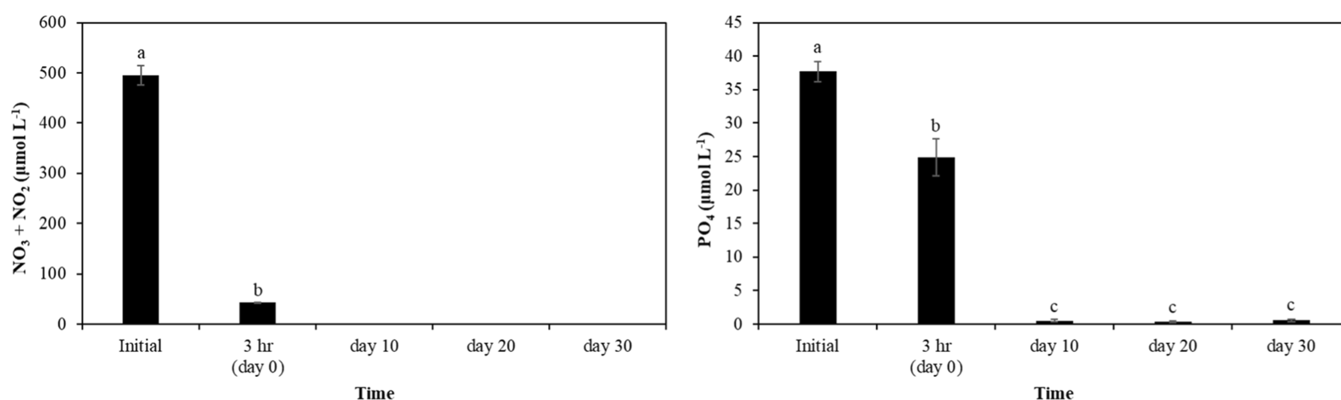

Fig. 2. Nitrite and nitrate (NO_3+NO_2), Phosphate (PO_4) concentration (μmol L^{-1}) in medium measured using a nutrient analyzer at initial and 3 hr (day 0), day 10, 20 and 30 after nutrient supply stopped. Each coordinate is the overall mean with standard deviation of 5 replicates. Different letters on the graph mean significant differences from others ($p < 0.05$).

$$Ik = ETRmax/\alpha$$

2.5. Statistical analysis

All Statistical analysis was conducted using SPSS Statistics 25.3.3 (IBM, Armonk, NY, USA). The result of nutrient (PO_4 and NO_3+NO_2), pigments (Chl *a* and carotenoids) and toxins (OA and DTX-1) was performed the analysis to compare according to period of nutrient deficiency. All analysis was Tukey's tests and One-way ANOVA were used to confirm post-hoc investigation as significant differences ($p < 0.05$).

