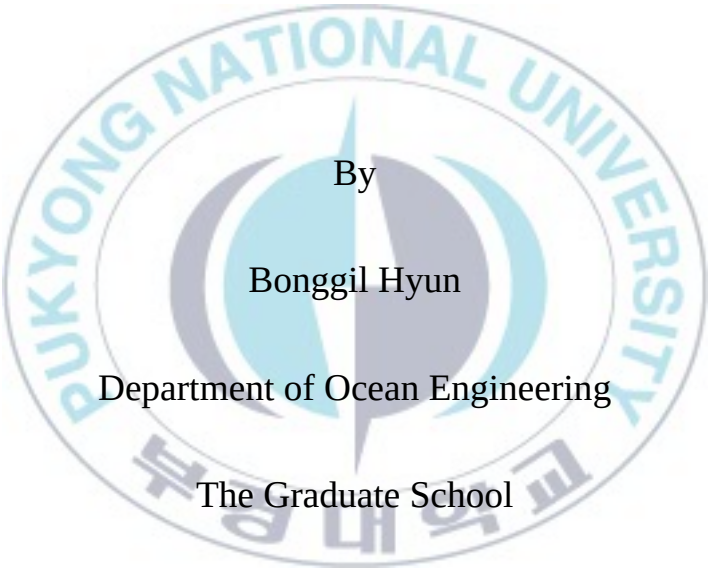
Thesis for the Degree of Doctor of Philosophy

Effects of the rising CO_2 and temperature on marine phytoplankton physiology



By

Bonggil Hyun

Department of Ocean Engineering

The Graduate School

Pukyong National University

February 2015

Effects of the rising CO_2 and temperature on marine phytoplankton physiology

(이산화탄소 및 수온 증가가 해양 식물플랑크톤의 생리에 미치는 영향)

Advisor: Prof. Chang Ho Moon

by

Bonggil Hyun

A thesis submitted in partial fulfillment of the requirements

for the degree of Doctor of Philosophy

in Department of Ocean Engineering, The Graduate School, Pukyong National University

February 2015

Effects of the rising CO_2 and temperature on marine phytoplankton physiology

A dissertation

by

Bonggil Hyun

Approved by:

(Chairman) Seok Jin Oh

(Member) Yongsik Sin

(Member) Kyoungsoon Shin

(Member) Keun-Hyung Choi

(Member) Chang-Ho Moon

February, 2015

CONTENTS

LIST OF TABLES

LIST OF FIGURES

이산화탄소 및 수온 증가가 해양 식물플랑크톤의 생리에 미치는 영향

현 봉 길

부경대학교 대학원 해양학과

요 약

해양 이산화탄소 농도 증가는 해수중의 pH 를 낮추고 표층 수온을 증가시킨다. 현재까지 선행된 많은 연구 결과들은 이러한 해양 환경 변화가 해양 식물플랑크톤의 생리상태에 지대한 영향을 미칠 것으로 예측하고 있다 (e.g. Hays et al., 2005). 예를 들면, Riebesell (2004)은 해수중 CO_2 농도 증가는 해양 식물플랑크톤의 광합성, 미량원소 구성 및 석회화 작용에 심대한 영향을 끼친 다고 보고하였다. Tortell et al. (2002)도 증가된 CO_2 는 식물플랑크톤 종 천이에 영향을 끼친 다고 보고하였다. 식물플랑크톤은 증가된 표층 수온에 대해서 분포 범위, 세포 크기 및 군집 조성의 변화등을

통해서 민감하게 반응 하는 것으로 알려져 있다 (Hays et al., 2005; Kudela et al., 2006). 또한 Eppley (1972)의 연구에 따르면 식물플랑크톤의 대사 작용은 증가된 수온에 의해서 가속화 될 수 있다고 하였다. 이와 같이, 많은 연구들이 전지구적 기후 변화가 식물플랑크톤에 영향을 끼친다고 보고 하였지만, 아직까지 식물플랑크톤 군집의 변화 정도와 방향에 대해서는 이해가 부족한 실정이다.

따라서 본 학위 논문 연구에서는 기후변화의 두가지 주요한 요소인 이산화탄소와 수온에 대해서 식물플랑크톤 종 반응 뿐만 아니라 전체 식물플랑크톤 군집반응도 알아 보고자 하였다. 이러한 연구 목적에 도달하기 위해, 첫번째는 실험실 실험과 부유 중형생태계를 이용해서 다양한 이산화탄소와 수온 조건에서 식물플랑크톤 그룹 및 종, 영양염 이용능력, 탄소 소모율 변화등을 조사하였으며, 두번째는 유해적조 생물의 성장률, 세포 부피 및 지방산 농도 및 조성 변화를 통해서 미래 해양에서의 HABs 의 성장 및 독성 정도를 예측해 보고자 하였다. 본 논문 연구에서

현재와 미래의 해양 조건은 A2 Scenarios of the Intergovemmental Panel on Climate Change Special Report on Emissions Scenarios (IPCC, 2007) 의 모델 결과를 기반으로 해서 설정하였다. 다음 아래의 문단에서 본 논문의 방법들과 결과들을 가지고 식물플랑크톤 생리 및 생태적 관점에서 토론하였다.

Study 1 에서는 미래의 증가된 이산화탄소와 수온이 4 종 (*Skeletonema costatum*, *Chaetoceros debilis*, *Chaetoceros didymus*, *Thalassiosira nordenskioldii*) 의 규조류 성장에 미치는 영향을 알아 보고자 다음과 같은 조건하에서 배양 실험을 실시 하였다: present pCO_2: 400ppm, temperature: 20°C; acidification pCO_2: 1000ppm, temperature: 20°C; global warming pCO_2: 400ppm, temperature: 25°C; greenhouse pCO_2: 1000ppm, temperature: 25°C. 수온이 높은 실험구에서 *S. costatum* 은 낮은 성장률을 보인 반면, *C. didymus* 는 높은 성장률을 보였고, *C. debilis* 와 *T. nordenskioldii* 는 수온 조건에 관계 없이 비슷한 성장률을 보였다. 조사되어진 4 종 모두 이산화탄소 농도

증가에 따른 성장률 변화는 관찰되지 않았다. *Chaetoceros* spp.는 증가된 이산화탄소 실험구에서 세포당 pH 변화율이 증가하는 경향을 보인 반면, *S. costatum* 과 *T. nordenskioldii* 는 현재 조건 대비 이산화탄소 농도가 높은 실험구에서 뚜렷한 pH 변화를 관찰할 수 없었다. 이는 해수 중 이산화탄소 농가가 증가하면 *Chaetoceros* spp. 가 다른 2 종에 비해 더 많은 CO_2 를 흡수 할수 있음을 나타낸다. 따라서 세포의 성장과 세포당 pH 변화 결과를 고려하였을 때 *C. debilis* 와 *C. didymus* 가 *S. costatum* 과 *T. nordenskioldeii* 보다 이산화탄소와 수온이 증가하는 미래 해양 환경 조건에서 보다 잘 적응 할 수 있을 것으로 예측된다.

Study 2 에서는 증가된 이산화탄소와 수온이 연안 해양 환경에 어떠한 부가적인 영향을 미치는 지에 대해서 알아 보고자 하였다. 본 연구는 메조코즘을 이용해서 수행하였다. 각각의 메조코즘 백(bag)은 장목만 연안수를 이용해서 해수를 채웠으며, CO_2 와 수온을 이용해서 다음과 같은 조건을 설정하였다: present: 380ppm, acidification: 980ppm,

greenhouse:980ppm and 주변수온 +3°C. 주요 연구 결과를 보면, 미래의 전 지구적 기후 변화는 온대기후지역의 연안 환경에 매우 심대한 영향을 미치는 것으로 나타났다. 보다 자세하게 보면, 전 지구적 기후변화는 식물플랑크톤의 영양염 이용능력을 바꾸며, 이는 식물플랑크톤의 종 천이 변화를 유도하였다. 또한 해양 산성화와 해수온 상승이 식물플랑크톤 그룹 및 종의 성장에 직접적으로 영향을 미친다는 사실을 발견했다. 특히 크기가 작은 식물플랑크톤과 와편모조류 몇몇 종은 현재 해양 환경 보다 미래 해양 환경에서 보다 빠르게 성장하는 것으로 나타났으며, 규조류 중에서는 크기가 큰 규조류 보다는 크기가 작고 체인을 형성하는 종(*Skeletonema costatum*)이 보다 미래 해양 환경에 잘 적응 하는 것으로 나타났다. 이러한 크기가 작은 식물플랑크톤의 성장은 와편모조류와 섬모충류와 같은 상위 영양단계의 포식자(microzooplankton)의 포식압을 증가시키는 요인으로 나타났다. 본 연구 결과는 추가적인 확인 연구가 필요하지만, 크기가 작은 식물플랑크톤과 와편모조류로 이루어진 대발생의 발생 빈도와 세포의

개체수 밀집 정도가 미래 해양 환경 조건에서 증가할 것으로 예측되며, 이는 해양 생태계와 양식산업에 심대한 영향을 미칠수 있을 것으로 예측된다.

Study 3 에서는 수온 변화가 유해 적조 식물플랑크톤 (HABs) 4 종 (*Akashiwo sanguinea*, *Alexandrium tamarense*, *Chattonella ovata*, and *Prorocentrum minimum*)의 성장율, 세포의 부피, 지방산 농도 및 조성에 미치는 영향을 알아 보았다. 이들 종들은 영양염의 과포화된 상태의 배지에 각각 주입 후, 4 개의 서로 다른 수온 조건에서 배양을 하였다. *Alexandrium tamarense* 을 제외한 3 개 종은 실험 대상 온도 내에서 잘 성장을 하는 것으로 나타났다. 세포의 부피는 수온 변화에 따라서 거의 변하지 않았다. 총 지방산 농도변화를 보면, *A. sanguinea, A. tamarense, C. ovata* 는 수온이 증가하면서 감소하는 경향을 보인 반면, *P. minimum* 의 총지방산 농도는 아무런 변화를 보이지 않았다. 세포의 세포막 유동성 및 독성에 관계가 있는 중요한 생화학적 구성 요소인 PUFAs (polyunsaturated fatty acids)의 농도와 세포내 함량은 *A. sanguinea* 가 수온이 증가할수록 총 지방산 함량에 대해서 PUFAs

함량이 증가한 경우를 제외하면 수온이 증가할수록 모두 감소하는 경향을 보였다. 이러한 PUFAs 농도 및 세포내 함량 감소는 세포막 유동성을 떨어뜨려서 광합성등과 같은 생합성 과정에 영향을 미치며, 또한 세포가 보다 빨리 성장하거나 혹은 세포가 상처를 입어 보다 독성이 강해져서 감소된 세포의 독성을 보완해주지 않는다면 이들 종의 독성은 감소 될 것으로 판단된다. 이러한 현상은 해양 생태계와 양식 산업에 영향을 줄 것으로 예측된다.

요약하면, 증가된 이산화탄소와 수온의 개별적 혹은 복합적인 영향은 식물플랑크톤 군집 및 종에 따라서 다르게 나타났다. 메조코즘 연구 결과를 보면, 이산화탄소와 수온이 증가한 미래 해양 환경은 크기가 작은 식물플랑크톤과 와편모조류의 성장에 더 적합한 환경으로 나타났다. 이러한 결과는 크기가 작은 식물플랑크톤과 와편모조류로 이루진 대발생이 발생 빈도와 세포의 개체수 밀집 정도가 미래 해양 환경 조건에서 증가될 것으로 예측된다. 그리고 수온 증가는 와편모조류 (HABs) 의 성장을

촉진시키지만 세포의 활성과 독성에 영향을 주는 PUFAs 의 농도를 낮추는 것으로 나타났다. 따라서 미래 해양 환경 조건에서 현재조건 대비 와편모조류는 잘 성장을 할 것으로 예측되지만, 세포의 독성은 낮아 질 것으로 예측된다. 또한 최근 연구결과에 따르면, 식물플랑크톤은 요각류의 중요한 먹이 공급원이며, EPA 와 DHA 를 포함하는 PUFAs 농도는 요각류의 난 생산력과 부화율에 중요한 역활을 담당한다고 보고 되고 있다 (Shin et al., 2003; Evjemo et al., 2008). Kleppel et al. (1991)의 연구에 따르면 규조류 보다 와편모조류의 생체량의 요각류의 난생산력에 더 영향을 미친다고 보고 하였다. 따라서 미래 해양 환경 조건에서 잘 적응 할 것으로 예측되어지는 와편모조류의 PUFAs 농도 감소는 이를 포식하는 요각류의 난 생산력과 부화율을 저하시킬 뿐만 아니라 요각류를 섭식하는 상위 영양단계의 생물에게도 영향을 미칠 것으로 판단된다. 결론적으로, 미래 해양 환경 변화는 해양 생태계에 심대한 영향을 미칠 것으로 예측된다.

1. GENERAL INTRODUCTION

1.1 Climate changes and phytoplankton

Over the past 250 years, atmospheric carbon dioxide (CO_2) levels have increased by nearly 40%, from preindustrial levels of approximately 280 ppmv (parts per million volume) to nearly 380 ppmv in 2007 (Solomon et al., 2007). This rates of increases, driven by humans fossil-fuel burning, cement manufacturing, and deforestation, is more and more, and will therefore atmospheric CO_2 level reached 750-800 ppmv by 2100. This will have several repercussions on the marine environment. As a direct effect the carbonate chemistry of seawater will be altered (Fig. 1.1.1). It is predicted that by the year 2100, seawater CO_2 concentration will increase by about 30umol kg^{-1} and, as a result, seawater pH will decrease to about 7.8, roughly 0.3 units lower than today's value (Wolf-Gladrow et al., 1999). In addition, CO_2 gas released into the atmosphere absorbs infrared energy radiated from the earth, causing the ocean surface temperature to increase. Over the next 50 to 100 years, greenhouse warming will increase average sea surface temperatures (SST) by as much as 5 ^{o}C, and increased

precipitation, runoff, stratification, and ice melting will lower surface salinities in many parts of the ocean (Bopp et al., 2001; Sarmiento et al., 2002). As a consequence of these marine environmental disturbances, marine ecosystems all over the world are currently changing at an alarming rate.

Ocean acidification involves increases in pCO_2 and bicarbonate ion concentration (HCO_3^-) and decreases in pH and carbonate ion concentration (CO_3^{2-}) in seawater (Fig. 1.1.1). Changes in carbon chemistry can influence phytoplankton physiology, because photosynthesis and respiration are affected by increases in CO_2 and HCO_3^- concentration. Generally, most phytoplankton groups, including dinoflagellates, possess a type I Rubisco (ribulose-1, 5-bisphosphate carboxylase-oxygenase; Tortell, 2000). Type I Rubisco has a high affinity for carboxylation and is therefore extremely efficiently at present day atmospheric concentration. But, some bloom-forming dinoflagellates have a type II Rubisco, which is considered a low-affinity CO_2-fixing system compared to type I Rubisco. To overcome this limitation, low-affinity CO_2-fixing systems may compensate for efficient carbon-concentration mechanisms (CCMs) such as various forms of

carbonic anhydrase (CA), which facilitate the uptake of HCO_3^- and its conversion to CO_2 (Giordano et al., 2005). This mechanism allows some dinoflagellates to grow rapidly under the present-day conditions of pCO_2 levels (Fu et al., 2012). Thus, the physiological response of phytoplankton is contingent on increases in pCO_2 levels dependent on species-specific physiological characteristics.

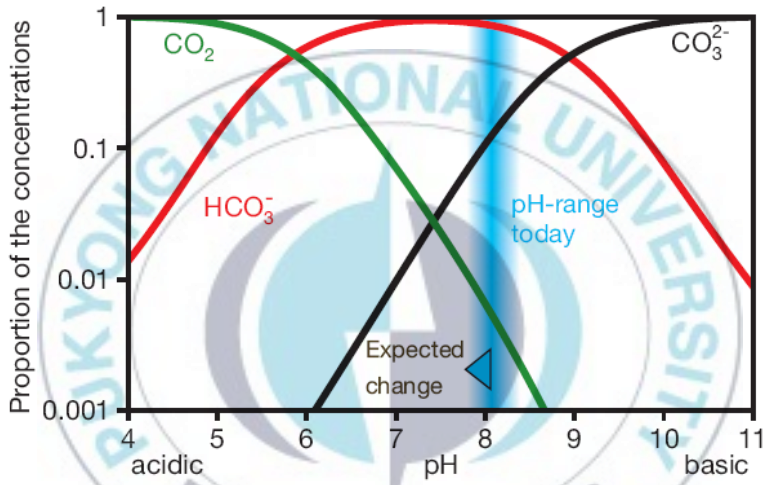


Fig. 1.1.1. Carbonate system of seawater. Relative proportions of the three inorganic components CO_2, HCO_3^- and CO_3^{2-}. The blue shaded area shows schematically the pH range in today's ocean. The arrow shows the expected shift of the average pH value when the atmospheric CO_2 concentration reached about 750ppm. Source: Raven et al., 2005.

Another issue related to the increase in atmospheric CO_2 level is ocean warming. Increase in sea surface temperature (SST) not only accelerates photosynthesis and respiration-related metabolic processes based on Q_{10} values in light-saturated productive areas (Sommer and Lengfellner, 2008), but also promotes a more stable layering of the water column, which reduces the nutrient supply to support plankton growth. The stronger stratification can also destabilize the dynamics of phytoplankton production (Huisman et al., 2006). Additionally, the types of lipids and fatty acids produced by phytoplankton were significantly affected by rising sea surface temperature (SST) (Ackman et al., 1968; Satu and Murata, 1980). Previous studies suggest that rising SST could decrease fatty acid unsaturation, and thus lead to reduction in cell membrane fluidity, which would affect on biosynthetic activity such as photosynthesis (Thompson et al., 1992b; Renaud et al., 1995). Especially, some free fatty acids such as PUFAs are associated with toxicity in harmful algal bloom species (HABs) (Yasumoto et al., 1990). Therefore, increased temperature can also be an important factor in the regulation of toxin concentration produced by HABs in future environmental conditions.

Many studies suggest that global climate changes will have major effects on the physiology of marine phytoplankton, including alteration of growth, carbon fixation rates, and metabolic activity (Eppley, 1972; Riebesell, 2000; Fu et al., 2007, 2008a, b; Rost et al., 2008; Feng et al., 2010; Gao et al., 2012a). However, until now, research on phytoplankton has predominantly focused on monoculture, and only a few studies have applied a community approach to test varying CO_2 concentrations on the natural phytoplankton community (Hein and Sand-Jensen, 1997; Tortell et al., 2000; Tortell et al., 2002). Furthermore, growth in the same phytoplankton species was inconsistent with increasing CO_2. For instance, while an increase in short-term photosynthesis and growth has been demonstrated for natural assemblages in the Northern Atlantic Ocean (Hein and Sand-Jensen, 1997), photosynthesis and growth rate of coastal phytoplankton communities did not respond to CO_2 enrichment (Tortell et al., 2002). In addition, pelagic mesocosm studies with combined effects of rising CO_2 and temperature on phytoplankton species have rarely been addressed. Therefore, the main objective of this thesis is to investigate the responses of the phytoplankton community and species with respect to rising CO_2 and temperature conditions.

In addition, most studies on the influence of rising SST on fatty acid composition and concentration in phytoplankton, until now, have focused on diatoms and chlorophyta, because they are of interest for biofuel production (e.g., Becker, 2007; Deng et al., 2009) and are the primary foods of herbivorous copepods (Peters et al., 2007; Evjemo et al., 2008). Dinoflagellates and raphidophytes comprise a substantial part of harmful algal blooms that affect fish and humans (Marshall and Hallegraeff, 1998; Heil et al., 2005). Recent studies have shown that PUFAs and some free fatty acids play a key role in the ichthyotoxicity of HABs (Cho et al., 2001). Suzuki and Matsuyama (1995) also reported that when PUFAs are actively released due to cell damage, they can be lethal to fish. Despite numerous researches that shows the close relationship between fatty acid production and their toxicity, changes in the fatty acid production in dinoflagellates and raphidophytes with rising temperature have not previously been studied. Therefore, the second goal of this thesis was to examine the potential impacts of changes in temperature on HAB species from a physiological perspective.

This dissertation will help to understand species-specific differences in the physiology of marine phytoplankton under future oceanic conditions.

1.2 The seawater carbonate system

In this part, a short description of the main parameters and equations of the seawater carbonate system are introduced, which are important for understanding carbon availability and its significance for phytoplankton carbon acquisition in marine systems.

When CO_2 dissolves in seawater, it reacts with H_2O to form carbonic acid (H_2CO_3), a weak acid:

$$[CO_2] + [H_2O] = [H_2CO_3]$$

Carbonic acid dissociates to hydrogen ions (H^+) and bicarbonate ions (HCO_3^-):

$$[H_2CO_3] = [H^+] + [HCO_3^-]$$

The extra hydrogen ions combine with carbonate ions to form bicarbonate ions:

$$[2H^+] + [CO_3^{2-}] = [HCO_3^-] + [H^+]$$

These reactions are reversible and near equilibrium (Millero et al., 2002); for surface seawater with a pH of ~ 8.1, approximately 90% of the inorganic carbon is bicarbonate ion, 9% is carbonate ion, and only 1% is dissolved CO_2. Increasing dissolved CO_2 in seawater results in higher concentrations of carbonic acid (H_2CO_3), hydrogen ions (H^+), and bicarbonate ions (HCO_3^-) and lower carbonate ions (CO_3^{2-}); the latter has a lower pH because pH = - $\log_{10}[H^+]$. Carbonate ion concentration declines, however, because of the increasing H^+ concentration. The projected 0.3 ~ 0.4 pH drop for the 21st century is equivalent to approximately a 150% increase in H^+ and 50% decrease in CO_3^{2-} concentration (Orr et al., 2005).