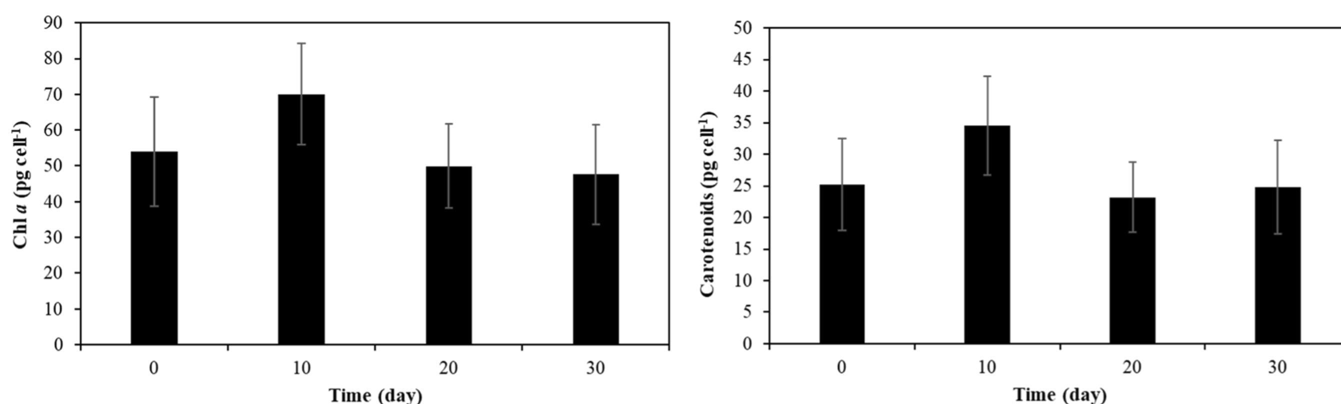

Fig. 3. Chl *a* and carotenoids content in *Prorocentrum lima* (pg cell^{-1}) measured on 0, 10, 20 and 30 day after nutrient supply stopped. Each coordinate is the overall mean with standard deviation of 5 replicates. There are no significant differences ($p < 0.05$).