1.3 Outline of the thesis

This thesis investigates the response of phytoplankton species to rising CO_2 and temperature. Through the monoculture experiments, (1) the response of four diatom species under increasing CO_2 and temperature was examined; (2) the changes in fatty acid production in HABs at four different were examined. In

mesocosm experiments, the response of both plankton groups from bacteria to ciliate and dominant phytoplankton species under future climate conditions was monitored.

Study 1 Effects of increased CO_2 and temperature on the growth of four diatom species (*Chaetoceros debilis*, *Chaetoceros didymus*, *Skeletonema costatum* and *Thalassiosira nordenskioeldii*) in laboratory experiments.

Study 2 Effects of increased pCO_2 and temperature on the coastal marine phytoplankton community structure.

Study 3 Thermal effects on the growth and fatty acid composition of four harmful algal bloom species: possible implications for ichthyotoxicity in future marine environments.

1.4 Reference

Ackman, R.G., C.S. Tocher and J. McLachlan. 1968. Marine phytoplankter fatty acids. J. Fish. Res. Can. 25: 1603~1620.

Becker, E.W. 2007. Microalgae as a source of protein. Biotechnol. Adv. 25: 207~210.

Cho, E.S., B.D. Choi, Y.C. Cho, T.J. Kim and H.G. Kim. 2001. Discrimination of three highly toxic *Alexandrium tamarense* (Dinophyceae) isolates using FITC-conjugated lectin probes. J. Plankton Res. 23(1): 89~95.

Deng, X., Y. Li and X. Fei. 2009. Microalgae: a promising feedstock for biodiesel. African J. Microbiol. Res. 3(13): 1008~1014.

Eppley, R.W. 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. 70: 1063~1085.

Evjemo, J.O., N. Tokle, O. Vadstein and Y. Olsen. 2008. Effect of essential dietary fatty acids on egg production and hatching success of the marine copepod *Temora longicornis*. J. Exp. Mar. Biol. Ecol. 365: 31~37.

Feng, Y., C. Hare, J. Rose, S. Handy and others. 2010. Interactive effects of iron, irradiance and CO_2 on Ross Sea phytoplankton. Deep-Sea Res. I. 57: 368~383.

Fu, F.X., M.E. Warner, Y. Zhang, Y. Feng and D.A. Hutchins. 2007. Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine Synechococcus and Prochlorococcus (Cyanobacteria). J. Phycol. 43: 485~496.

Fu, F.X., Y. Zhang, M.E. Warner, Y. Feng and D.A. Hutchins. 2008b. A comparison of future ncreased CO_2 and temperature effects on sympatric *Heterosigma akashiwo* and *Prorocentrum minimum.* Harmful Algae. 7: 76~90.

Fu, F.X., A.O. Tatters and D.A. Hutchins. 2012. Global change and the future of harmful algal blooms in the ocean. Mar. Ecol. Prog. Ser. 470: 207~233.

Gao, K., J. Xu, G. Gao, Y. Li and others. 2012. Rising CO_2 and increased light exposure synergistically reduce marine primary productivity. Nat. Clim. Change. 2: 519~523.

Giordano, M., J. Beardall and J.A. Raven. 2005. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Ann. Rev. Plant. Biol. 56: 99~131.

Hays, G.C., A.J. Richardson and C. Robinson. 2005. Climate change and marine plankton. Trends. Ecol. Evol. 20: 337~344.

Heil, C.A., P.M. Glibert and C. Fan. 2005. *Prorocentrum minimum* (Pavillard) Schiller A review of a harmful algal bloom species of growing worldwide importance. Harmful algae. 4: 449~470.

Hein, M. and K. Sand-Jense. 1997. CO_2 increase oceanic primary production. Nature. 388(6642): 526~527.

Huisman, J., N. Nga, P. Thi, D.M. Karl and B. Sommeijer. 2006. Reduced mixing generates oscillations and chaos in the oceanic deep chlorophyll maximum. Nature 439: 322~325.

Kleppel, G.S., D.V. Holiday and R.E. Pieper. 1991. Trophic interactions between copepods and microplankton: a question about the role of diatoms. Limnol. Oceanogr. 36:172~178.

Kudela, R.M., W.P. Cochlan, T.D. Peterson and C.W. Trick. 2006. Impacts on phytoplankton biomass and productivity in the Pacific Northwest during the warm ocean conditions of 2005. Geophys Res Lett 33:L22S06, doi:10.1029/2006GL026772.

Marshall, J.A. and G.M. Hallegraeff. 1998. Comparative ecophysiology of the harmful alga Chattonella marina (Raphidophyceae) from South Australian and

Japanese waters. J. Plankton Res. 21: 1809~1822.

Millero, F.J., D. Pierrot, K. Lee, R. Wanninkhof, R. Feely, et al. 2002. Dissociation constants fro carbonic acid determined from field measurements. Deep Sea Res. I. 49: 1705~1723.

Orr, J.C., V.J. Fabry, O. Aumont, L. Bopp and S.C. Doney, et al. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature. 437: 681~86.

Peters, J., J. Dutz and W. Hagen. 2007. Role of essential fatty acids on the reproductive success of the copepod *Temora longicornis* in the North Sea. Mar. Ecol. Prog. Ser. 341: 153~163.

Raven, J., K. Caldeira, H. Elderfield, O. Hoegh-Guldberg, P. Liss, U. Riebesell, J. Shepherd, C. Turley and A. Watson. 2005b. Ocean Acidification Due to Increasing Atmospheric Carbon Dioxide. The Royal Society, London. 68 pp.

Renaud, S.M., H.C. Zhou, D.L. Parry, T. Loung-Van and K.C. Woo. 1995. Effects of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae *Isochrysis* sp., *Nitzschia closterium*, *Nitzschia paleacea*, and commercial species *Isochrysis* sp. (clone

T.ISO). J. Appl. Phycol. 7: 595~602.

Riebesell, U., I. Zondervan, B. Rost, P.D. Tortell, R.E. Zeebe and F.M.M. Morel. 2000. Reduced calcification of marine plankton in response to increased atmospheric CO_2. Nature. 407: 364~367.

Riebesell, U. 2004. Effects of CO_2 enrichment on marine phytoplankton. J. Oceanogr. 60: 719~729.

Rost, B., I. Zondervan and D. Wolf-Gladrow. 2008. Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions. Mar. Ecol. Prog. Ser. 373: 227~237.

Sarmiento, J.L., J. Dunne, A. Gnanadesikan, R.M. Key, K. Matsumoto and R. Slater. 2002. A new estimate of the $CaCO_3$ to organic carbon export ratio. Glob. Biogeochem. Cycles. 16: 1107.

Satu, N. and N. Murata. 1980. Temperature shift-induced responses in lipids in the blue-green alga, *Anabaena variabilis*. The central role of diacylmonoalactosylglycerol in thermo adaption. Biochim. Biophys. Acta. 619: 353~365.

Shin, K., M.-C. Jang, P.-K. Jang, S.-J. Ju, T.-K. Lee and M. Chang. 2003.

Influence of food quality on egg production and viability of the marine planktonic copepod Acartia omorii. Prog. Oceanography. 57: 265~277.

Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis and others. 2007. Climate Change 2007: The Physical Science Basis: Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. New York: Cambridge Univ. Press.

Sommer, U. and K. Lengfellner. 2008. Climate change and the timing, magnitude, and composition of the phytoplankton spring bloom. Glob. Change Biol. 14: 1199~1208.

Suzuki, T. and Y. Matsuyama. 1995. Determination of free fatty acids in marine phytoplankton causing red tides by fluorometric high-performance liquid chromatography. JAOCS. 72: 1211~1214.

Thompson, P.A., M. Guo, P.J. Harrison and J.N.C. Whyte. 1992b. Effects of variation in temperature: II. On the fatty acid composition of eight species of marine phytoplankton. J. Phycol. 28: 488~497.

Tortell, P.D., G.H. Rau and F.M.M. Morel. 2000. Inorganic carbon acquisition in coastal Pacific phytoplankton communities. Limnol. Oceanogr. 45: 1485~1500.

Tortell, P.D. and F.M.M. Morel. 2002. Sources of inorganic carbon for phytoplankton in the eastern Subtropical and Equatorial Pacific Ocean. Limnol. Oceanogr. 47: 1012~1022.

Tortell, P.D., G.R. DiTullio, D.M. Sigman and F.M.M. Morel. 2002. CO_2 effects on taxonomic composition and nutrient utilization in an equatorial Pacific phytoplankton assemblage. Mar. Ecol. Prog. Ser. 236: 37~43.

Wolf-Gladrow, D.A., U. Riebesell, S. Burkhardt and J. Bijma. 1999. Direct effects of CO_2 concentration on growth and isotopic composition of marine plankton. Tellus. B. Chem. Phys. Meterol. 51: 461~476.

Yasumoto, T., B. Inderdal, T. Aune, V. Hormazabal, O.M. Skulberg and Y. Oshima. 1990. Screening for hemolytic and ichthyotoxic components of *Chrysochromulina polyepsis* and *Gyrodinium aureolum* from Norwegian coastal waters. In: Graneli, E., Sundstron, B., Edler, L., Anderson, D.M., (eds.), Toxic Marine Phytoplankton. Elsevier, USA. 436~440 pp.

2. STUDIES

2.1 Effects of increased CO_2 and temperature on the growth of four diatom species (*Chaetoceros debilis, Chaetoceros didymus, Skeletonema costatum and Thalassiosira nordenskioeldii*) in laboratory experiments

2.1.1 Introduction

Global warming gases (greenhouse gases; mainly carbon dioxide) are continuously being emitted into the atmosphere, with carbon dioxide increasing in the atmosphere by 0.4% each year. Its concentration has increased by about 30% since the Industrial Revolution (Feng et al., 2009), and the CO_2 level in 2100 is predicted to be twice the current CO_2 level (Houghton et al., 2001; Alley et al., 2007). If the current rate of increase atmospheric carbon dioxide continues, it will lower ocean surface pH about 0.35 units (Caldeira and Wickett, 2003), and increase the surface temperature by at least 1.1°C (low CO_2 emission scenario B1) and possibly up to 6.4°C, the high CO_2 emission scenario "A1FI " suggested

by the year 2100 (Huertas et al., 2011). Such ocean acidification and seawater temperature increases can affect the biogeochemical cycles of the ocean, including the physiology, species composition, and interspecific competition of marine organisms (Fu et al., 2007).

Diatoms account for approximately 25% of global primary production in marine ecosystems, playing an important role in the marine carbon cycle (Hu and Gao, 2008). Diatoms typically require larger amounts of carbon dioxide than is in solution in the oceans, as in general their growth in seawater has been proved to be CO_2-limited. To cope with this deficiency, diatoms are known to have carbon-concentrating mechanisms (CCMs) (Rost et al., 2003; Giordano et al., 2005). Operation of the CCMs ensures that internal carbon dioxide concentrations are close to saturating the photosynthetic capacity for its fixation (Engel et al., 2005). Kim et al. (2006) reported that enhanced CO_2 concentration can stimulate the abundance of chain forming diatom *Skeletonema costatum*, and Tortell et al. (2008) suggested that elevated CO_2 enhanced the growth of *Chaetoceros* spp. in the Southern Ocean. The diatom *Phaeodactylum tricornutum* showed faster

growth and greater carbon fixation at higher concentrations of carbon dioxide (Matsuda et al., 2001, Wu et al., 2010). Therefore, atmospheric CO_2 rise and associated increase in seawater pCO_2 may not adversely affect marine diatom production (Wu et al., 2010). However, CCMs can be down-regulated by increasing CO_2 levels, reducing their CO_2-transfer capacity (Giordano et al., 2005). Moreover, recent studies have demonstrated that phytoplankton species and functional groups differ regarding the efficiency and regulation of their CCMs, indicating that changes in CO_2 availability might affect competition and succession of phytoplankton species (Burkhardt et al., 2001; Rost et al., 2003). For instance, Tortell et al. (2002) showed that increasing CO_2 concentration changed the relative success of phytoplankton groups, with the prymnesiophyte *Phaeocystis* sp. favored at pCO_2 150 ppm and diatoms at 750 ppm. However, it is likely that CO_2 dependence variations are not necessarily consistent even among diatoms.

Until recently, most studies have focused singly on the effects of CO_2 or water temperature on phytoplankton growth, and studies of the combined effects have

been limited to dinoflagellates (Fu et al., 2008) and certain blue-green algae (Fu et al., 2007). Few studies have been performed for dominant diatoms. Our study was performed to examine growth of the marine diatoms *Skeletonema costatum, Chaetoceros debilis, Chaetoceros didymus,* and *Thalassiosira nordenskioeldii* under current and three simulated future conditions of water temperature and CO_2 level: acidification, global warming, and intense greenhouse conditions. The consumption of carbon dioxide under these conditions was indirectly estimated by determining *p*H in the culture media.

2.1.2 Materials and methods

From diatom cultures maintained at the Korea Institute of Ocean Science and Technology (KIOST), four species (*S. costatum, C. debilis, C. didymus,* and *T. nordenskioeldii*) were used in this study. Seawater for culturing diatoms was collected from the Korea Strait (salinity range, 29.9-34.8 psu from 2006 to 2008) 18.5 km from the southern coast of Korea. The seawater was passed through a membrane filter (pore size 0.2μm, Advantec), and was enriched with f/2 medium with SiO_4. The experimental culture media were autoclaved (15 min, 121°C).

Crystal rising dishes (diameter 115mm x height 65mm, DURAN^{TM} borosilicate glass) were used, and the incubator (Lab-tech co.) was modified to facilitate the inflow of carbon dioxide gas required for the experiment. We used CO_2 gas at high precision concentrations of commercially prepared air/CO_2 mixtures (400 ppm and 1000 ppm, air balance). The salinity was maintained at about 31 psu, light intensity at 60 μmol m $^{-2}s^{-1}$, and the photoperiod at a cycle of 12h light: 12h dark (Table 2.1.1).

Table 2.1.1. Initial experimental conditions for four diatom species grown under present, acidification, global warming and greenhouse conditions

Environmental parameters	Initial Incubation conditions			
	Present	Acidification	Global warming	Greenhouse
pCO_2 (ppm)	400	1000	400	1000
Temperature (°C)	20±1.0	20±1.0	25±1.0	25±1.0
pH	8.17±0.1	7.81±0.1	8.17±0.1	7.81±0.1
Salinity (psu)	31.4±0.1			
Photoperiod (hr)	12light : 12 dark			
Medium	Filtered (0.2μm) and sterilized seawater			
Nutrient	Adding the F/2 media 132μL and silicate (10mM) 1mL			
Chamber	0.5 liter volume Duran Borosilicate Glass, ø115mm×h65mm			

A control group (present condition- pCO_2: 400ppm, water temperature: 20°C) and three experimental groups (acidification condition - pCO_2: 1000ppm, water temperature: 20°C; global warming condition- pCO_2: 400ppm, water temperature: 25°C; greenhouse condition - pCO_2: 1000ppm, water temperature: 25°C) were set up to simulate three possible future conditions of ocean water based on the IPCC

A2 Scenarios (IPCC, 2007) (Table 2.1.1). Each level of CO_2 gas was injected into the incubation dishes until their pH values stabilized, and then phytoplankton were inoculated at 50 to 100 cells mL^{-1} for each dish. The culture dishes (treatments replicated in triplicate), with their tops open to allow the air-CO_2 mixtures to exchange with their interiors, were arranged in a air-tight sealed transparent acrylic container (l400mm X 250mm X 400mm) for each treatment inside the incubators set at 20°C (i.e., containers for present and acidification condition) and 25°C (i.e., containers for global warming and greenhouse condition), respectively. The concentration of carbon dioxide in each dish was maintained by continuously injecting the air-CO_2 mixture into each container at 400 ppm and 1,000 ppm.

Growth of the phytoplankton cultures was checked by measuring *in vivo* fluorescence (FSU) (Turner Designs 10-AU) daily after the third day of incubation, along with the pH changes in the medium with a pH meter (Orion Inc.). The experiments were terminated when the populations were in late exponential phase or early stationary phase. A sample of 15 mL was taken from

each bottle for each measurement of FSU and pH, and the growth rates, as doubling per day (μ), were calculated by applying

$$\mu = (\log_2 N_t - \log_2 N_o) / t$$

where t is length of incubation (days), N_0: initial value of FSU, N_t : FSU value at the end of the experiment.

With uptake of CO_2 by phytoplankton, pH in the culture medium can increase substantially. To maintain the pH values more or less at the initial levels of the experiments, small amounts of (1-5 mL) freshly sterilized culture medium hypersaturated with CO_2 (10,000 ppm) were added to the culture dishes. The volume of culture medium in the culture dishes was maintained > 70% of the initial volume until the end of experiment.

The FSU normalized pH values for the last two samplings (generally between Day 6 and 7 or Day 7 and 8) of the incubation were calculated to examine species-specific capability of chlorophyll synthesis (i.e., growth) per unit uptake of CO_2 concentration (i.e., pH increase as a proxy for CO_2 uptake).

One-way analysis of variance (n=2 or 3) and Tukey's honestly significant difference tests (Tukey's HSD) were performed to compare the mean growth rates among the four conditions. Normality of the variations was examined from the distribution of values around the means.

2.1.3 Results and discussion

All of the four diatom species showed exponential growth in the control (i.e., present condition) dishes, but with growth rates ranging from 0.54 doublings d^{-1} (*C. debilis*) to 0.96 doublings d^{-1} (*T. nordenskioeldii*). A lag phase was observed in the control dishes for all species; growth was generally slower in the first 3 days of incubation.

The four diatom species exhibited different growth responses to various simulated conditions. *Skeletonema costatum* showed repressed growth under global warming and greenhouse conditions (0.65 doublings d^{-1} and 0.68 doublings d^{-1}, respectively) compared with present and acidification conditions (0.87 doublings

d^{-1} and 0.85 doublings d^{-1}, respectively) (Fig. 2.1.1a and 2.1.2a, Tukey's HSD, $p<0.05$). Its growth was not different between present and acidification conditions or between global warming and greenhouse conditions (Tukey's HSD, $p>0.05$). Increased temperature, but not elevated CO_2 level in the culture, affected the cell growth of this diatom (Table 2.1.2).

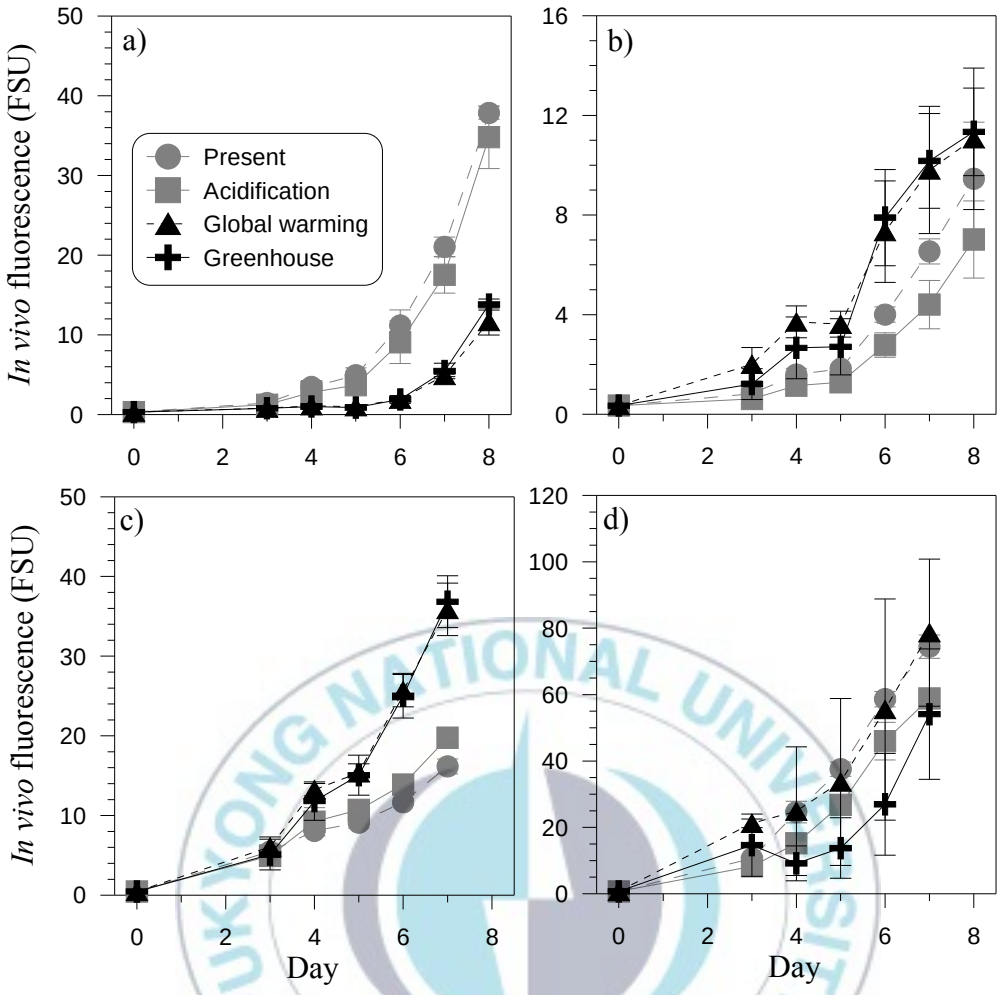


Fig. 2.1.1. Changes of fluorescence during experimental periods for four diatom species under present, acidification, global warming and greenhouse conditions. (a) *Skeletonema costatum*, (b) *Chaetoceros debilis*, (c) *Chaetoceros didymus*, (d) *Thalassiosira nordenskioeldii*.