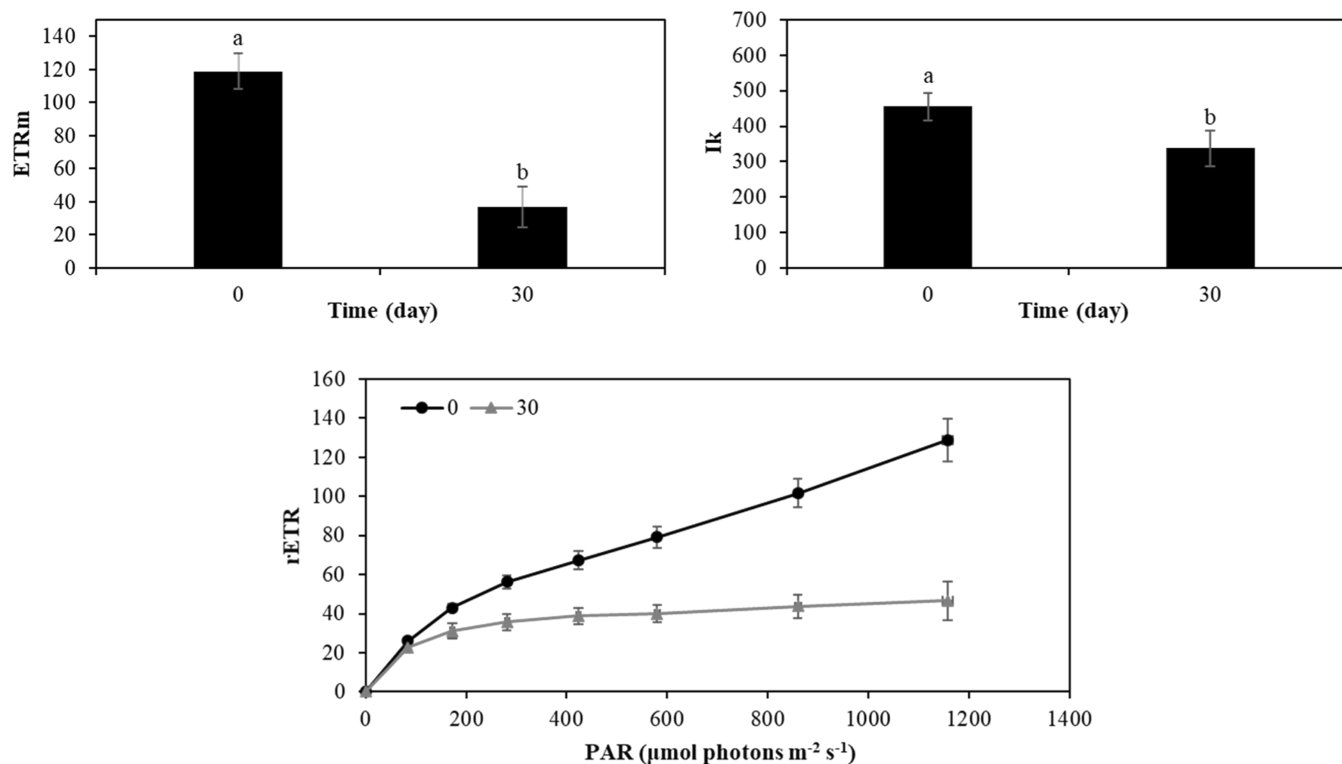

Fig. 4. Photosynthetic analysis results of *Prorocentrum lima* using a Diving-PAM on day 0 and 30 after nutrient supply stopped (ETRmax; maximum electron transfer rate, Ik; light requirement for saturation of photosynthesis, rETR: relative electron transfer rate). Each coordinate is the overall mean with standard deviation of 5 replicates. Different letters on the graph mean significant differences from others ($p < 0.05$).

3. Results

3.1. *Prorocentrum lima* culture

Prorocentrum lima started growing from an initial concentration of about 3000 cells ml^{-1} (Fig. 1.). Cell counting was performed every 4 days. The growth rate was approximately 14.5 % day^{-1} during the exponential growth phase. By day 40 of cultivation, the algae entered the stationary phase as the growth rate declined. At this point, cell concentration reached about 20×10^4 cells ml^{-1}, with the growth rate deceasing to 2.3 % day^{-1}. Nevertheless, the cell concentration increased slightly over an additional 30 days without nutrient supply after Day 40 (Fig. 1.).

Before nutrient addition, the phosphate concentration of natural seawater was 1.3 μM, and the nitrate concentration was 8 μM. By adding f/2 medium, the levels were adjusted to 35 μM and 500 μM, respectively. On the first day, sampling was conducted 3 hours after medium replacement to compare with the initial medium (Fig. 2.). Nitrate and nitrite in the medium were depleted more than 90 % within 3 hours ($p < 0.05$). On day 10, nitrate and nitrite concentration were depleted ($p < 0.05$), and phosphate had already been absorbed by *P. lima*, < 0.5 μmol L^{-1}. Nutrient depletion continued until day 30 (Fig. 2.).

3.2. Photosynthetic pigments (Chl a and carotenoids) and efficiency (Fv/Fm, ETRmax, Ik and rETR)

Chl *a* and carotenoids contents (pg $cell^{-1}$) showed no significant effect from nutrient deficiency ($p < 0.05$) (Fig. 3.). However, photosynthetic efficiency significantly decreased when measured at the beginning (day 0) and end (day 30) of the experiment ($p < 0.05$). Photosynthetic efficiency decreased as the nutrient depletion period lengthened. ETRm, Ik and rETR were significantly lower at day 30 than

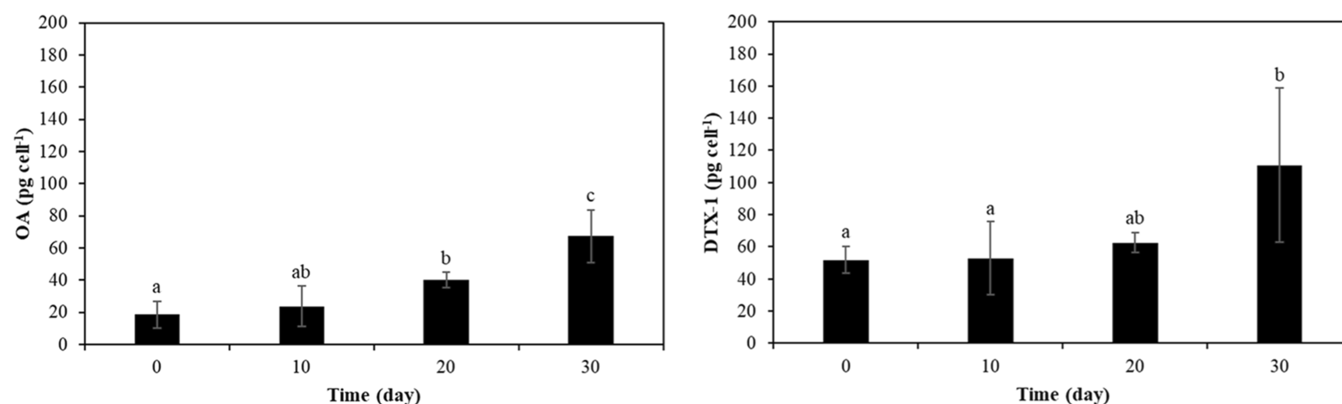

Fig. 5. Toxins (OA and DTX-1) contents in *Prorocentrum lima* (pg $cell^{-1}$) measured on 0, 10, 20 and 30 day after nutrient supply stopped. Each coordinate is the overall mean with standard deviation of 5 replicates. Different letters on the graph mean significant differences from others ($p < 0.05$).

day 0 (Fig. 4.).

3.3. Toxins in Prorocentrum lima (OA and DTX-1)

The OA content in *Prorocentrum lima* significantly increased on day 20 after the nutrient supply was stopped ($p < 0.05$). The highest OA content was observed on day 30. At day 0, the OA content was 18.5 pg cell^{-1}, but increased more than 3 times to 67.3 pg cell^{-1} by day 30 (Fig. 5.). Similarly, DTX-1 content showed no significant differences until day 20 but increased significantly on day 30 ($p < 0.05$). Compared to 51.9 pg cell^{-1} on day 0, the DTX-1 content rose to 110.8 pg cell^{-1} on day 30, more than doubling (Fig. 5.).

4. Discussion

4.1. Cell growth continues after nutrient supply was stopped

Prorocentrum sp. is known to exhibit slower growth compared to other dinoflagellates (López-Rosales et al., 2014; Nascimento et al., 2005), with reported growth rates ranging from 1.4 to 27 % day^{-1} (Ben-Gharbia et al., 2016; Bravo et al., 2001; Holmes et al., 2001; Morlaix and Lassus, 1992a,b; Varkitzi et al., 2010). Microalgae grow in competitive resource environments and often absorb more nutrients than required to meet steady-state demands (Fogg and Thake, 1987). In the present study, *Prorocentrum lima* absorbed more than 90 % of the nitrate and nitrite in the medium within 3 hours, and continued to increase its cell density even under nutrient-depleted conditions. These findings are similar to a previous study conducted with *Prorocentrum donghaiense*, where growth was maintained after entering the stationary phase in 3 days under dissolved inorganic phosphorus (DIP) depleted conditions (Li et al., 2015). The cell division rate was decreased, but the cells did not die and were maintained during experimental period. This is because the *P. donghaiense* is able to recycle the phosphorus in their body (Li et al., 2015). Moreover, *P. lima* thrives from 3.05×10^4 to 4.83×10^4 cells ml^{-1} in environments where both nitrate and phosphate are depleted to less than 1 μM (Nascimento et al., 2005). *P. lima* was able to maintaine its cell density for up to 7 weeks without additional nutrient supply (Varkitzi et al., 2010). This sustained growth may result from the utilization of nutrients accumulated within the cells after the exponential growth phase (Nascimento et al., 2005).

4.2. Physiological responses to nutrient depletion

Nitrogen (N) and phosphorus (P) are the most important factors for phytoplankton growth and its biochemical compositions (Hallegraeff, 1993). Especially, nitrogen is involved in metabolic processes to create essential components such as proteins and chlorophyll within microalgae (Lai et al., 2011). Microalgae normally produce certain metabolisms to overcome and acclimatize in biotic and abiotic stress conditions (Gauthier et al., 2020). For example, the algae such as Dunaliella sp. and Neochloris sp. is known to increase the lipid production in nitrogen depletion condition (Chen et al., 2011; Guevara et al., 2005; Sun et al., 2014). In this study, when the *Prorocentrum* lima was cultured for 30 days without a nutrient supply, Chl a and carotenoids were maintained at approximately 50 and 25 pg cell^{-1}, respectively. This is likely because N in the cells was recycled, or metabolism promoting chlorophyll production was stimulated, as observed in previous studies (Gauthier et al., 2020; Li et al., 2015).