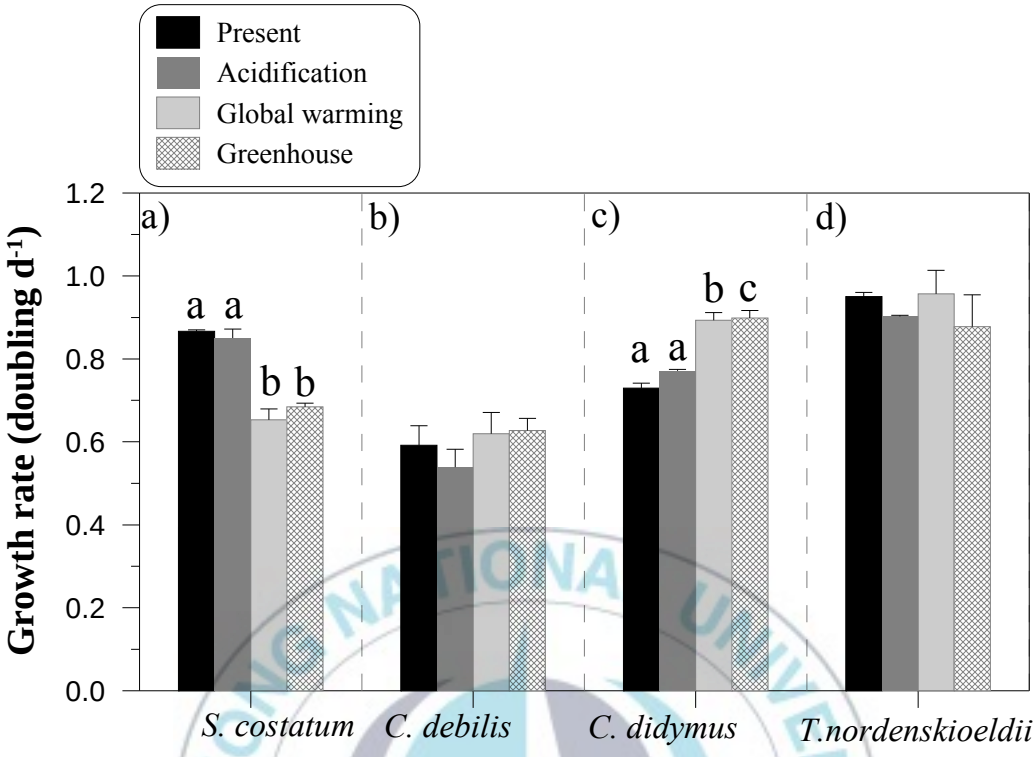


Fig. 2.1.2. Comparison of growth rate of four diatom species for four different growth conditions. The vertical bars represent 95% confidence interval (n=2 for *Thalassiosira nordenskioeldii* culture in acidification and greenhouse condition, other all species culture conditions in n=3).

Chaetoceros debilis grew more or less similarly in all simulated conditions (Fig. 2.1.1b and 2.1.2b), whereas *C. didymus* grew much faster under global warming

and greenhouse condition (Fig. 2.1.1c and 2.1.2c), quite the opposite from the results for *S. costatum*. *Chaetoceros didymus* also showed slightly better growth in the acidification condition (0.77 doublings d^{-1}) than under the present condition (0.73 doublings d^{-1}). Its growth was not different between global warming and greenhouse condition, which suggests that water temperature increase had a greater effect on the growth than did the elevated CO_2 level (Table 2.1.2).

Thalassiosira nordenskioldii grew the fastest among the four diatom species (0.88-0.96 doublings d^{-1}). However, fluorescence for *T. nordenskioeldii* increased at similar rates for all simulated conditions over the incubation period, resulting in no significant difference in the growth rate (Fig. 2.1.1d and 2.1.2d).

The pH in the culture media varied quite differently among species over the incubation period (Fig. 2.1.3), with the least variation observed in *C. debilis* cultures that grew the slowest and the most in *T. nordenskioeldii* cultures that grew the fastest. The pH quickly rose for all treatments in the *T. nordenskioeldii* cultures (Fig. 2.2.3d) such that addition of concentrated CO_2 solution was

necessary to bring down the pH in the cultures to the level at the beginning. Such rises in pH in the cultures of *T. nordenskioeldii* and other phytoplankton cultures were likely due to cellular uptake of CO_2 from the media, uptake which took place mostly in the later days (e.g., Day 4 or 5 for *T. nordenskioeldii*) of the incubation. The variation differed among the simulated conditions for each species, but generally was positively correlated with cell growth under each condition (Fig. 2.1.1 vs. Fig. 2.1.3).

The FSU normalized pH changes for the last two sampling times (generally between Days 6 and 8 or Days 7 and 8, Fig. 2.1.4), during which the cells grew exponentially, followed the same patterns of pH change over the incubation period (Fig. 2.1.4). The ratio, along with the results of pH change (Fig. 2.1.3), could indicate higher cell growth under future marine conditions of higher concentration of CO_2, and thus those species with higher values might be better suited to future conditions. Cultures of *S. costatum* and *T. nordenskioeldii* were not different in pH increase per FSU (pH/FSU) among the four conditions (Fig. 2.1.4a and 2.1.4d). For *C. debilis*, increased pH/FSU was observed only in the

greenhouse treatment (Fig. 2.1.4b). *Chaetoceros didymus* showed more a complex pattern, the greenhouse condition having a significantly greater pH/FSU increase than the global warming condition, and the pH/FSU of the acidification condition was greater than for both the greenhouse and the present conditions (Fig. 2.1.4c).

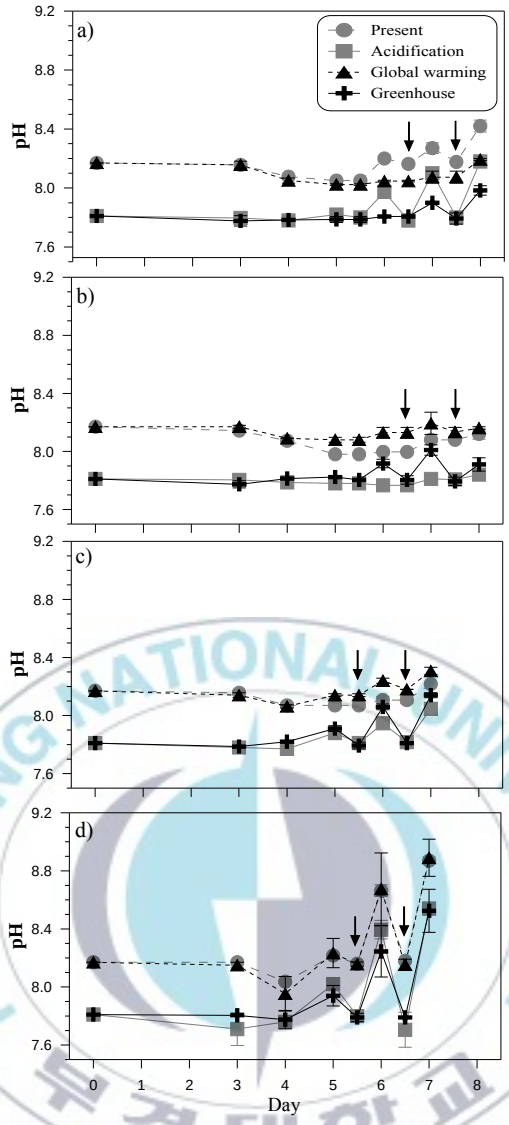


Fig. 2.1.3. Changes of pH concentration of four diatom species under present, acidification, global warming and greenhouse conditions during the experimental periods. (a) *Skeletonema costatum*, (b) *Chaetoceros debilis*, (c) *Chaetoceros didymus*, (d) *Thalassiosira nordenskioeldii*. The arrows indicate the time points when concentration CO_2 was added to phytoplankton culture.

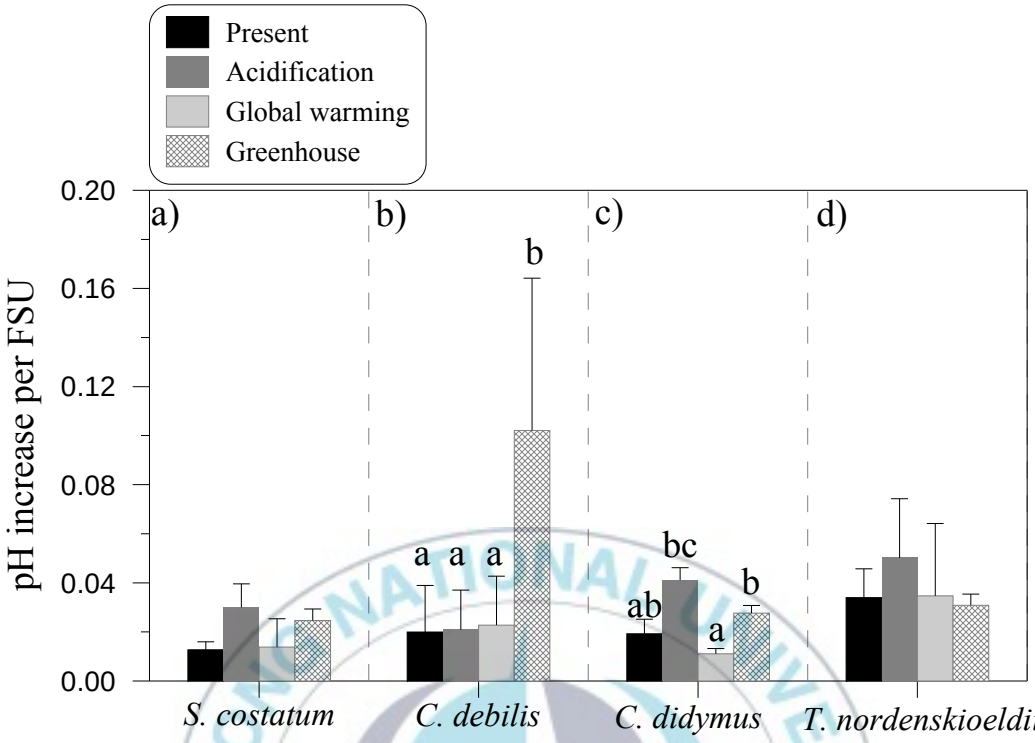


Fig. 2.1.4. Changes in the pH value per fluorescence units (FSU) during last two days for four diatom species under present, acidification, global warming and greenhouse conditions. Characters indicate significant difference at α =0.05 level for Tukey's honestly significant difference tests. The vertical bars represent 95% confidence interval (n=2 for *Thalassiosira nordenskioeldii* culture in acidification and greenhouse condition, other all species culture conditions in n=3).

Table 2.1.2. Predicted outcome in future ocean for four diatom species in response to combined impacts of CO_2 and temperature increase

Condition	*Skeletonema costatum*	*Chaetoceros debilis*	*Chaetoceros didymus*	*Thalassiosira nordenskioeldii*
CO_2	Neutral	Neutral	Neutral	Neutral
Temperature	Negative	Neutral	Positive	Neutral
Future ocean	Negative	Positive	Positive	Neutral

Our results show that diatoms respond in various ways to increased water temperature and CO_2 level, ranging from suppression in growth, to no response, to enhanced growth. More specifically, the diatoms studied appeared to be more affected by water temperature than by CO_2 level. Growth of *S. costatum* is generally unaffected by elevation of CO_2 concentration (Chen and Gao 2003). No changes in its growth were observed even at very low CO_2 concentration (<4 μmol L^{-1}) (like results of Goldman, 1999). Cultures of the diatom *Chaetoceros muelleri* showed lower growth and/or no response in elevated CO_2 concentrations (Gao et al., 2012). In contrast, in a mesocosm study, both *S. costatum* and

Chaetoceros spp. showed increased growth rates with increased pCO_2 (Kim et al., 2010). The reduced growth of *S. costatum* at 25°C (Fig. 2.1.1a) is consistent with the observation of Montagnes and Franklin (2001). Their *S. costatum* cultures grew faster with temperature increase up to 20°C, but reduced growth at 25°C. However, *S. costatum* is a eurythermal species consisting of many sub-strains (Sarno et al., 2007), growing at water temperatures from 2.0°C to 30°C (Hitchcock, 1980), strains appearing through the year off the coasts of Korea and Japan (Oh et al., 2008; Shikata et al., 2008; Park et al., 2009). Maximal growth rates of different strains are found between 20 and 30°C (Yoder, 1979), and thus more studies for various strains of this species are necessary fully to characterize their responses to temperature and CO_2 increases.

The *Chaetoceros* species annually occur around in coastal waters around the world (Guiry and Guiry, 2012), and they mainly occur in spring and autumn in Korean seawaters, frequently being the dominant phytoplankton species (Park et al., 2009). However, recent studies show that they usually occur massively in summer (Baek and Kim, 2010) and grow better in the laboratory when grown at

temperatures higher than the *in situ* temperature (Karentz and Smayda, 1984). Our results of higher growth rates for both *C. debilis* and *C. didymus* at high temperature are consistent with previous studies.

Thalassiosira nordenskioeldii is reported to occur at high density in cold seasons (winter and spring) in Long Island Sound and Narragansett Bay (Popovich and Gayoso, 1999). It is also known as a cold-water species in Korean waters, present at high density in seasons of low temperature (Choi et al., 1997). Durbin (1974) reported that the species grew slower as the culture temperature rose from 10°C to 15°C, but no such growth repression at higher temperature was detected in our study (Fig. 2.1.1d).

Phytoplankton groups and species greatly vary in their requirements for the carbon dioxide that is essential for their growth (Riebesell, 2004). Previous studies indicate that diatoms may respond in various ways to increased CO_2 as ocean acidification progresses, from better growth (positive response) (Schippers et al., 2004; Kim et al., 2006; Egge et al., 2009), to no effects (neutral response)

(Gao et al., 2012), to reduced growth (negative response) (Wu et al., 2010). In this study, enhanced carbon dioxide concentration seemed to have no adverse effects on the growth for the four species examined. For *Chaetoceros* spp., future rise in CO_2 may not only increase their growth but also their uptake of CO_2, leading to potentially faster growth rates and greater carbon fixation by this genus. Thus, in the likely warmer, higher-CO_2 oceans of the future, *Chaetoceros* spp. are apparently better adapted for survival and growth than are the other two species.

2.1.4 Conclusion

Growth rate determinations in elevated CO_2 and temperature showed no negative effects of more CO_2 in any of four diatom species studied; in fact it stimulated their growth. All of the adverse effects were from temperature increase. With their higher carbonate consumption capacity, *Chetoceros* spp. seem to be better adapted than other two species studied to coastal waters of increased CO_2 and temperature.

2.1.5 Reference

Alley, R.B., T. Berntsen, N.L. Bindoff, Z. Chen and others. 2007. Summary for policymakers, In: Solomon, S, D Qin, M Manning, Z Chen and others (eds). Climate change. 2007: The physical science basis. Contribution of Working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge and New York.

Baek, S.H. and Y.O. Kim. 2010. The study of summer season in Jinhae Bay-Short-term changes of community structure and horizontal distribution characteristics of phytoplankton. Kor. J. Environ. Biol. 28(3): 115~124. (in Korean)

Burkhardt, S., G. Amoroso, U. Riebesell and D. Sultemeyer. 2001. CO_2 and HCO_3- uptake in marine diatoms acclimated to different CO_2 concentrations. Limnol. Oceanogr. 46: 1378~1391.

Caldeira, K. and M.E. Wickett. 2003. Anthropogenic carbon and ocean pH. Nature. 425: 365.

Chen, X. and K. Gao. 2003. Effect of CO_2 concentrations on the activity of photosynthetic CO_2 fixation and Extracellular carbonic anhydrase in the marine.

Chi. Sci. Bull. 48(23): 2616~2620.

Choi J.K., E.H. Lee, J.H. Noh and S.H. Huh. 1997. The study on the phytoplankton bloom and primary productivity in lake Shihwa and adjacent coastal areas. J. Kor. Soc. Oceanogr. 2(2): 78~86. (in Korean)

Durbin, E.G. 1974. Studies on the autecology of the marine diatom *Thalassiosira nordenskioeldii* Cleve. 1. The influence of day-length, light intensity and temperature on growth. J. Phycol. 10: 220~225.

Egge, J.K., T.F. Thingstad, A. Larsen, A. Engel, J. Wohlers, R.G.J. Bellerby and U. Riebesell. 2009. Primary production during nutrient-induced blooms at elevated CO_2 concentrations. Biogeosciences. 6: 877~885.

Engel, A., I. Zondervan, K. Aerts, L. Beaufort, A. Benthien, L. Chou, B. Delille, J.P. Gattuso, J. Harlay, C. Heemann, L. Hoffmann, S. Jacquet, J. Nejstgaard, M.D. Pizay, E.R. Newall, U. Schneider, A. Terbrueggen and U. Riebesell. 2005. Testing the direct effect of CO_2 concentration on a bloom of the coccolithophorid *Emiliania Huxleyi* in mesocosm experiments. Limnol. Oceanogr. 50(2): 493~507.

Feng, Y.Y., C.E. Hare, K. Leblanc, J.M. Rose, Y.H. Zhang, G.R. DiTullio, P.A.

Lee, S.W. Wilhelm, J.M. Rowe, J. Sun, N. Nemcek, C. Gueguen, U. Passow, I. Benner, C. Brown and D.A. Hutchins. 2009. Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. I. The phytoplankton community and biogeochemical response. Mar. Ecol. Prog. Ser. 388: 13~25.

Fu, F.X., Y. Zhang, M.E. Warner, Y. Feng, J. Sun and D.A. Hutchins. 2008. A comparison of future increased CO_2 and temperature effects on sympatric *Heterosigma akashiwo* and *Prorocentrum minimum*. Harmful algae. 7: 76~90.

Fu, F.X., M.E. Warner, Y. Zhang, Y. Feng and D.A. Hutchins. 2007. Effects of increased temperature and CO_2 on photosynthesis, growth and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria). J. Phycol. 43: 485~496.

Gao, K., E.W. Helbling, D.P. Hader and D.A. Hutchins. 2012. Response of marine primary producers to interactions between ocean acidification, solar radiation, and warming. Mar. Ecol. Prog. Ser. 470: 167~189.

Giordano, M., J. Beardall and J.A. Raven. 2005. CO_2 concentrating mechanisms in algae: Mechanisms, environmental modulation, and evolution. Annual Review of Plant Biol. 56: 99~131.

Goldman, J.C. 1999. Inorganic carbon availability and the growth of large marine diatoms. Mar. Ecol. Prog. Ser. 180: 81~91.

Guiry, M.D. and G.M. Guiry. 2012. AlgaeBase. World-wide electronic publication. National University of Ireland. Galway. http://www.algaebase. org.

Hitchcock, G.L. 1980. Influence of temperature on the growth rate of *Skeletonema costatum* in response to variations in daily light intensity. Mar. Biol. 57: 261~269.

Hu, H. and K. Gao. 2008. Impact of CO_2 enrichment on growth and photosynthesis in freshwater and marine diatom. Chi. J. Oceanogr. Limnol. 26(4): 407~414.

Houghton, J.T., Y. Ding, D.J. Griggs, M. Noguer and others. 2001. Climate change 2001: the scientific basis. Cambridge University Press. Cambridge.

Huertas, I.E., R. Monica, V. Lopez-Rodas and E. Costas. 2011. Warming will affect phytoplankton differently: evidence through a mechanistic approach. Proceedings of the Royal Society B. 278: 3534~3543.

IPCC Fourth Assessment Report 2007. Climate Change: The Physical Science Basis; Intergovernmental Panel on Climate Change; edited by: Solomon, S.,

Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., and Miller, H.L. Cambridge University Press. New York.

Karentz, D. and T.J. Smayda. 1984. Temperature and seasonal occurrence patterns of 30 dominant phytoplankton species in Narragansett Bay over a 22-year period (1959-1980). Mar. Ecol. Prog. Ser. 18: 277~293.

Kim, J.M., K. Lee, K. Shin, J.H. Kang, H.W. Lee, M. Kim, P.G. Jang and M.C. Jang. 2006. The effect of seawater CO_2 concentration on growth of a natural phytoplankton assemblage in a controlled mesocosm experiment. Limnol. Oceanogr. 51: 1629~1636.

Kim, J.M., K. Lee, E.J. Yang, K. Shin, J.H. Noh, K.T. Park, B. Hyun, H.J. Jeong, J.H. Kim, K.Y. Kim, M. Kim, H.C. Kim, P.G. Jang and M.C. Jang. 2010. Enhanced production of oceanic dimethylsulfide resulting from CO_2-induced grazing activity in high CO_2 world. Environ. Sci. Technol. 44(21): 8140~8143.

Matsuda, U., T. Hara and B. Colman. 2001. Regulation of the induction of bicarbonate uptake by dissolved CO_2 in the marine diatom *Phaeodactylum tricornutum*. Plant Cell Environ. 24: 611~620.

Montagnes, D.J.S. and D.J. Franklin. 2001. Effect of temperature on diatom

volume, growth rate, and carbon and nitrogen content: Reconsidering some paradigms. Limnol. Oceanogr. 46(8): 2008~2018.

Oh, S.J., J.S. Lee, J.S. Park, I.H. Noh and Y.H. Yoon. 2008. Environmental factors on the succession of phytoplankton community in Jinju Bay, Korea. J. Kor. Soc. Mar. Environ. Engin. 11(2): 98~104. (in Korean)

Park, J.S., Y.H. Yoon and S.J. Oh. 2009. Variational characteristics of phytoplankton community in the mouth parts of Gamak Bay, Southern Korea. Kor. J. Environ. Biol. 27(2): 205~215. (in Korean)

Popovich, C.A. and A.M. Gayoso. 1999. Effects of irradiance and temperature on the growth rate of *Thalassiosira curviseriata* Takano (Bacillariophyceae), a bloom diatom in Bahía Blsanca estuary (Argentina). J. Plank. Res. 21(6): 1101~1110.

Riebesell, U. 2004. Effects of CO_2 enrichment on marine phytoplankton. J. Oceanogr. 60: 719~729.

Rost, B., U. Riebesell, S. Burkhardt and D. Sultemeyer. 2003. Carbon acquisition of bloom-forming marine phytoplankton. Limnol. Oceanogr. 48: 55~67.

Sarno, D., W.H.C.F. Kooistra, S. Balzano, P.E. Hargraves and A. Zingone. 2007.

Diversity in the genus *Skeletonema* (BACILLARIOPHYCEAE): III. Phylogenetic position and morphological variability of *Skeletonema costatum* and *Skeletonema grevillei*, with the description of *Skeletonema ardens* sp. NOV.[1], J. Phycol.. 43: 156~170.

Schippers, P., M. Lűrling and M. Scheffer. 2004. Increase of atmospheric CO_2 promotes phytoplankton productivity. *Ecology Letters*. 7: 446~451.

Shikata, T., S. Nagasoe, T. Matsubara, S. Yoshikawa, Y. Yamasaki, Y. Shimasaki, Y. Oshima, I.R. Jenkinson and T. Honjo. 2008. Factors influencing the initiation of blooms of the raphidophyte *Heterosigma akashiwo* and the diatom *Skeletonema costatum* in a port in Japan. Limnol. Oceanogr. 53(6): 2503~2518.

Tortell, P.D., G.R. Ditullio, D.M. Sigman and F.M.M. Morel. 2002. CO_2 effects on taxonomic composition and nurtrient utilization in an equatorial Pacific phytoplankton assemblage. Mar. Ecol. Prog. Ser. 236: 37~43.

Tortell, P.D., C.D. Payne, Y. Li, S. Trimborn, B. Rost, W.O. Smith, C. Riesselman, R.B. Dunbar, P. Sedwick and G.R. Ditullio. 2008. CO_2 sensitivity of Southern Ocean phytoplankton. Geophysical Research Letters. 35, L04605, doi:10.1029/2007GL032583.

Wu, Y., K. Gao and U. Riebesell. 2010. CO_2-induced seawater acidification affects physiological performance of the marine diatom *Phaeodactylum tricornutum*. Biogeosciences. 7: 2915~2923.

Yoder, J.A. 1979. Effect of temperature on light-limited growth and chemical composition of *Skeletonema costaturm* (Bacillariophyceae). J. Phycol. 15: 362~370.

2.2 Effects of increased pCO_2 and temperature on the coastal marine phytoplankton community structure

2.2.1 Introduction

Human-induced ocean acidification and global warming are emerging as the greatest threat facing the earth's ecosystem. The atmospheric partial pressure of CO_2 (pCO_2) is predicted to nearly double (i.e., 750ppm) in the next century (Houghton et al., 2001). The resulting increase of the dissolved CO_2 concentration in the world's oceans will reduce the pH of the sea surface by 0.5 units, which is equivalent to a threefold increase in the concentration of hydrogen ions, by 2100 (Wolf-Gladrow et al., 1999; Caldeira and Wickett, 2003; Tanaka et al., 2008). At the same time, warming associated with the release of greenhouse gases into the atmosphere has been predicted to raise sea surface temperature (SST) by 1 to 4˚C over the next 100 years (Feng et al., 2009). The pace of climate change is unprecedented in our geological history, and some coastal waters will experience much higher shifts than 2˚C or so (Beardall and Stojkovic, 2006).

These altered oceanographic conditions might impact the primary marine producers. Phytoplankton in particular account for approximately 50% of global primary production, playing an important role in the marine carbon cycle (Hu and Gao, 2008). Many phytoplankton groups possess a type I Rubisco (ribulose-1, 5-bisphosphate carboxylase-oxygenase; Tortell, 2000). Type I Rubisco has a high affinity for carboxylation and is therefore extremely efficiently at present-day atmospheric concentration, compared to the type II Rubisco found in some bloom-forming dinoflagellates (Fu et al., 2012). To overcome this limitation, the low-affinity CO_2-fixing system may be compensated for by efficient carbon-concentration mechanisms (CCMs) such as various forms of carbonic anhydrase (CA), which facilitate the uptake of bicarbonate and its conversion to CO_2 (Giordano et al., 2005). However, their efficiencies can differ between groups (Badger et al., 1998; Tortell, 2000) and, hence, carbon fixation could be stimulated by rising CO_2 for some species, but not for others.

One of the many changes associated with global climate change is ocean

acidification. Ocean acidification can change in cell growth and primary production, as well as in the community structure of the phytoplankton assemblages (Riebesell, 2004; Kim et al., 2013). Results from previous studies indicate that one response to elevated CO_2 concentration could enhance the phytoplankton primary production (Hein and Sand-Jensen, 1997). For instance, elevated pCO_2 can enhance primary production (Zondervan et al., 2001; Leonardos and Geider, 2005) and also modify phytoplankton species composition and succession (Tortell et al., 2002; Tanaka et al., 2008). Additionally, a recent model suggests that the growth of marine phytoplankton will increase by 40% between current CO_2 levels and 700ppm CO_2 (Schippers et al., 2004; Fu et al., 2008). In contrast, other studies have reported that increased pCO_2 has no significant effects on the primary production (Sciandra et al., 2003; Delille et al., 2005). According to this perspective, response of phytoplankton parameters was not necessarily consistent with increasing pCO_2. Moreover, previous studies were mostly conducted in the laboratory and short-term field incubation experiments, which do not reflect the natural marine ecosystem. Thus, in order to obtain available information on the response of natural phytoplankton assemblages with

respect to increasing CO_2 concentration, large-scale field mesocosm studies are needed.

Ocean warming is probably the most widely recognized consequence of climate change. Therefore, numerous studies have reported that increases in sea surface temperature (SST) can impact the growth rate, pigment content, light-harvesting capacity, and photosynthetic carbon fixation of many phytoplankton (e.g. Fu et al., 2012). Likewise, increase in SST accelerates the physiological rates of phytoplankton. For instance, nitrate uptake and reduction in diatoms is rapid at elevated temperatures (Loams and Gilbert, 1999). Additionally, recently studies have suggested that increase in SST can influence the production of phytoplankton more significantly than increases in CO_2 concentration (Hare et al., 2007; Kim et al., 2013). Although increased temperature can be an important factor to regulate the phytoplankton physiology and ecology in future ocean environments, pelagic mesocosm studies have rarely addressed the effects of temperature on the response of phytoplankton communities and species-specific responses.

In this study, we focused on the individual and combined effects of increased pCO_2 and temperature on natural phytoplankton structure with emphasis on species response. We also examined the nutrient availability in phytoplankton and the response of other plankton groups such as bacteria and ciliates. To do this, we set up in-situ pelagic mesocosm facilities because this approach can evaluate well-established hypotheses and determine responses from the level of the organism to the ecosystem to future climatic conditions (Kim et al., 2006; Riebesell et al., 2008). The results of this large collaborative experiment are presented in two companion papers. Therefore, this paper mainly discusses the response of the phytoplankton community included in a species-specific response. The companion paper by Kim et al. (2010) focused on the mechanisms for dimethylsulfide (DMS) production (2010, Environmental Science and Technology) and on the shift in biogenic carbon flux (2011, Geophysical Research Letters). Another study by Kim et al. (2013, Biogeosciences) addressed the enhancement of photosynthetic carbon assimilation efficiency.

2.2.2 Materials and Methods

2.2.2.1 Experimental Section

To evaluate the response of phytoplankton communities to the CO_2 concentration and CO_2 concentration + temperature, nine marine vertical mesocosms were immersed in the water column at the South Sea of Korea (Jangmok Bay, Geoje Island; 34.6 ^{o}N, 128.5 ^{o}E) from 21 November to 11 December 2008. Each cylinder-shaped mesocosm (1m in diameter and 3 m in depth) with transparent caps comprised a 3,000-L enclosure that contained 1,500 L of seawater, and was made of polyethylene material reinforced with a polyester grid (Fig. 2.2.1). To supply identical mass of water to each mesocosm enclosure, a single body of seawater with a volume of 1,500 L filtered through a net with a 100 um mesh to eliminate the large-sized grazer was supplied slowly to all mesocosm bags using by flowmeter. To set-up the present and future oceanic conditions, we designed three experimental conditions based on model projections under the A2 Scenarios of the Intergovemmental Panel on Climate Change Special Report on Emissions Scenarios (IPCC, 2007): the present ocean condition (~400 ppmv CO_2/ ambient temperature), the acidification condition (~900 ppmv CO_2/ ambient temperature),

and the greenhouse conditions (~900 ppmv CO_2/~3°C warmer than ambient temperature. The initial target p CO_2 level was achieved by injecting the CO_2 oversaturated seawater into natural seawater through peristaltic pump in the nine enclosures.

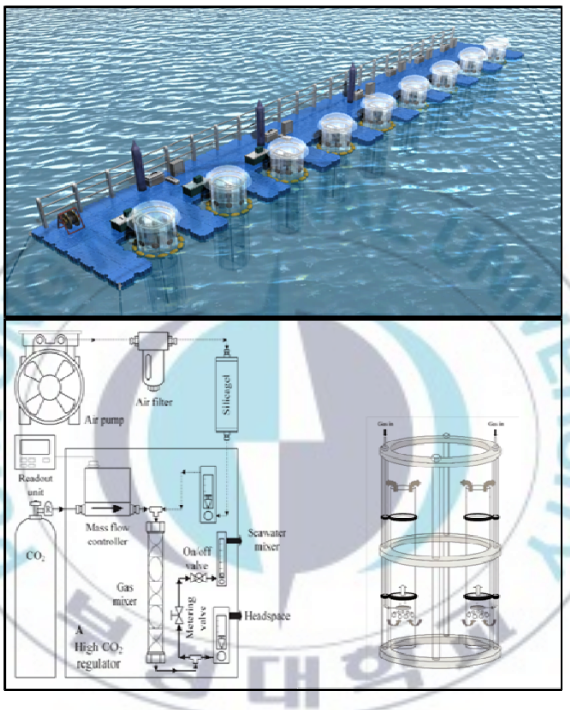


Fig. 2.2.1. Photograph of the floating raft, pCO_2 concentration regulation units, and enclosures of the mesocosm facility. The lower diagram shows the pathways of target pCO_2 air generated from a pCO_2 regulation units and mixer to an enclosure.

To manipulate the greenhouse conditions (~3^{o}C warmer than ambient temperature), high heat conduction rate tube was fixed in mesocosm bags, where it was fixed at 1.5m~2m water depth to create increased temperature treatment, and heated water was circulated within the tube that was coiled around the inside of mesocosm system. On day 0, nutrient was added to each enclosure to development of phytoplankton bloom. At the initial of experiment, nutrients were added to the initial development of a phytoplankton bloom (nitrate: ~33 μmol kg^{-1}, phosphate: ~2.5 μmol kg^{-1}, silicate: ~50 μmol kg^{-1}). All seawater samples collected at 1m water depth by fluid metering pump without cell damage after the particulates and solutes inside in each enclosure were homogeneously mixed for 20 min using bubble-mediated mixers (Kim et al., 2008). The more detailed description for mesocosm system was described at the series of published papers (Kim et al., 2008).

2.2.2.2 Measurement of Parameters

Samples were taken daily from each mesocosm enclosure at 13:00 PM. Water temperature and salinity was directly measured by a multiparameter sonde (YSI-

6600). To analyze the nutrient concentration [dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP), and dissolved silica (DSi)], samples from each enclosure were filtered through 25mm syringe filter with 0.45 pore size and frozen in 50mL acid-cleaned polyethylene (PE) bottle at -80 ^{o}C. Nutrient concentrations were analyzed using an autoanalyzer (Flow Injections Analyzer, Quickchem 8000, LACHAT Co.), following the methods of Parsons et al. (1984).

The growth of phytoplankton was monitored by determining the phytoplankton biomass (chlorophyll-*a*) using 10-AU fluorometer (Turner-Desings Co.). To analyze the phytoplankton community, 500mL-seawater samples taken and preserved immediately with lugol's solution at a final concentration of 5%, and concentrated for 24h by sedimentation. Phytoplankton species compositions were identified under a light microscope (Zeiss, Axioplane II) at a magnification of x200-1,000 following the books of Cupp (1943), Rines and Hargraves (1988), and Tomas (1997). And their cell numbers were estimated by direct counting using a Sedgewick-Rafter chamber under same light microscope.

2.2.2.3 Statistical analysis

Results are given as the mean and standard deviation (SD) of the raw data. Analysis of variance (ANOVA) was used to determine the effects of CO_2 and combined effects of CO_2 and temperature on the phytoplankton group and species. All datasets met assumptions of normality and homogeneity of variance. When the ANOVA identified a significant difference ($p<0.05$), Scheffe's post hoc test was used. *p* values less than 0.05 were considered significant. This analysis was performed using SPSS 17.0 (SPSS Inc., USA).

2.2.3 Results

2.2.3.1 Carbonate parameters

Temperature and pCO_2 differ significantly among the treatment groups (Table 2.2.1; $F=33.15$, $p<0.001$; $F=50.49$, $p<0.001$ respectively). At the beginning of the experiment, seawater temperature and seawater pCO_2 were adjusted to ambient seawater temperature and to 366 ± 5.3 ppm under present conditions, to ambient seawater temperature and to 856 ± 13.2 ppm under acidification conditions, and to 2.3 ^{o}C higher than ambient seawater temperature and 862 ± 24.7 ppm under

greenhouse conditions. Although seawater temperature decreased steadily over the course of the experiment because of change of the season from fall to early winter, differences between each treatment were consistent (Fig. 2.2.2a). Because of biological activity, seawater pCO_2 also decreased toward the end of the experiment, and this phenomenon was most under acidification conditions (Fig. 2.2.2b).

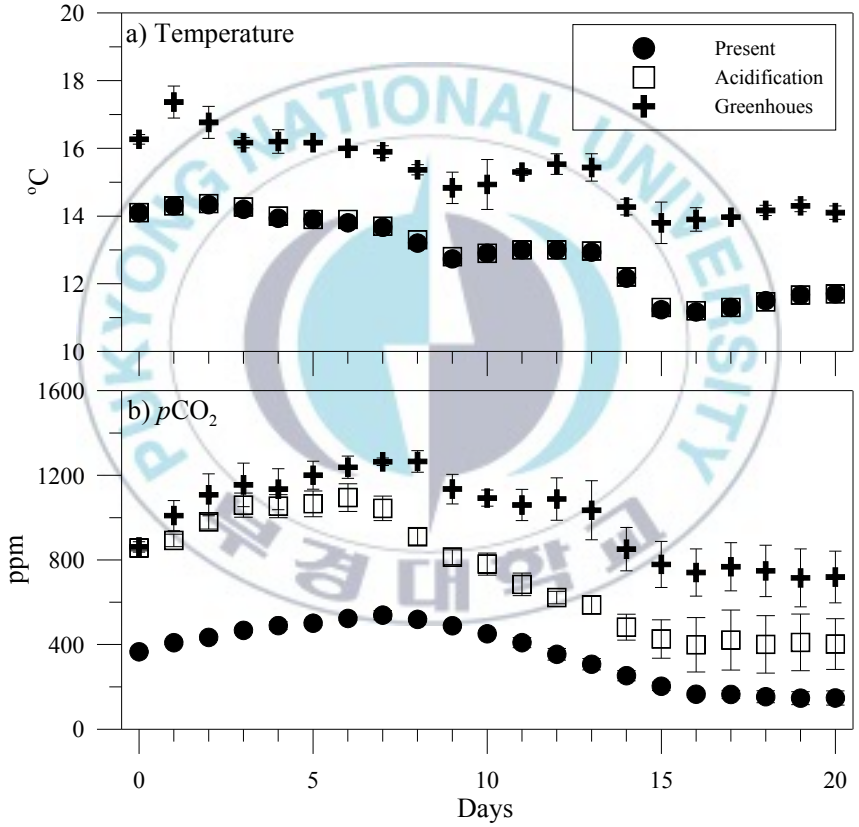


Fig. 2.2.2. Changes in temperature (a) and pCO_2 (b) under present, acidification and greenhouse conditions during the experimental periods.

Table 2.2.1. Summary of changes in abiotic and biotic parameters in mesocosm subjected to different treatments.

Variable	Present	Acidification	Greenhouse	F value (p)
Temperature (°C)	12.9±1.1 ^{B}	12.9±1.1 ^{B}	15.3±1.0 ^{A}	33.15***
Salinity (psu)	34.7±0.5	34.6±0.5	34.4±0.5	N.S.
pH	8.1±0.2 ^{A}	7.8±0.2 ^{B}	7.7±0.1 ^{C}	42.46***
pCO_2 (ppm)	357±144 ^{C}	733±266 ^{B}	998±196 ^{A}	50.49***
DSi (μmol kg^{-1})	32.4±17.3	33.1±15.3	34.6±13.6	N.S.
DIP (μmol kg^{-1})	1.4±1.0	1.2±1.1	1.2±1.1	N.S.
DIN (μmol kg^{-1})	21.0±16.5	18.4±15.9	17.6±16.7	N.S.
DOC (μmol kg^{-1})	124.9±24.6	133.3±31.4	131.9±31.1	N.S.
Chlorophyll-*a* (μg L^{-1})	11.22±9.95 ^{A}	9.68±6.73 ^{A,B}	7.17±4.54 ^{B}	4.56*
Diatom (10^3 cells L^{-1})	510±308 ^{B}	1266±986 ^{A}	287±262 ^{B}	14.61***
Dinoflagellate (DF) (10^3 cells L^{-1})	35.3±41.4	31.9±32.7	40.6±50.7	N.S.
HNF (10^4 cells L^{-1})	382±231 ^{B}	664±548 ^{A}	293±168 ^{B}	6.19**
ANF (10^4 cells L^{-1})	95.8±91.7	167.4±159.1	89.0±84.1	N.S.
Picophytoplankton (PP) (10^4 cells L^{-1})	408±319	578±546	541±684	N.S.
Heterotrophic bacteria (10^4 cells L^{-1})	196±116	185±102	185±100	N.S.
Ciliates (cells L^{-1})	46444±76839	44958±76784	37162±54952	N.S.
Cerataulina dentata (10^3 cells L^{-1})	60.9±74.1 ^{A}	7.55±13.6 ^{B}	4.84±8.18 ^{B}	11.64***
Chaetoceros decipiens (10^3 cells L^{-1})	43.8±61.4 ^{A}	13.3±21.7 ^{B}	5.02±10.4 ^{B}	17.38***
Chaetoceros socialis (10^3 cells L^{-1})	64.9±77.7 ^{B}	333±435 ^{A}	4.82±4.06 ^{B}	9.82***
Cylindrotheca closterium (10^3 cells L^{-1})	11.5±14.1 ^{A}	3.49±6.49 ^{B}	6.63±9.83 ^{B}	8.83***
Detonula pumila (10^3 cells L^{-1})	7.79±10.3 ^{A}	17.6±24.0 ^{B}	8.49±12.4 ^{A}	6.43**
Eucampia zodiacus (10^3 cells L^{-1})	27.2±39.2 ^{A}	6.52±9.91 ^{B}	1.85±2.49 ^{B}	20.03***
Skeletonema spp. (10^3 cells L^{-1})	80.9±59.2 ^{B}	584±834 ^{A}	160±197 ^{B}	6.27**
Thalassiosira spp. (10^3 cells L^{-1})	66.9±68.7 $^{A, B}$	80.3±61.7 ^{A}	24.6±34.0 ^{B}	5.51**
Akashiwo sanguinea (10^3 cells L^{-1})	3.64±5.68 ^{A}	1.50±1.71 ^{B}	1.89±2.40 ^{B}	5.73**
Alexandrium spp. (10^3 cells L^{-1})	1.83±3.96	0.57±0.95	2.05±4.92	N.S.
Gyrodinium spp. (10^3 cells L^{-1})	6.74±8.34 ^{A}	17.9±21.0 ^{B}	12.7±12.8 ^{A,B}	8.36***
Nematodinium armatum (10^3 cells L^{-1})	2.77±5.67 ^{A,B}	2.33±3.37 ^{A}	5.79±11.1 ^{B}	3.85*
Prorocentrum dentatum (10^3 cells L^{-1})	1.46±1.48 ^{A}	2.15±1.37 ^{B}	3.09±2.58 ^{B}	11.19***
Protoperidinium bipes (10^3 cells L^{-1})	4.43±6.38 ^{A}	4.38±6.67 ^{A}	9.49±19.2 ^{A}	3.42*

Data show the mean±S.D. N.S., not significant; *,P<0.05, **P<0.01, ***P<0.001. Results were analyzed by one-way ANOVA and Scheffe's post-hoc test. Letters ($^{A, B, and C}$) represent significant differences.