Photosynthesis is critical to the primary productivity of phytoplankton (Jakob et al., 2005; Li and Sun, 2016; Strzepek and Harrison, 2004). Previous studies have shown the interaction between nutrient concentration and the photosynthetic activity of microalgae. The light utilization and electron transport rate were decreased under nutrient limited or depleted conditions (Cleveland and Perry, 1987; García-Cañedo et al., 2016; Rodríguez-Román and Iglesias-Prieto, 2005; Roleda et al., 2008). This study also showed that the maximum electron transfer rate (ETRmax), light requirement for saturation of photosynthesis (Ik) and the saturation light were significantly lower on day 30 compared to day 0. The nutrient depletion may affect the calvin cycle, causing the algae to bloom or crash (Glibert et al., 1988).

4.3. Effects of nutrient depletion on toxins of Prorocentrum lima

The toxin contents in cell changes with environmental conditions such as nutrient, temperature, salinity and light intensity (Granéli and Flynn, 2006; Legrand et al., 2003; Lehtimäki et al., 1994). Under nutrient-depleted conditions, some micro-algae such as *Prorocentrum lima*, Dinophysis sp., Prymnesium sp., and Crysochromulina sp. increased their toxin content (Granéli and Flynn, 2006; Johansson et al., 1996; Li et al., 2009). However, the response to toxin content to nutrient depletion varies depending on the concentration or ratio of nitrogen to phosphorus. In the dinoflagellate Protogonyaulax tamarensis, the total toxin content of GTX-1,2,3,4 per cells was lower under the 10 % N added condition compare to the control (nutrient-rich condition) (Boyer et al., 1987). Under the 0 % N condition, the total toxin contents were below the detection limit. On the other hand, under the P-depleted condition, the total toxin content per cell increased up to 8 times higher after approximately 24 days compared to the initial level (Boyer et al., 1987).

In another study, the cell concentration and toxin production of *P. lima* were examined under different conditions of temperature, nutrients, photoperiod and salinity (Wang et al., 2015). The maximum OA and DTX-1 contents were 12.731 and 16.587 pg cell^{-1}, respectively. Regarding nutrient conditions (f/8, f/4, f/2, f and 2f), the growth rate and DTX-1 content per cell were highest in the f/2 medium, whereas OA content per cell was higher under the low nutrient condition of f/8 (Wang et al., 2015). In addition, when *P. lima* was cultured for 35 days under optimal growth conditions (20°C, f/2 medium, 12L:12D photoperiod and salinity of 30 psu), the contents of both OA and DTX-1 increased by more than two times on day 35 compared to day 5 (Wang et al., 2015). The toxin content was also significantly increased under phosphate and nitrate depleted conditions (McLachlan et al., 1994; Sohet et al., 1995). In a study comparing toxin levels under nitrogen and phosphorus depletion, OA and DTX-1 contents ranged from 6.69 to 15.80 pg cell^{-1} and 0.12 to 0.39 pg cell^{-1} respectively (Vanucci et al., 2010). However, all of these studies were conducted from the exponential growth phase to the stationary phase. No results are available for conditions where nutrient supply is halted during the stationary phase. In this study, on day 0, the toxin content was already more than 4 times higher than in previous studies, and by day 30, OA and DTX-1 contents were 100 times higher.

4.4. Correlation between cell density and toxin content

When microalgae divide, their cellular components are duplicated and distributed into two cells (Arellano and Moreno, 1997; Nurse, 1994). Therefore, during the exponential growth phase, cells divide rapidly, and toxin concentration per cell generally decreases (Granéli and Flynn, 2006). However, as cell enters the stationary phase, the division rate of cell decreases, leading to an increase in toxin content per cell (Flynn et al., 1994; Morlaix and Lassus, 1992a,b; Quilliam et al., 1996; Wang et al., 2015). In this study, when a nutrient depleted environment was maintained for 30 days, OA concentration significantly increased from day 20 compared to day 0. By day 30, both OA and DTX-1 contents showed 2-3 times higher. This may be due to the slowed growth rate in the stationary phase, which prevented the division of cells and allowed toxins to accumulate within the cells.