2.2.3.2 Bloom development

The mesocosm experiment was divided by chlorophyll–*a* (chl–*a*) and nutrient concentration (pre-bloom period: days 0-5, bloom period: days 6-15, post-bloom period: days 16-20) (Fig. 2.2.3). In all enclosures, the phytoplankton biomass (measured as the chlorophyll-*a* concentration; chl-*a*) increased slowly during the pre-bloom period and started to increase exponentially on day 6 (Fig. 2.2.3a). Maximum chl-*a* was observed under present and acidification conditions on day 14 during the bloom period and under greenhouse conditions on day 20 during the post-bloom period. The highest chl-*a* concentration was observed under present conditions among the treatments, and its mean value reached about 28.4 μg L^{-1}. During the experimental period, the highest mean production of phytoplankton biomass was also observed under present conditions (Table 2.2.1).

The development of the phytoplankton bloom was characterized by a rapid decline in dissolved inorganic nutrients (Fig. 2.2.3b~3d). Nitrite + Nitrate decreased from an initial concentration of 40.94 ± 1.22 μmol to below the limiting concentration (~2.0 μmol) for cell growth after day 15 (Fig. 2.2.3b).

Phosphate started at 2.55 ± 0.06 μmol and began to decrease drastically below the limiting concentration on day 13 under acidification and greenhouse conditions and on day 15 under present conditions (Fig. 2.2.3c). The initial concentration of silicate was 48.25 ± 0.71 μmol and started to gradually decrease toward the end of the experiment to a final value of 5.98 ± 4.38 μmol. Silicate concentration was not below the limiting concentration for cell growth under all treatment conditions during the entire experiment (Fig. 2.2.3d). After the bloom period, we observed that both nitrite + nitrate and phosphate concentrations were below the limiting concentration for autotrophic phytoplankton cell growth (Fig. 2.2.3b and 3c). Interestingly, although each nutrient was not significantly different among the treatments (Table 2.2.1), there were different patterns of the decrease: nitrite + nitrate and phosphate decreased in order of greenhouse, acidification and present conditions, but silicate showed the reverse trend in order of present, acidification and greenhouse conditions (Fig. 2.2.3b~3d).

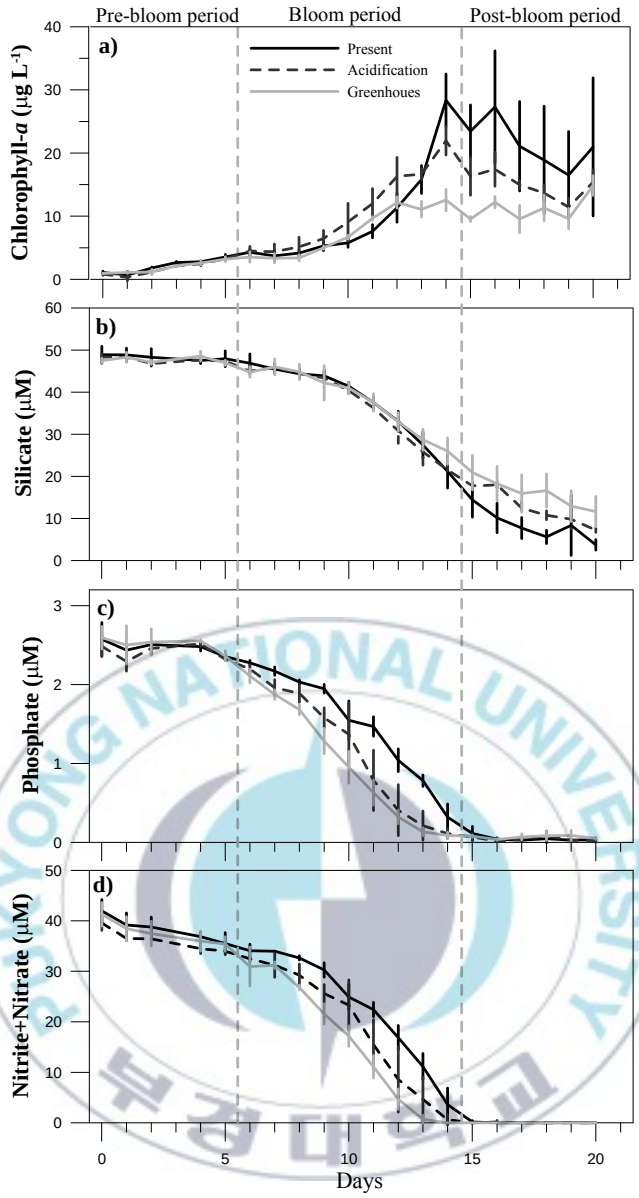


Fig. 2.2.3. Chlorophyll-*a* and nutrient curves under present, acidification and greenhouse conditions during experimental periods. Based on chlorophyll-*a* concentration, three phases can be distinguished (pre-bloom period, bloom period, post-bloom period).

2.2.3.3 Variation of autotrophic parameters

The groups present in the autotrophic community based on the data were identified as picophytoplankton (PP), autotrophic nanoflagellate (ANF) and diatoms (Fig. 2.2.4). PP abundance started to increase exponentially after the pre-bloom period, and the highest cell numbers were observed in all treatments on day 12 during the bloom period (Fig. 2.2.4a). After day 12, PP abundance decreased until the end of the experiment. During the bloom period, PP was well growth under both acidification and greenhouse conditions relative to present conditions (Fig. 2.2.4c). ANF started to increase in all treatments after the pre-bloom period, and the highest values were observed under acidification conditions on day 13 during the bloom period, with an average value of 4.9 x cells L^{-1} (Fig. 2.2.4e). During the bloom and post-bloom periods, ANF was well growth under acidification conditions relative to both present and greenhouse conditions (Fig. 2.2.4g and 2.2.4h). The growth response of PP and ANF exhibited no significant relationship between the treatments (Table 2.2.1).

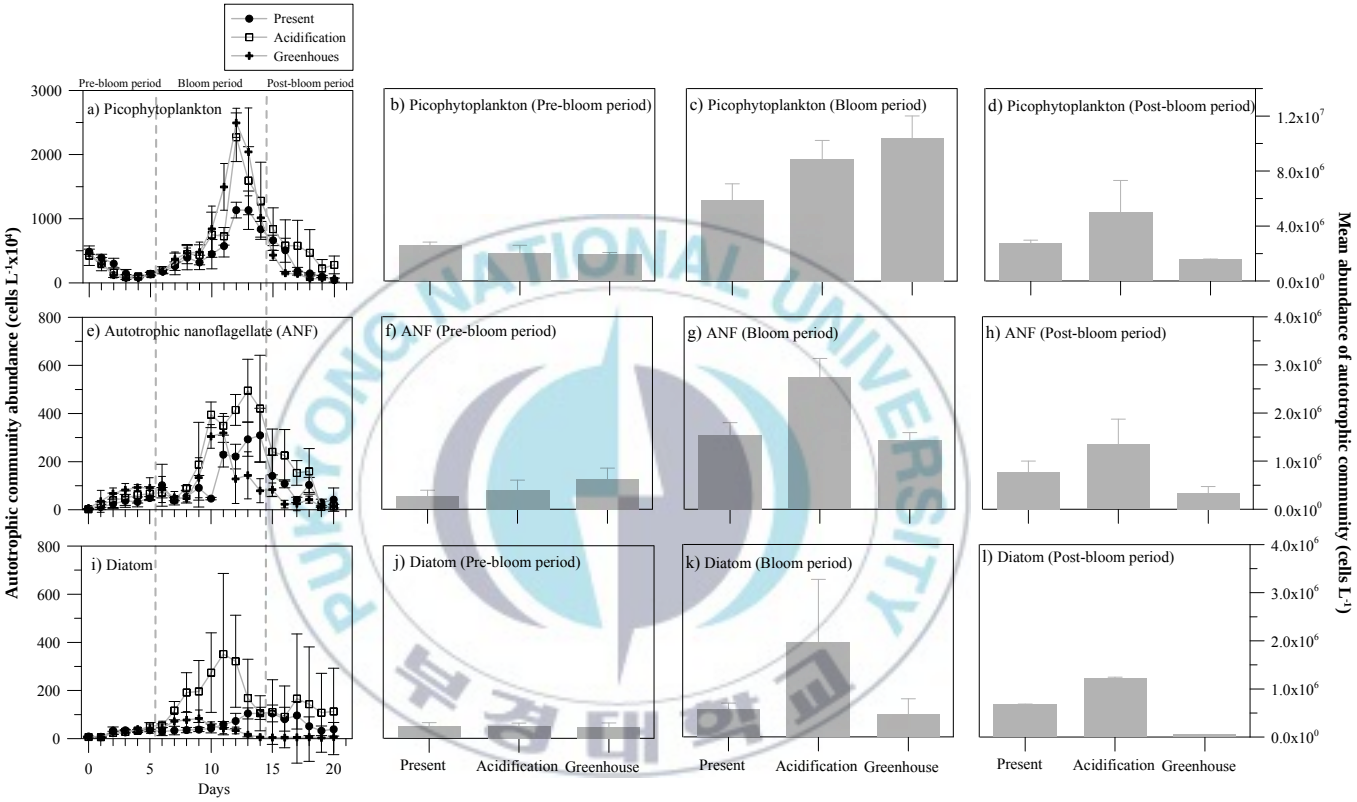


Fig. 2.2.4. Temporal variations of autotrophic phytoplankton community under present, acidification and greenhouse conditions during the experimental periods.

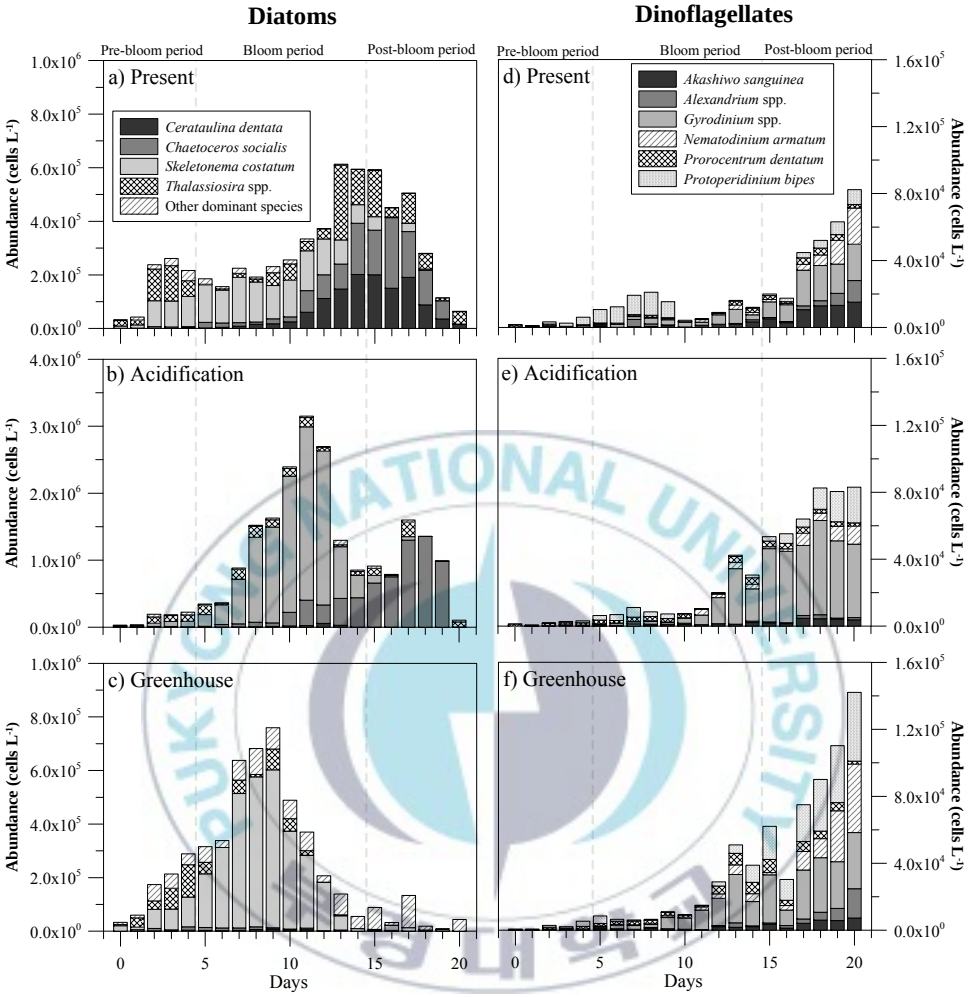


Fig. 2.2.5. Temporal variations of dominant phytoplankton species (>2x10^5 cells L^{-1}) under present, acidification and greenhouse conditions during the experimental periods.

Diatom abundance was similar in all treatments during the pre-bloom period with a value of $1x10^6$ cells L^{-1} and increased until day 9 under greenhouse conditions, until day 11 under acidification conditions and until day 13 under present conditions (Fig. 2.2.4i). The increase was most pronounced under the acidification conditions with a peak value of $3x10^6$ cells L^{-1}. Diatom abundance was significant among the treatments (Table 2.2.1; $F=14.61$, $p<0.001$). Among the diatom species, *Cerataulina dentata* (*C. dentata*), *Chaetoceros socialis* (*C. socialis*), *Skeletonema costatum* and *Thalassiosira* spp. were prominent and had a different peak between the treatments during the experimental period. *Cerataulina dentata* exhibited significant growth under present conditions during the bloom period compared to other treatments and was highest on day 14, with an average value of $2.0x10^5$ cells L^{-1} (Fig. 2.2.5a). The growth of *C. socialis* was highest on day 14 under present conditions and on day 18 under acidification conditions, with an average value of $1.4x10^6$ cells L^{-1} (Fig. 2.2.5b). However, under greenhouse conditions, cell growth was not observed (Fig. 2.2.5c). *Skeletonema costatum*, which was the predominant

species among the diatoms during the experiment, appeared to bloom prior to other dominant species under both acidification and greenhouse conditions (Fig. 2.2.5b and 2.2.5c). The highest abundance was observed on day 11 under acidification conditions, with an average value of $2.6x10^6$ cells L^{-1}. *Thalassiosira* spp. did not show a distinctive growth pattern between the treatments, and the highest abundance was observed on day 13, with an average value of $2.8x10^5$ cells L^{-1} (Fig. 2.2.5a). ANOVA results showed that dominant diatom species were significantly different among the treatment groups (Table 2.2.1). For changes in abundance of dominant species, their importance, in terms of their contribution to cell number, changed according to the treatments and time.

2.2.3.4 Variation of DOC and heterotrophic parameters

Dissolved organic carbon (DOC), heterotrophic bacteria (HB), heterotrophic nanoflagellates (HNF), dinoflagellates (DF) and ciliates are considered major components of the microbial loop shown in Figure 2.2.6. DOC production started at 94.85±2.20μmol and was sustained during the pre-

bloom period and began to gradually increase toward the end of the experiment (Fig. 2.2.6a). DOC production in all treatments gradually increased toward the end of the experiment in order of greenhouse, acidification, and present conditions (Fig. 2.2.6a~6d). The highest DOC production was observed on day 18 under greenhouse conditions, with an average concentration of 193.2μmol kg^{-1}, on day 20 under acidification and present conditions, with an average concentration of 185.4 and 166.5μmol kg^{-1}. HB abundance started at 3.51 $x10^{6}$cells L^{-1} and rapidly decreased until day 4 and began to gradually increase toward the end of the experiment. After the pre-bloom period, trends in bacteria abundance showed similar DOC concentrations, but differences between the treatments were not observed (Fig. 2.2.6e). For the trend in HNF abundance, two peaks occurred during the experiment. The first peak was observed during the pre-bloom period, with an average value of 2.2 $x10^{8}$cells L^{-1}, and the second peak was observed at the end of the bloom period (Fig. 2.2.6i). Generally, the secondary peak was suppressed relative to the first peak. During the experimental periods, HNF abundance was higher under acidification

conditions than under other simulated conditions (Fig. 2.2.6j~6l). Increase of ciliate abundance was not observed until day 12 during the middle of the bloom period and thereafter rapidly increased toward the end of experiment (Fig. 2.2.6q). Ciliate abundance was not different between the treatments (Fig. 2.3.6r~6t). For DF abundance, there were different growth trends from that of the other autotrophic communities (Fig. 2.2.4a, 4e, 4i), and there was significant growth under acidification and greenhouse conditions compared to the present conditions (Fig. 2.2.6m~6p). Trends in DF abundance did not differ significantly between the treatments (Table 2.2.1). Among DF species, *Akashiwo sanguinea, Alexandrium* spp., *Gyrodinium* spp., *Nematodinium armatum*, *Prorocentrum dentatum*, *Protoperidinium bipes* was dominant and mainly occurred between the end of the bloom and post-bloom periods (Fig. 2.2.5d, 5e, 5f). During the experimental period, dominant DF species showed different growth patterns; *Akashiwo sanguinea* was higher under the present conditions, but *Gyrodinium* spp., *P. dentatum* was higher under both acidification and greenhouse conditions. The growth of *N. armatum* was high under greenhouse conditions relative to other simulated conditions.

Although abundance of dominant DF species was lower relative to other autotrophic communities, they showed a significantly statistical difference during the experimental periods except *Alexandrium* spp. (Table 2.2.1).

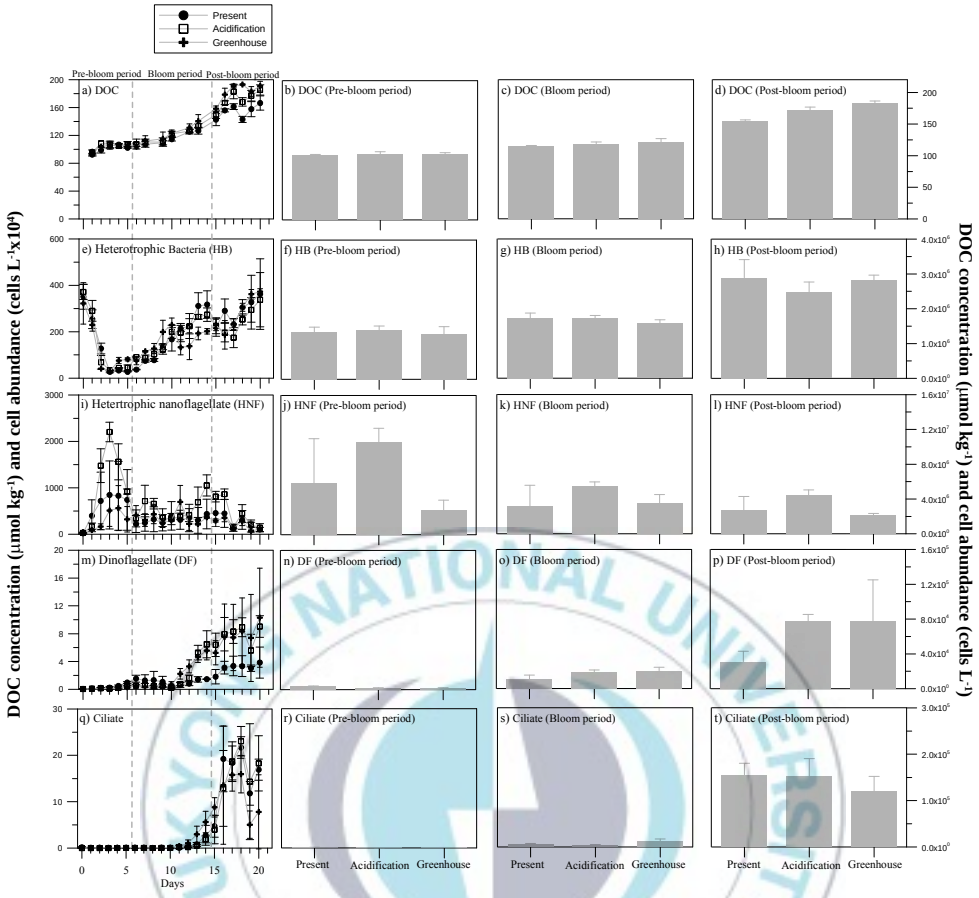


Fig. 2.2.6. Temporal variations of microbial-loop parameters under present, acidification and greenhouse conditions during the experimental periods.

2.2.4 Discussion

2.2.4.1 Implication of microbial community response

During our experiment, elevated pCO_2 and temperature effects were superimposed on the autotrophic community that was first structured by inorganic nutrient availability in all enclosures. Therefore, in order to increase phytoplankton growth, we intentionally supplied inorganic nitrate, phosphate and silicate. These adjusted inorganic nutrient concentrations in all enclosures were typical of those we observed throughout the study area at the time of study. Thus, inorganic nutrient influence on autotrophic community structure in our experiments should have been similar to those on the in situ community of the late fall bloom. In our study, as expected, addition of inorganic nutrient resulted in a rapid increase in autotrophic community abundance and a correspondingly enhanced phytoplankton biomass (mainly autotrophic community), but their response was not always statistically significant (Table 2.2.1). More specifically, during the late bloom period, the highest phytoplankton biomass was observed under

present conditions, but the highest abundance in the autotrophic community was observed with diatoms and ANF under acidification conditions and with PP under acidification and greenhouse conditions. Previous studies have shown similar results, at that temperature, and pCO_2-dependent taxonomic group shifts can occur (Raven, 1991; Tortell et al., 2002; Feng et al., 2009).

As discussed above, our results also show the discrepancy between phytoplankton biomass and autotrophic community abundance. Considering the cell volume and abundance of each autotrophic community groups studied (data not shown), diatoms are major contributors to total phytoplankton biomass. In this study, various dominant diatom species responded differently to future oceanic conditions. For example, during the bloom period, *S. costatum* growth was significant in both future oceanic conditions relative to the present conditions and, especially, growth of *S. costatum* by over four-fold was observed under acidification conditions. In contrast, growth of *C. dentata* and other dominant diatom species such as *C.*

decipiens and *E. zodiacus* showed highest growth under present conditions compared to future oceanic conditions during the period from late bloom to post-bloom (Fig. 2.2.5a~5c). According to the report of the Baltic Sea Environment Proceedings (2006), these species had a higher volume by over 20-fold than *S. costatum* and, thus, might lead to the highest peaks of phytoplankton biomass under present conditions during the period from late bloom to post-bloom.

In this study, ANF and PP apparently profit from elevated CO_2, reflected by enhanced abundance under acidification. Similar to our result, Schneider (2004) suggested that the smaller phytoplankton apparently profited from elevated CO_2. However, while growth in PP also stimulated significant growth under greenhouse conditions during the bloom period, growth in ANF was similar compared to the present conditions. During the period from late bloom to post-bloom, their abundance rapidly declined under future oceanic conditions compared to the present condition. Rapid depression in growth of ANF and PP under future oceanic conditions with

respect to present conditions might be affected by grazing activity such as DF and ciliates. That is, their grazing activity was higher under future oceanic conditions compared to present conditions. The detailed discussion of grazing activity is presented in the following paragraph. Therefore, the significant growth in small phytoplankton will influence the other group organisms including grazers and bacteria in the future environment.

Inorganic nutrient availability was one of the most important parameters for intensity and duration of phytoplankton bloom. In our study, concomitant with increased phytoplankton biomass, nutrient concentration in DIN and DIP was decreased in order of greenhouse, acidification and present conditions during the blooming period. This pattern was affected by diatom species *S. costatum*, which was fast-growing in order of greenhouse, acidification and ambient conditions. Previous studies explained that the growth rate of *S. costatum* was higher under acidification conditions than under present conditions (Gervais and Riebesell, 2001; Kim et al., 2006), and it was shown to have a higher uptake rate for nutrients (DIN and DIP)

(Conway and Harrison, 1977; Stolte et al., 1994). In addition, nutrient uptake rate in *S. costatum* was enhanced when water temperature increased from under 6 ^{o}C to 17 ^{o}C (Takabayashi et al., 2006). During the blooming period, our study also showed that silicate concentration was similar between the treatments, although abundance of the most dominant species *S. costatum* was significantly different between treatments. This result showed indirect effects of elevated temperature and pCO_2 on silicate availability of *S. costatum*, although we could not calculate silicate availability. During the post-bloom period, in contrast to the pattern of DIN and DIP, the lowest silicate concentration observed under present conditions indicates that some different diatom species with a higher half-saturation constant compared to *S. costatum* were dominant. In this study, large diatoms such as *C. dentata, C. decipiens and E. zodiacus* exhibited significant growth under present conditions compared to future oceanic conditions during the period from late bloom to post-bloom. Our results suggest that elevated temperature and pCO_2 can change phytoplankton species composition through different growth and nutrient availability in phytoplankton species, although further

works will be needed to determine the exact mechanisms by which phytoplankton species acquire nutrients under future climate conditions.

Enhanced pCO_2 and temperature significantly influenced grazing activity. Hare et al. (2007) explained that both phytoplankton community shift and decrease in algal biomass were significantly influenced by grazers, and changing top-down control, thus, could modify or even negate the effects of bottom-up global change variables. In our experiment, dinoflagellates are considered main grazers (~90% of the total carbon biomass of microzooplankton); they feed largely on the autotrophic community species. All autotrophic community groups showed significant growth under future oceanic conditions from the bloom period and thereafter a rapid decline toward the end of the experiment (Fig. 2.2.4a~4l). As a result, DF abundance was higher under future oceanic conditions (Fig. 2.2.6m and 6p), and the grazing rate was also significantly higher under future climate conditions than under present conditions (Figure S4) (Kim et al., 2010), suggesting that increased grazing rate in DF may incorporate both intensity

and duration of phytoplankton blooms under future oceanic conditions.

The important HB parameters in the microbial process include converting particle organic carbon (POC) to dissolved organic carbon (DOC). After the pre-bloom period, DOC production and abundance in HB become closely linked with each other and increase in all treatments, but DOC production was higher under future climate conditions than under present conditions, while differences in HB abundance between the treatments were not observed. Therefore, the processes alone involved in the transformation of POC to DOC by HB do not adequately explain the difference in DOC production between the treatments. Previous studies have indicated that elevated pCO_2 generally increases bacterial productivity, whereas bacterial abundance could not change with pCO_2 (e.g. Allgaier et al., 2008). These studies imply that elevated pCO_2 might enhance HB productivity and lead to the enhancement of DOC production under future oceanic conditions much more than under present conditions. However, this cannot explain why DOC production was higher under the greenhouse conditions than under

acidification conditions. According to the paper already published by my co-workers in this experiment (Kim et al., 2011), another possible mechanism of DOC production is extracellular release by phytoplankton, and these processes were enhanced under warm-water conditions.

2.2.4.2 Growth response of dominant species

Diatoms were the dominant taxonomic group during the experimental period, and dinoflagellates showed higher abundance during the period from late bloom to post-bloom in this study. More specifically, eight dominant diatom species (*Cerataulina dentata, Chaetoceros decipiens, Chaetoceros socialis, Cylindrotheca closterium, Detonula pumila, Eucampia zodiacus, Skeletonema costatum, Thalassiosira* spp.) and six dominant dinoflagellate species (*Akashiwo sanguinea, Alexandrium* spp., P*rorocentrum dentatum, Protoperidinium bipes, Gyrodinium* spp., *Nematondinium armatum*) were most abundant and comprised more than 90% of each group. For all of these species, abundance markedly differed in respect to CO_2 and temperature change, indicating that there are diverse strategies utilized by these species

under future climate conditions.

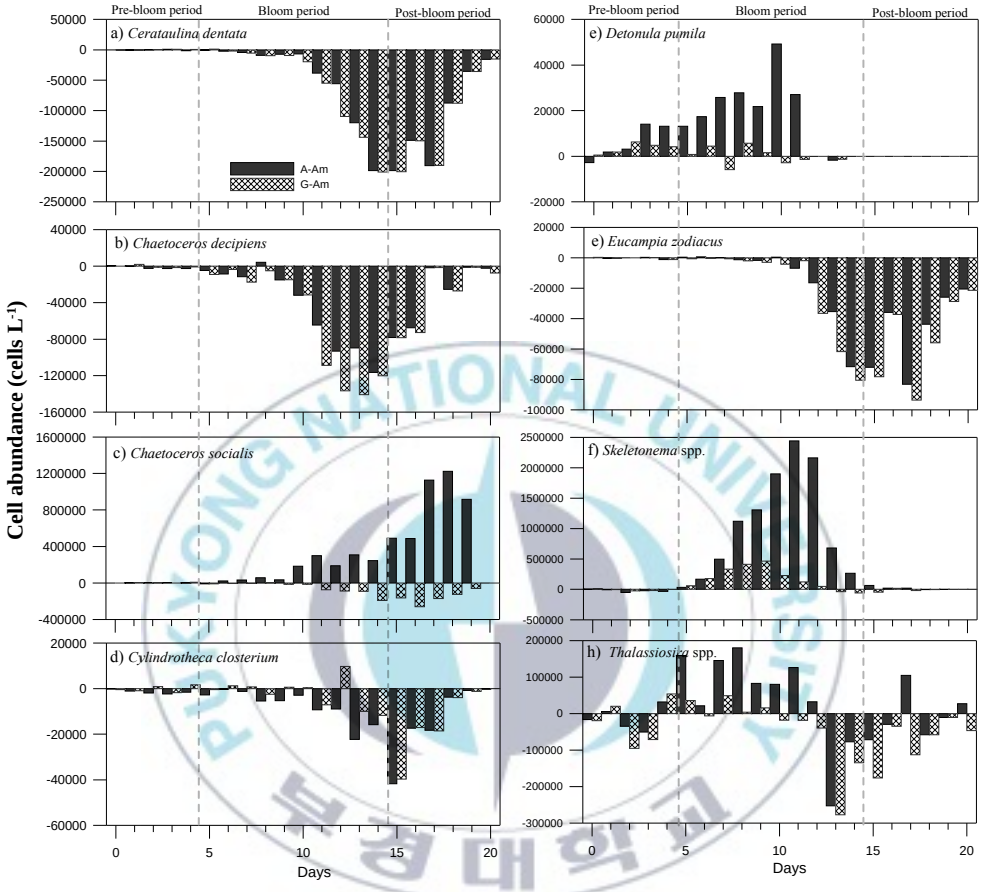


Fig. 2.2.7. Temporal variations of dominant diatom species abundance under acidification and greenhouse conditions relative to present conditions.

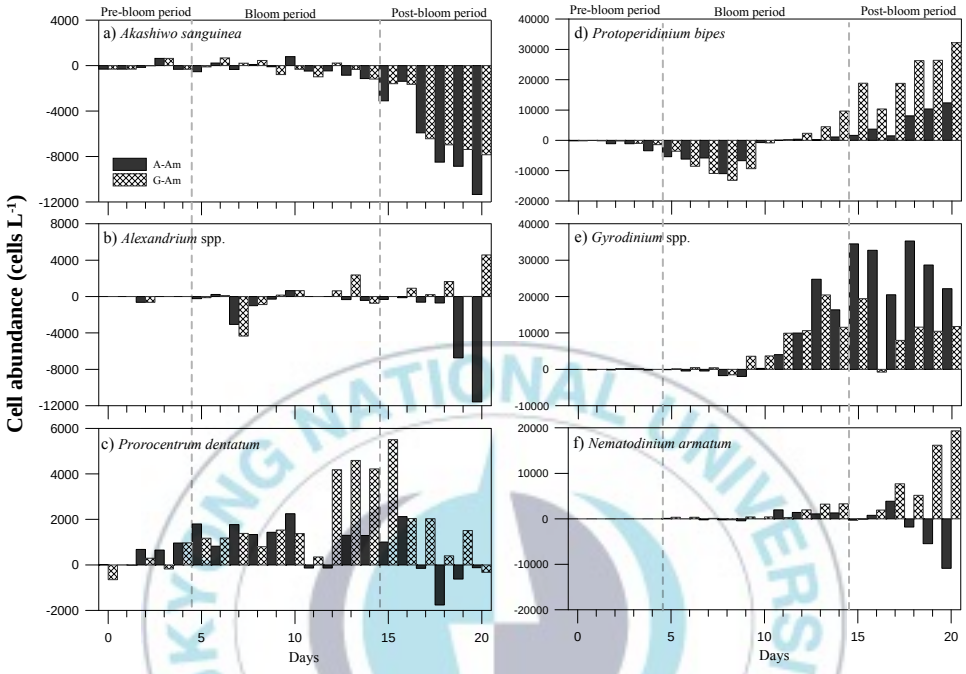


Fig. 2.2.8. Temporal variations of dominant dinoflagellate species abundance under acidification and greenhouse conditions relative to present conditions.

Previous studies have suggested that growth in diatom species could not be stimulated by increasing CO_2 concentration (Burkhardt et al., 1999, 2001; Rost et al., 2003). However, in this study, growth of dominant diatom species significantly differed between the treatments (Fig. 2.2.7). *Skeletonema costatum* showed positive growth under future oceanic conditions during the period from pre-bloom to bloom (Fig. 2.2.7f). The significant growth in *S. costatum* is consistent with the observation of Kim et al. (2006). Their results showed that *S. costatum* exhibited increased growth rates with increased pCO_2. However, abundance of *S. costatum* under greenhouse conditions was not much higher than under present conditions. This phenomenon was also observed in the growth of *D. pumila* and *C. socialis*. During the pre-bloom and bloom periods, although the growth response in *D. pumila* and *C. socialis* showed a positive response under acidification conditions (Fig. 2.2.7c and 7e), abundance of these species under greenhouse conditions was similar and/or lower compared to the present conditions. We easily understand why abundance of *C. socialis* was lower under greenhouse condition than under present conditions

because this species is known to prefer cold-water conditions. However, growth limitations of *S. costatum* and *D. pumila* at elevated temperatures are not fully understood, since maximal growth rates in *S. costatum* were identified at higher water temperature ranges (20~30^{o}C) compared to greenhouse conditions in this study (Yoder, 1979). According to the study by Fu et al. (2007), two HAB species in *Heterosigma akashiwo* and *Prorocentrum minimum* that commonly bloom together in the same body of water showed a different response under individual and combined warming and acidification conditions: growth of *H. akashiwo* was in order of greenhouse, high temperature, high CO_2 and present conditions, but growth in *P. minimum* showed better growth under high CO_2 and greenhouse than high temperature and present conditions. Moreover, we can exclude the grazing activity because the abundance of dinoflagellates and ciliates, which are considered main grazers in this study, increased on day 13 at the end of the blooming period. Taken together, the growth limitation in *S. costatum* and *D. pumila* under greenhouse conditions was affected by temperature. In contrast, the pennate diatom species *C. closterium* and large centric diatom

species, *C. dentata, C. decipiens* and *E. zodiacus*, did not exhibit significant growth under future oceanic conditions (Fig. 2.3.7a, 7b, 7d, 7e). Similar to our study, Schneider (2004) found that abundance in large *Corataulina pelagica* and *Chaetoceros* sp. was the highest under low-CO_2 conditions and low when CO_2 levels increased. These four species are known to be cosmopolitan species, easily found in temperate regions throughout the year. Therefore, growth with changing water temperature in this study was not observed, suggesting that CO_2 concentration severely depressed their growth.

Until recently, although recent extensive research has been directed toward understanding the effects of rising CO_2 levels in concert with rising temperature, only a handful of studies were conducted to catalog the responses of various coastal dinoflagellate species included in harmful algal bloom species despite their blooms seriously injuring fisheries and humans with their toxins (Fu et al., 2007). According to previous studies, the frequency and intensity of HABs have been increasing globally since the

mid-1970s (Marshall et al., 2001; Hallegraeff, 2010). Thus, defining the niche of key HAB species is crucial when trying to predict winners or losers of climate change because certain HAB species will dominate than others (Hallegraeff, 2010).

Many phytoplankton groups including DF possess a type I Rubisco (ribulose-1, 5-bisphosphate carboxylase-oxygenase; Tortell, 2000). Type I Rubisco has a high affinity for carboxylation and is therefore extremely efficient at present-day atmospheric concentration, compared to the type II Rubisco found in some bloom-forming dinoflagellates (Fu et al., 2012). To overcome this limitation, low-affinity CO_2-fixing systems may be compensated for by efficient carbon-concentration mechanisms (CCMs) such as various forms of carbonic anhydrase (CA), which facilitate the uptake of bicarbonate and its conversion to CO_2 (Giordano et al., 2005). These mechanisms allow some DF species to grow rapidly at present-day pCO_2 levels (Fu et al., 2012). In this study, only one DF species (*A. sanguinea*) reflected these trends (Fig. 2.2.8a). This result suggests that *A.*

sanguiena alone might possess the CCMs, and their growth, therefore, could be significant under present conditions. In contrast, *Gyrodinium* spp. and *P. dentatum* exhibited significant growth under future oceanic conditions compared to present conditions (Fig. 2.2.8c and 8e). The growth response in *P. bipes* differed during the experimental period. Under future oceanic conditions, their growth was negative, but after 11 days, the growth response changed towards the positive direction (Fig. 2.2.8d). Our results show that DF species, *Gyrodinium* spp., *P. dentatum*, and *P. bipes*, may not possess the CCMs and/or possess less efficient CCMs, and might enhance their growth rate under future oceanic conditions.

In conclusion, we set up the mesocosm facility according to the IPCC scenario, and showed the possibility that future global climate changes could significantly impact coastal ecosystems in temperate regions. More specifically, global climate change can alter the phytoplankton species succession and nutrient availability to phytoplankton species. We also found that ocean warming and acidification directly impact growth of

phytoplankton community groups and species. In particular, small phytoplankton and some dinoflagellates will benefit in terms of their growth under future oceanic conditions. Among diatom species, small species can profit in terms of their growth more than large diatom species under future oceanic conditions. These significant growth trends in small phytoplankton under future oceanic conditions, in this study, will lead to enhancement of microzooplankton grazing activity. In addition, from our results, the frequency and intensity of blooms are consistent with small phytoplankton and dinoflagellates and may increase in future oceanic environments. Our studies will require further confirmation, but may suggest that rising CO_2 and temperature in the future ocean might be significant impacts on the future marine ecosystem.

2.2.5 References

Allgaier, M., U. Riebesell, M. Vogt, R. Thyrhaug and H.P. Grossart. 2008. Coupling of heterotrophic bacteria to phytoplankton bloom development at different pCO_2 levels: a mesocosm study. Biogeosciences. 5: 1007~1022.

Badger, M.R., T.J. Andrews, S.M. Whitney, M. Ludwig, D.C. Yellowlees, W. Leggat, G.D. Proce. 1998. The diversity and co-evolution of RUBISCO, plastids, pyrenoids and chloroplast-based CO2- concentrating mechanisms in the algae. Can. J. Bot. 76, 1052~ 1071.

Beardall, J. and S. Stojcovik. 2006. Microalgae under global environmental change: Implications for growth and productivity, populations and trophic flow. Science Asia. 32(1): 1~10.

Burkhardt, S., I. Zondervan, U. Rievesell. 1999. Effects of CO_2 concentration on C:N:P ratio in marine phytoplankton: A species comparison. Limnol. Oceanogr. 3: 683~690.

Caldeira, K. and M.E. Wickett. 2003. Anthropogenic carbon and ocean pH.

Nature. 425: 365.

Conway, H.L. and P.J. Harrison. 1997. Marine diatoms grown in chemostats under silicate or ammonium limitation. 4. Transient response of *Chaetoceros debilis, Skeletonema costatum,* and *Thalassiosira gravida* to a single addition of the limiting nutrient. Mar. Biol. 43: 33~43.

Cupp, E.E. 1943. Marine Plankton Diatoms of the West Coast of North America. University of California Press. Berkeley and Los Angeles.

Delille, B, J. Harlay, I. Zondervan, S. Jacquet and others. 2005. Response of primary production and calcification to changes of pCO_2 during experimental blooms of the coccolithophorid *Emiliania huxleyi.* Global. Biogeochem. Cycles. 19:GB2023, doi: 10.1029/2004GB002318.

Edwards, M. and A.J. Richardson. 2004. Impact of climate change on marine pelagic phenology and trophic mismatch. Nature. 430: 881~884.

Engel, A., I. Zondervan, K. Aerts, L. Beaufort, A. Benthien, L. Chou, B. Delille, J.P. Gattuso, J. Harlay, C. Heemann, L. Hoffmann, S. Jacquet, J. Nejstgaard, M.D. Pizay, E. Rochelle-Newall, U. Schneider, A. Terbrueggen and U. Riebesell. 2005. Testing the direct effect of CO_2

concentration on a bloom of the coccolithophorid *Emiliania huxleyi* in mesocosm experiments. Limnol. Oceanogr. 50: 493~507.

Eppley, R. W. 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. 70: 1063~1085.

Feng, Y., M.E.Warner, Y. Zhang, J. Sun and others. 2008. Interactive effects of increased pCO_2, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae). Eur. J. Phycol. 43: 87~98.

Feng, Y., C.E. Hare, K. Leblanc, J.M. Rose and others. 2009. Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. I. The phytoplankton community and biogeochemical response. Mar. Ecol. Prog. Ser. 388: 13~25.

Fu, F.X., M.E. Warner, Y. Zhang, Y. Feng, D.A. Hutchins. 2007. Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine Synechococcus and Prochlorococcus (Cyanobacteria). J. Phycol. 43: 485~496.