The cell density of dinoflagellates during the stationary phase varies depending on the strains, nitrogen and phosphorus sources, and it has a significant impact on toxin content (Lee et al., 2016). For example, the dinoflagellate, *Alexandrium ostenfeldii* showed a positive correlation ($p < 0.01$) between the production of paralytic shellfish toxins (PSTs) and cell density (Savela et al., 2016), with higher PST concentrations at higher

Table 1
Cell growth rate (d^{-1}), Maximum cell density (cells ml^{-1}) and OA content (pg $cell^{-1}$) of *Prorocentrum lima* were observed under different temperature (°C), light intensity (μmol m^{-2} s^{-1}) and salinity (psu).

Species	Growth rate (d^{-1})	Temp (°C)	Culture medium	Light intensity (Light:Dark)	Salinity (psu)	Maximum cell density (cells ml^{-1})	OA content (pg $cell^{-1}$)	References
P. lima	0.05-0.18	5-30	f/2	30-70 μmol m^{-2} s^{-1} (12L:12D)	28-31	0.06-8.8 $\times 10^3$	0.4-17.1	(Aquino-Cruz et al., 2018)
P. lima	0.22-0.23	20	f/2	90 μmol m^{-2} s^{-1} (16L:8D)	25	33-70.4 $\times 10^3$	6.69-15.80	(Vanucci et al., 2010)
P. lima	0.014-0.16	20	f/2	100 μmol m^{-2} s^{-1} (12L:12D)	30	200-340 $\times 10^3$	18.5-67.3	This study

cell densities. Under stressful conditions for cell growth, specific metabolism such as toxins, lipids and antioxidants production of microalgae become active, leading to an increase in the toxin content of *Prorocentrum lima* (Gauthier et al., 2020; Wang et al., 2015). In the present study, the OA content was up to 100 times higher than reported in previous studies (Table 1), likely due to the significantly higher cell density observed, which was up to 5,600 times greater than in previous studies (Table 1). These results suggest that nutrient depletion, along with higher cell density may lead to both rapid growth inhibition and the accumulation of toxin contents within the cells of *P. lima*.

5. Conclusion

In this study, over 90 % of nitrate and nitrite were removed within 3 hours after the nutrient supply was stopped at the stationary phase. Despite the continued depletion of phosphate and nitrate for 30 days, *Prorocentrum lima* exhibited sustained cell growth. As the duration of nutrient depletion increased, a decline in photosynthetic efficiency was observed, accompanied by a marked increased in toxin production of *P. lima*. Notably, the okadaic acid (OA) content was up to 100 times higher than reported in previous studies (Aquino-Cruz et al., 2018; Vanucci et al., 2010). This suggests that toxin production per cell may increase under prolonged nutrient depletion, with a more pronounced effect at higher cell concentrations (Lee et al., 2016; Savela et al., 2016). These finding offer insights into the physiological responses of *P. lima* to nutrient depletion stress, highlighting the potential for DSP risk under prolonged reversal of eutrophication scenarios or oligotrophic conditions. This work provides a foundation for improving the prediction and risk assessment of DSP related harmful algal blooms (HABs). Future studies should explore how other environmental stressors such as temperature shifts, light limitation, and salinity changes interact with nutrient dynamics to influence toxins production in dinoflagellates.

CRediT authorship contribution statement

Jeong Hwa Hwang: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Ji-Sook Park:** Writing – review & editing, Writing – original draft. **Young-Seok Han:** Writing – review & editing, Writing – original draft. **Youn-Jung Kim:** Writing – review & editing, Writing – original draft. **Mungi Kim:** Writing – review & editing, Formal analysis, Data curation. **Seongjin Hong:** Writing – review & editing, Formal analysis, Data curation. **Jang K. Kim:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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