Fu, F.X., Y. Zhang, M.E. Warner, Y. Feng and D.A. Hutchins. 2008b. A

comparison of future ncreased CO_2 and temperature effects on sympatric *Heterosigma akashiwo* and *Prorocentrum minimum*. Harmful Algae. 7: 76~90.

Fu, F.X., A.O. Tatters and D.A. Hutchin. 2012. Global change and the future of harmful algal blooms in the ocean. Mar. Ecol. Prog. Ser. 470: 207~233.

Giordano, M., J. Beardall, J.A. Raven. 2005. CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Ann. Rev. Plant Biol. 56:99~131.

Hallegraeff, G.M. 2010. Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. J. Phycol. 46: 220~235.

Hare, C.E., K. Leblanc, G.R. DiTullio, R.M. Kudela and others. 2007. Consequences of increased temperature and CO_2 for phytoplankton community structure in the Bering Sea. Mar. Ecol. Prog. Ser. 352: 9~16.

Hein, M., K. Sand-Jense. 1997. CO_2 increase oceanic primary production. Nature. 388 (6642):526~527.

Houghton, J.T., Y. Ding, D.J. Griggs, M. Noguer, P.J. Van der Linden, X. Dai, K. Maskell and C.A. Johnston. 2001. Climate Change 2001: The Scientific Basis: Contribution of Working Group I to the third Assessment Report of the Intergovernmental Panel of Climate Change. Cambridge Univ. Press. Cambridge. UK and New York. USA.

Hu, H. and K. Gao. 2008. Impact of CO_2 enrichment on growth and photosynthesis in freshwater and marine diatom. Chi. J. Oceanogr. Limnol. 26(4): 407~414.

Hutchins, D.A., F.X. Fu, Y. Zhang, M.E. Warner and others. 2007. CO_2 control of *Trichodesmium* N_2 fixation, photosynthesis, growth rates, and elemental ratios: implications for past, present, and future ocean biogeochemistry. Limnol. Oceanogr. 52: 1293~1304.

IPCC Fourth Assessment Report: Climate Change: The Physical Science Basis; Intergovernmental Panel on Climatic Change; edited by: Solomon, S., Qin, S., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., and Miller, H. L. Cambridge University Press. New York. 2007.

Kim, J.M., K. Lee, K. Shin, J.H. Kang, H.W. Lee, M. Kim, P.G. Jang and

M.C. Jang. 2006. The effect of seawater CO_2 concentration on growth of a natural phytoplankton assemblage in a controlled mesocosm experiment. Limnol. Oceanogr. 51: 1629~ 1636.

Kim, J.M., K. Shin, K. Lee and B.K. Park. 2008. In situ ecosystem based carbon dioxide perturbation experiments: Design and performance evaluation of a mesocosm facility. Limnol. Oceanogr. Meth. 6: 208~217.

Kim, J.M., K. Lee, E.J. Yang, K. Shin, J.H. Noh, K.T. Park, B. Hyun, H.J. Jeong, J.H. Kim, K.Y. Kim, M. Kim, H.C. Kim, P.G. Jang and M.C. Jang. 2010. Enhanced production of oceanic dimethylsulfide resulting from CO_2-induced grazing activity in a high CO_2 world. Environ. Sci. Technol. 44: 8140~8143.

Kim, J.M., K. Lee, K. Shin, E.J. Yang, A. Engel, D.M. Karl and H.C. Kim. 2011. Shifts in biogenic carbon flow from particulate to dissolved forms under high carbon dioxide and warm ocean conditions. Geophys. Res. Lett. 38: doi:10.1029/2011GL047346.

Kim, J.H, K.Y. Kim, E.J. Kang, K. Lee, J.-M. Kim, K.-T. Park, K. Shin, B. Hyun, and H.J. Jeong, 2013. Enhancement of photosynthetic carbon

assimilation efficiency by phytoplankton in the future coastal ocean. Biogeosciences, 10, 7525~7535.

Leonardos, N. and R.J. Geider. 2005. Elevated atmospheric carbon dioxide increases organic carbon fixation by *Emiliania huxleyi* (Haptophyta), under nutrient-limited high-light conditions. J. Phycol. 41: 1196~1203.

Lomas, M.W., P.M. Gilbert. 1999. Interactions between NH_4^+ and NO_3^- uptake and assimilation: comparison of diatoms and dinoflagellates at several growth temperatures. Mar. Biol. 133: 541~551.

Marshall, J.A., B. Munday, Y. Yoshizawa and G.M. Hallegraeff. 2001. Effect of irradiance on superoxide production by *Chattonella marina* (Raphidophyceae) from South Australia and Japan. In Hallegraeff, G.M., Balckburn, S.I., Bolch, C.J.S., Lewis, R. (Eds). Harmful Algal Blooms 2000. IOC UNESCO, Paris. 316~319 pp.

Olenina, I., S. Hajdu, L. Edler, A. Andersson, N. Wasmund, S. Busch, J. Göbel, S. Gromisz, S. Huseby, M. Huttunen, A. Jaanus, P. Kokkonen, I. Ledaine and E. Niemkiewicz. 2006. Biovolumes and size-classes of phytoplankton in the Baltic Sea HELCOM Balt. Sea Environ. Proc. No.

106: 144 pp.

Raven, J. 1991. Physiology of inorganic C acquisition and implications for resource use efficiency by marine phytoplankton: relation to increased CO_2 and temperature. Plant Cell Environ. 14: 779~794.

Riebesell, U. 2004. Effects of CO_2 enrichment on marine phytoplankton, J. Oceanogr., 60, 719~729.

Riebesell, U., R.G.J. Bellerby, H.-P. Grossart and F. Thingstad. 2008. Mesocosm CO_2 perturbation studies: from organism to community level, Biogeosciences, 5, 1157~1164.

Rines, J.E.B. and P.E. Hargraves. 1988. The Chaetoceros Ehrenberg (Bacillariophyceae) Flora of Narragansett Bay, Rhode Island, USA. J. Cramer Press. Berlin.

Roessler, P.G. 1990. Environmental control of glycerolipid metabolism in microalgae: commercial implications and future research directions. J. Phycol. 26: 393~399.

Rost, B., U. Riebesell, S. Burkhardt and D. Sültemeyer. 2003. Carbon acquisition of bloom-forming marine phytoplankton. Limnol. Oceanogr.

48: 55~67.

Schippers, P., M. Lűrling and M. Scheffer. 2004. Increase of atmospheric CO_2 promotes phytoplankton productivity. Ecol. Lett. 7: 446~451.

Schneider, U. 2004. Influence of carbonate chemistry and light intensity on natural phytoplankton assemblages with emphasis on species composition. Doctorial thesis.

Sciandra, A., J. Harlay, D. Lefèvre, R. Lemée, P. Rimmelin, M. Denis and J.P. Gattuso. 2003. Response of coccolithophorid *Emiliania huxleyi* to elevated partial pressure of CO_2 under nitrogen limitation. Mar. Ecol. Prog. Ser. 261: 111~122.

Sommer, U. and K. Lengfellner. 2008. Climate change and the timing, magnitude, and composition of the phytoplankton spring bloom. Glob. Change Biol. 14: 1199~1208.

Stolte, W., T. Mccollin, A.A.M. Noordeloos and R. Riegman. 1994. Effect of nitrogen source on the size distribution within marine phytoplankton populations. J. Exp. Mar. Biol. Ecol. 184: 93~97.

Takabayashi, M., K. Lew, A. Johnson, A. Marchi, R. Dugdale and F.P.

Wilkerson. 2006. The effect of nutrient availability and temperature on chain length of the diatom, *Skeletonema costatum*. J. Plankton Res. 28: 831~840, doi:10.1093/plankt/ fbl018.

Tanaka, T., T.F. Thingstad, T. Løvdal, H.P. Grossart, A. Larsen, M. Allgaier, M. Meyerhőfer, K.G. Schulz, J. Wohlers, E. Zőllner and U. Riebesell. 2008. Avaiability of phosphate for phytoplankton and bacteria and of glucose for bacteria at different pCO_2 levels in a mesocosm study. Biogeosciences. 5: 669~678.

Tomas, C.R. 1997. Identifying Marine Phytoplankton. Academic Press, California.

Tortell, P.D., G.H. Rau and F.M.M. Morel. 2000. Inorganic carbon acquisition in coastal Pacific phytoplankton communities. Limnol. Oceanogr. 45: 1485~1500.

Tortell, P.D. and F.M.M. Morel. 2002. Sources of inorganic carbon for phytoplankton in the eastern Subtropical and Equatorial Pacific Ocean. Limnol. Oceanogr. 47: 1012~1022.

Wolf-Gladrow, D.A., U. Riebesell, S. Burkhardt and J. Bijma. 1999. Direct

effects of CO_2 concentrations on growth and isotopic composition of marine plankton. Tellus. 51(2): 461~476.

Yoder, J.A. 1979. Effect of temperature on light-limited growth and chemical composition of Skeletonema costatum (Bacillariophyceae). J. Phycol. 15: 362~370.

Zondervan, I., R.E. Zeebe, B. Rost, U. Riebesell. 2001. Decreasing marine biogenic calcification: a negative feedback on rising atmospheric pCO_2. Global Biogeochem. Cycles. 15: 507~516.

2.3 Thermal effects on the growth and fatty acid composition of four harmful algal bloom species: possible implications for ichthyotoxicity in future oceans

2.3.1 Introduction

Harmful algal blooms (HABs) have a negative impact on marine resources, water quality and human health (Laabir et al., 2011). The frequency and intensity of HABs has been increasing globally since the mid-1970s (Marshall et al., 2001; Hallegraeff, 2010). The southern coast of Korea and western coast of island of Japan, in particular, has experienced an increase in the number of potential toxin-producing algal species over the past several decades (Asakawa et al., 1993; Chang and Kim, 1997; Kim et al., 2004; Hiroishi et al., 2005). Some free fatty acids such as polyunsaturated fatty acids (PUFAs) are associated with toxicity in HAB species. Yasumoto et al. (1990) identified two haemolytic fractions during a massive fish kill in 1998, associated with a *Karenia mikimotoi* bloom along the Norwegian

coast. The fraction with the highest haemolytic activity against mouse blood cells corresponded to the free fatty acid octadecapentaenoic acid [18:5(n-3)]. Therefore, it is critical to clarify the fatty acids production of the HAB species.

Fatty acid profiles in phytoplankton are significantly affected by various environmental factors, especially light, salinity, temperature and nutrient availability (Roessler, 1990; Renaud et al., 1995). Of those, temperature is one of the most important factors influencing the types of fatty acid produced by phytoplankton (Ackman et al., 1968; Satu and Murata, 1980). Thompson et al. (1992b) and Renaud et al. (1995) found that 12 diatom species respond to a decrease in temperature by increasing the ratio of unsaturated to saturated fatty acids, which could affect the fluidity of the cell membrane. Jiang and Chen (2000) also reported that DHA concentration in *Crypthecodinium cohnii*, which was dinoflagellate species, sharply declined from 25°C to 30°C.

Most studies of fatty acid profiles have, until recently, focused on diatoms and chlorophyta, because they are of interest for biofuel production, (e.g., Becker, 2007; Deng et al., 2009) and are primary foods of herbivorous copepods (Peters et al., 2007; Evjemo et al., 2008). Dinoflagellates and raphidophytes comprise a substantial part of HABs that affect fish and humans (Marshall and Hallegraeff, 1998; Heil et al., 2005). There have been fewer studies of HAB species, even though they are among the most important phytoplankton groups in coastal ecosystems. In particular, the fatty acid compositions and concentrations in dinoflagellates and raphidophytes at different temperatures have not previously been studied. Therefore, the goal of the present study was to examine the potential impacts of changes in temperature on HAB species in a physiological perspective. Especially, we examined the effects of temperature on the growth rates, cell volumes, and fatty acid profiles of four such phytoplankton: *Akashiwo sanguinea*, *Alexandrium tamarense*, *Prorocentrum minimum* (which are dinoflagellates), and *Chattonella ovata* (a raphidophyte).

2.3.2 Materials and methods

2.3.2.1 Isolation and culture of HAB species

The coastal marine HAB species *A. sanguinea*, *A. tamarense*, *C. ovata*, and *P. minimum* were isolated, using a capillary method, from water collected off the southern coast of Korea (34.6° N and 128.5°E). The isolated strains were initially cultured in sterilized f/2 medium without a source of silica, as described by Guillard (1975), at 20 °C, pH 8.2, salinity 33 psu, light intensity of 50 μmol photons m^2 s^{-1} and a 12 h : 12 h :: light : dark cycle. Each HAB strain was cultured at temperatures of 15, 20, 25 and 30 °C to assess thermal effects on their growth rates, cell volumes and fatty acid compositions.

2.3.2.2 Measures of cell growth rates and cell volumes

Cells of each species in exponential growth phase were inoculated into 1.3 L polycarbonate bottles containing 1 L of f/2 medium to give an initial cell

density of approximately 50 cells mL^{-1}. Each experiment was performed in triplicate, and experiments were terminated in late exponential phase. A 10 mL subsample from each incubation bottle was collected every day and immediately fixed with acidic Lugol's solution (5% final concentration) for measurement of cell growth and volume. The numbers of cells in the subsamples were counted using a Sedgwick Rafter counting chamber with a light microscope (Axioplan2; Carl Zeiss, Germany) at ×10 magnification. The specific growth rates (μ) of each species under the controlled conditions were calculated as $\mu = (\ln Nt_2 - \ln Nt_1)/(t_2 - t_1)$, where N is the number of cells at time t in the exponential phase (Guillard, 1973). The cell volumes were measured using a Portable FlowCAM™ (Fluid Imaging Technologies, USA) equipped with a 100 μm flow tube and using ×10 magnification. At least 200 cells were measured in each sample.

2.3.2.3 Analysis of fatty acids in the HABs

To measure the dry weight of cultured cells, they were collected from the medium in late exponential or early stationary phase, using pre-weighed 47

mm Nuclepore track-etched membrane filters (pore size 0.4 μm; Whatman, Maidstone, UK), and the filters were dried at 60 °C for 12 h. Cells were prepared for fatty acid analysis by filtering 500 mL culture in late exponential or early stationary phase through 47 mm GF/F filters (Whatman pore size: 0.7 μm) that had been baked at 450 °C for 2 hrs. Lipids were extracted from the samples three times with a 1:1 (v/v) mixture of dichloromethane and methanol (Bligh and Dyer, 1959). The extract was dried under nitrogen, and the lipids were redissolved in a known volume of 2:1 (v/v) dichloromethane and methanol spiked with an internal standard (n-nonadecanoic acid; Sigma-Aldrich, St Louis, MO, USA). The extracts were dried again under nitrogen, and the extracted lipids were subjected to alkaline hydrolysis with 0.5N methanolic potassium hydroxide (KOH) at 70 °C for 30 min. The neutral lipid fraction (alcohols and sterols) was removed with a 9:1 (v/v) mixture of hexane and diethyl ether. Then the polar fraction (fatty acids) was acidified to pH <2 and extracted three times with a 9:1 (v/v) mixture of hexane and diethyl ether. The extracted fatty

acids were dried under nitrogen and converted to methyl esters (FAMEs) using boron trifluoride–methanol at 70 °C for 30 min. The FAMEs were isolated by extracting the sample three times with 9:1 (v/v) hexane and diethyl ether. The positions of the double bonds in FAMEs were determined with the dimethyl disulphide method (Nichols et al., 1986). FAMEs were analysed by gas chromatography (GC; Agilent 7890A; Agilent Technologies, Santa Clara, CA, USA) equipped with a ZB-5 ms coated, 5% phenyl–arylene and 95% dimethylpolysiloxane chromatography column (60 m long, 0.32 mm id, 0.25 μm film thickness; Phenomenex, Torrance, CA, USA) using helium as the carrier gas. The GC system had a flame ionization detector (FID). The splitless injection mode was used, the initial oven temperature was 50 °C, the injector temperature was 250 °C and the detector temperature was 320 °C. After injection, the oven temperature was increased at 10 °C/min to 120 °C and then 4 °C/min to 300 °C, where it was held for 5 min. The duration of the oven temperature programme was 57 min. Individual FAMEs were identified using an Agilent 7890A GC system equipped with a mass selective detector (Agilent 5975C MSD). The MSD

was operated in electron impact ionization mode, with 70 eV electron energy. The mass range acquired was 50–700 amu. The same conditions and chromatography column were used for the GC-MSD analyses as for the GC-FID analyses. The fatty acids were named using the IUPAC shorthand nomenclature A:B (n-X), where A is the number of carbon atoms in the individual fatty acid, B is the number of double bonds, and X (in n-X) is the position of the first double bond, where the numbering starts at the terminal methyl end ($–CH_3$) of the fatty acid.

2.3.2.4 Statistical analyses

The results are presented as the means and standard deviations of the raw data. All the data sets had normal distributions and homogeneous variances. The relationships of growth rate, volume, length, width, group, and individual fatty acid concentrations with temperature were fitted using simple linear regressions and analysed using one-way ANOVA followed by Tukey's post-hoc test, using SPSS 12.0 (SPSS, USA).

2.3.3 Results

2.3.3.1 Cell growth rates and cell volumes

The responses of specific cell growth rates to water temperature exhibited different patterns for the four HABs species (Fig. 2.3.1a). Growth either increased or remained steady up to 25 °C, but was substantially slower at 30 °C for all species. Growth rates of *A. sanguinea* and *P. minimum* doubled when the temperature was increased from 15 °C to 25 °C, while the rate of *P. minimum* at 30 °C was similar to that at 25 °C. *Alexandrium tamarense* had the lowest growth rate among the species studied, and it did not grow at 30 °C. Growth rate of *C. ovata* slightly increased from 15 to 25 °C, but it decreased at 30 °C. The cell volumes of all four species were little affected by the temperature (Fig. 2.3.1b).

2.3.3.2 Fatty acid profiles at 15 °C

The FA compositions found in the HABs are summarized in Figure 2.3.2, and individual components in each FA group are tabulated in Tables 2.3.1

and 4.3.2. Saturated fatty acids (SAFAs) contributed the largest proportion of the total fatty acids in *A. sanguinea* and *A. tamarense* at 15 °C (Fig. 2.3.2a and 2b), whereas SAFAs and PUFAs contributed almost equally for *C. ovata*, (Fig. 2.3.2c) and PUFAs were the dominant FAs in *P. minimum* at 15 °C (Fig. 2.3.2d). In particular, 16:0 was the predominant SAFA, contributing more than 20% of total fatty acid concentration in all four species (Table 2.3.1). EPA and DHA contributed substantially to the PUFA concentrations. *C. ovata* had distinctive EPA and DHA abundances, the mean EPA proportion being more than 16% of the total fatty acid concentration. The dinoflagellate species had mean EPA fractions that were 5% of total fatty acids. The contributions of DHA to the total fatty acid concentrations were complements to those for EPA: *C. ovata* having a mean DHA fraction less than 3% of total fatty acids, whereas the dinoflagellates had mean DHA fractions greater than 10% of total fatty acids. Branched fatty acids contributed only slightly to total fatty acids.

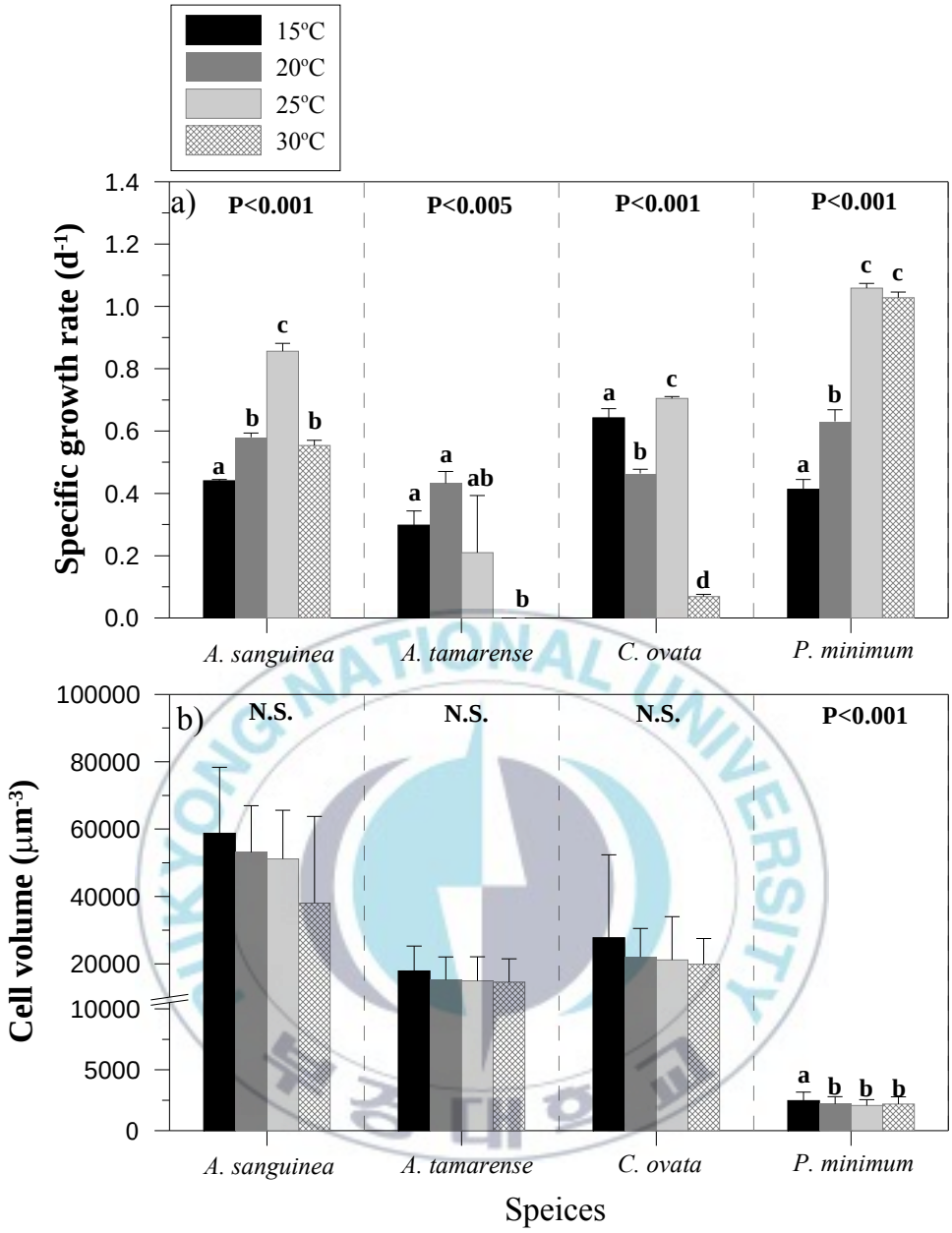


Fig. 2.3.1. The (a) growth rates and (b) cell volumes of four harmful-algal-bloom phytoplankton species cultured at four temperatures (N.S. = not significant).

2.3.3.3 Temperature effects on fatty acid concentration and composition

The four HAB species exhibited varying concentration and composition of fatty acid with water temperature due to differing responses of fatty acid of each species to increasing temperature (Fig. 2.3.2 and 2.3.3). Total fatty acid concentrations in *A. sanguinea*, *A. tamarense* and *C. ovata* decreased markedly, by more than 70%, when the culture temperature was increased from 15 to 30 °C (Fig. 2.3.2a~2c), whereas the total fatty acid concentration in *P. minimum* did not show clear thermal effects (Fig. 2.3.2d). Both SAFA and MUFA (monounsaturated fatty acid) cell concentrations decreased with increasing temperature in *A. sanguinea*, *A. tamarense* and *C. ovata*, but they increased in *P. minimum* (Fig. 2.3.2). PUFA concentrations markedly decreased with increasing temperature, except in *P. minimum* that showed no trend in this respect (Fig. 2.3.2d).

Table 2.3.1. Fatty acid compositions[1] (as percentages of the total fatty acid concentrations) in *Akashiwo sanguinea* and *Alexandrium tamarense* grown at four different temperatures.

Fatty acid	*Akashiwo sanguinea*				*Alexandrium tamarense*			
	15 °C	20 °C	25 °C	30 °C	15 °C	20 °C	25 °C	30 °C
n- Saturates								
12:0	-[2]	-	-	-	1.2	0.2	-	-
14:0	7.5	5.2	5.2	5.6	9.2	7.1	1.7	2.4
16:0	32.0	32.7	38.3	38.4	29.3	29.9	25.6	25.2
18:0	2.3	3.3	4.4	4.7	4.1	4.8	15.9	21.7
Total % SAFAs[a]	42.4	41.8	48.4	49.3	44.3	42.5	43.8	50.2
Monounsaturates								
16:1(n-9)	0.4	0.2	0.3	0.1	0.7	0.4	0.7	1.3
16:1(n-7)	2.6	2.0	1.7	2.1	2.5	1.8	3.1	2.2
16:1(n-5)	0.1	0.3	0.2	0.4	0.9	1.3	0.7	0.7
18:1(n-9)	27.0	16.6	15.7	8.4	10.7	9.0	15.8	10.3
18:1(n-7)	3.1	3.7	3.7	2.4	5.8	9.6	9.2	10.3
20:1	-	-	-	-	0.1	0.2	0.9	1.3
24:1(n-9)	0.1	0.2	0.1	0.1	0.9	1.4	-	-
Total % MUFAs[b]	33.7	23.2	21.9	13.8	21.9	24.5	31.5	26.3
Polyunsaturates								
18:2(n-6)	2.3	5.1	1.4	2.9	6.5	4.4	3.7	0.8
18:2(n-4)	0.9	0.8	0.8	0.7	3.6	1.8	3.5	1.8
18:4	1.4	3.6	6.3	8.6	-	-	-	-
18:4(n-?)	-	-	-	-	6.5	7.2	0.5	0.6
20:5(n-3) EPA[c]	5.4	10.4	9.0	11.7	1.4	4.5	3.5	0.8
22:6(n-3) DHA[d]	12.3	12.4	9.8	10.5	11.7	9.1	6.3	8.8
Total % PUFAs[e]	22.6	32.5	27.6	34.8	30.1	27.4	18.4	14.3
Branched & odd-chain								
15:1(?)	-	-	-	-	0.7	1.0	0.4	0.2
15:0i	0.9	1.7	1.0	1.1	1.2	1.9	0.9	1.1
17:0a	0.1	0.3	0.4	0.4	1.0	1.8	2.1	2.8
17:0n	-	0.1	0.2	0.3	0.3	0.4	1.9	3.8
Total % BrFAs[f]	1.3	2.5	2.0	2.2	3.7	5.7	6.4	9.3

(1) '-' indicates that the fatty acid contributed less than 0.1% of the total fatty acid concentration

(2) [a] SAFAs = saturated fatty acids; [b] MUFAs = monounsaturated fatty acids; [c] EPA = eicosapentaenoic acid [20:5(n-3)] ;[d] DHA = docosahexaenoic acid [22:6(n-3)];[e] PUFAs = polyunsaturated fatty acids; [f] BrFAs = branched fatty acids

Table 2.3.2. Fatty acid compositions[1] (as percentages of the total fatty acid concentrations) in *Chattonella ovata* and *Prorocentrum minimum* grown at four different temperatures.

Fatty acid	*Chattonella ovata*				*Prorocentrum minimum*			
	15 °C	20 °C	25 °C	30 °C	15 °C	20 °C	25 °C	30 °C
n- Saturates								
14:0	5.2	3.5	7.0	8.5	1.3	2.1	0.4	1.5
16:0	26.4	23.8	27.9	24.2	22.5	33.0	29.7	30.5
18:0	2.1	2.7	4.5	11.4	4.3	5.4	10.1	9.0
20:0	-[2]	-	-	-	1.6	1.9	1.4	1.1
Total % SAFAs[a]	33.8	30.2	39.5	44.2	29.6	42.3	41.5	42.1
Monounsaturates								
16:1(n-7)	10.8	4.9	3.1	9.8	1.8	2.7	3.5	3.3
16:1(n-3)?	1.4	2.1	1.2	-	-	-	-	-
18:1(n-9)	5.2	5.8	9.2	6.5	3.5	3.6	5.2	7.5
18:1(n-7)	3.5	4.6	2.1	6.9	5.0	8.0	19.8	14.0
20:1(n-11)	0.2	1.2	-	-	-	-	-	-
22:1(n-11)	0.5	0.6	1.0	3.2	-	-	-	-
Total % MUFAs[b]	22.4	20.0	16.9	26.4	10.8	14.8	29.1	25.6
Polyunsaturates								
16:2(n-4)	0.5	0.3	0.0	1.5	-	-	-	-
18:2(n-6)	2.9	4.4	7.1	3.8	22.3	14.5	7.4	8.9
18:2(n-4)	-	-	-	-	3.1	3.2	4.7	7.5
18:4(n-?)	-	-	-	-	6.5	6.6	7.1	7.0
20:4(n-6)	5.7	6.2	3.8	6.9	-	-	-	-
20:4(n-3)	0.6	1.2	0.9					
20:5(n-3) EPA[c]	19.5	20.5	16.0	8.1	4.7	3.6	1.9	1.5
22:5(n-6)	4.4	5.2	2.6	2.4	-	-	-	-
22:6(n-3) DHA[d]	1.8	1.8	5.0	0.6	23.0	15.0	8.3	7.4
Total % PUFAs[e]	35.8	40.2	36.3	23.3	59.5	42.9	29.4	32.4
Branched & odd-chain								
15:0i	0.4	0.3	0.2	1.3	-	-	-	-
16:0br	0.3			3.9	-	-	-	-
18:0br	6.6	8.8	6.6	0.6	-	-	-	-
Total % BrFAs[f]	8.0	9.6	7.3	6.1	0.0	0.0	0.0	0.0

(1) '-' indicates that the fatty acid contributed less than 0.1% of the total fatty acid concentration

(2) [a] SAFAs = saturated fatty acids; [b] MUFAs = monounsaturated fatty acids; [c] EPA = eicosapentaenoic acid [20:5(n-3)] ;[d] DHA = docosahexaenoic acid [22:6(n-3)];[e] PUFAs = polyunsaturated fatty acids; [f] BrFAs = branched fatty acids

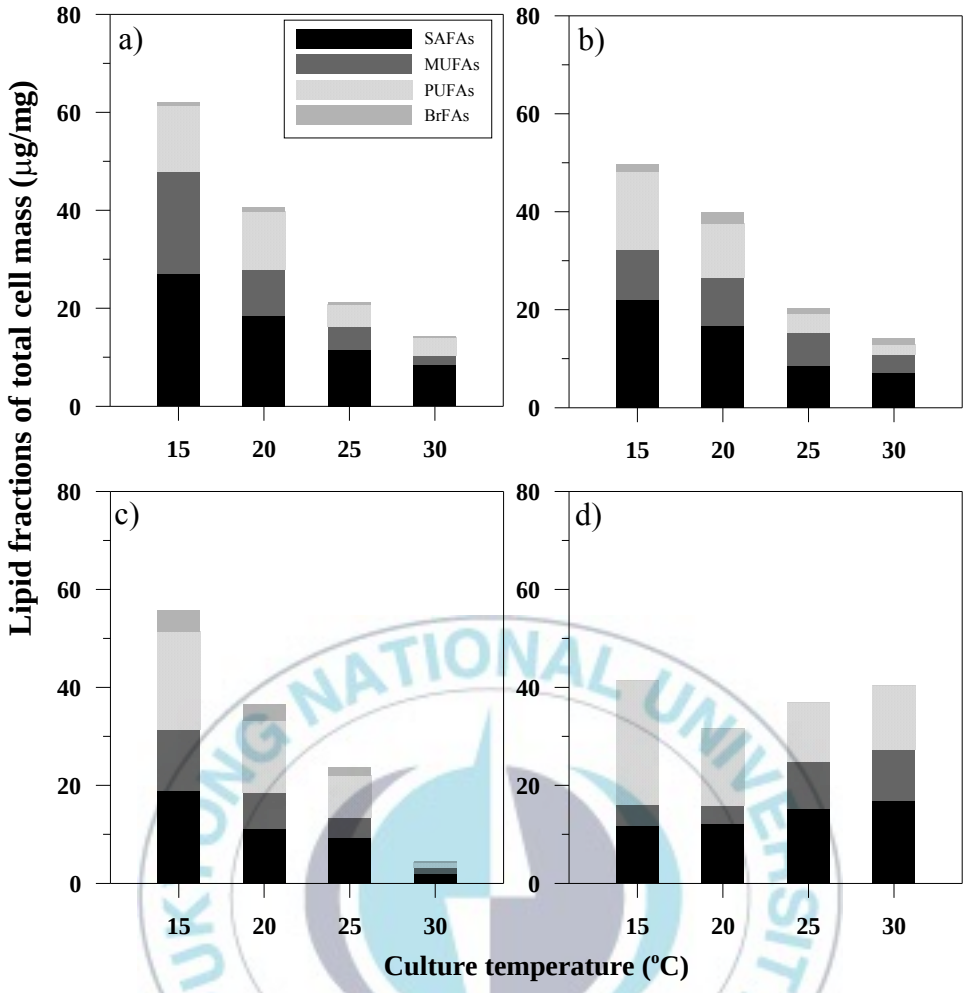


Fig. 2.3.2. Lipid fractions of total cell mass in (a) *Akashiwo sanguinea*, (b) *Alexandrium tamarense*, (c) *Chattonella ovata*, and (d) *Prorocentrum minimum* at four temperatures (SAFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids, and BrFAs = branched fatty acids).

The SAFA contributions to total fatty acids increased slightly with increasing temperature for all species. This trend reflected the positive linear regressions between percentages of 16:0 and 18:0 and culture temperature. Especially, *A. sanguinea* had a significant linear increase with increasing culture temperature (linear regression, $P<0.001$; Fig. 2.3.3a). Proportions of MUFAs and PUFAs showed opposite trends in respond to increasing temperature (Fig. 2.3.3). In *A. sanguinea*, proportions of MUFA were significantly linear decreased with increasing temperature ($P<0.001$; Fig. 2.3.3a), whereas proportions of PUFA was increased. In *A. tamarense*, *C. ovata,* and *P. minimum*, proportions of PUFA were significantly decreased with increasing temperature ($P<0.05$), but proportions of MUFA were decreased (Fig 2.3.3b~3d). Considering the BrFA (branched fatty acid) data in all species, there were low proportion of total fatty acids and did not change significantly with culture temperature. Only *A. tamarense* had a significant relationship between culture temperature and percentages of BrFA ($P<0.001$, Fig. 2.3.3b).

2.3.3.4 Temperature effects on EPA and DHA

The EPA and DHA are major components of PUFAs and there are showed different response with changes in culture temperature (Fig. 2.3.4a). The contributions of EPA to the total fatty acid concentrations in the four HABs were different at different temperatures (Fig. 2.3.4a). The relative contributions of EPA to the total fatty acid concentrations changed significantly with changes in temperature in *A. sanguinea* ($P<0.005$, ANOVA), *C. ovata* ($P<0.005$, ANOVA) and *P. minimum* ($P<0.05$, ANOVA). However, the trend of EPA in *A. sanguinea* was different in compare of *C. ovata* and *P. minimum* under different temperature; proportion of EPA in *A. sanguinea* increased, but in *C. ovata* and *P. minimum*, decreased (Fig. 2.3.4a). Only *P. minimum* showed a significant relationship between the DHA contribution to total fatty acid concentration and temperature (Fig. 2.3.4b, $P<0.005$, ANOVA); the DHA contributions in the other species showed no overall trends with temperature.

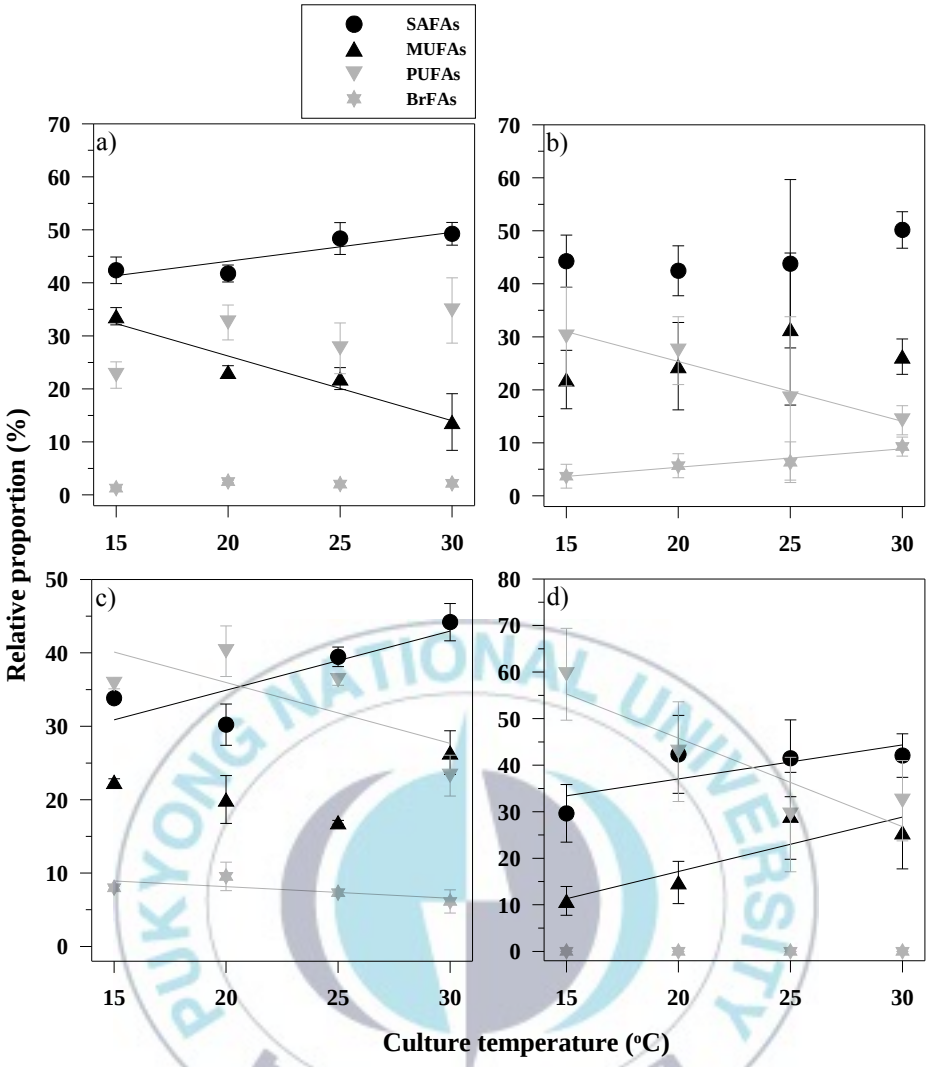


Fig. 2.3.3. Relative proportions of saturated fatty acids (SAFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and branched fatty acids (BrFAs) in (a) *Akashiwo sanguinea*, (b) *Alexandrium tamarense*, (c) *Chattonella ovata*, and (d) *Prorocentrum minimum* at four temperatures.

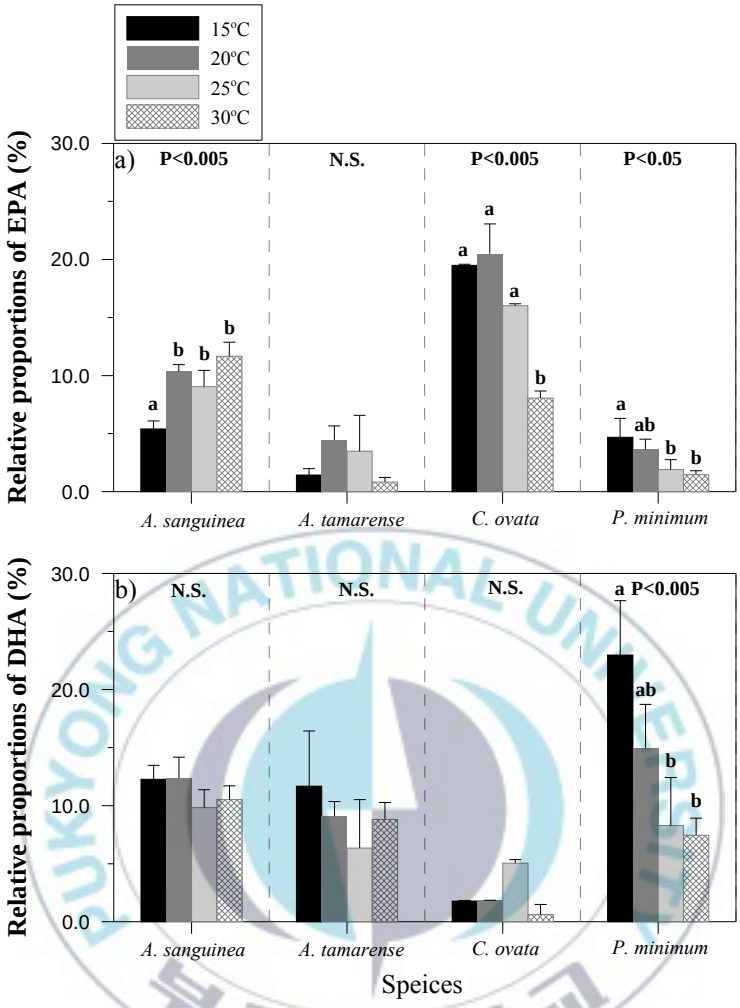


Fig. 2.3.4. Relative proportions of (a) eicosapentaenoic acid [EPA; 20:5(n-3)] and (b) docosahexaenoic acid [DHA; 22:6(n-3)] in *Akashiwo sanguinea*, *Alexandrium tamarense*, *Chattonella ovata*, and *Prorocentrum minimum* at four temperatures (N.S. = not significant).

2.3.4 Discussion

The cell volume of the four species changed little over the experimental temperature range, differing from reports that phytoplankton cell volumes generally decrease with increasing temperature (Montagnes and Franklin, 2001; Sayegh and Montagnes, 2011). Thus, the cell-abundance-based growth rates reflected no sacrifice in cell biomass for the four HAB species studied. The growth rates of phytoplankton based on abundance change are generally expected to increase with temperature and then to decrease rapidly above a critical temperature (Sayegh and Montagnes, 2011). Our data generally support this trend for each of the species studied, a trend previously described for *A. sanguinea* by Matsubara et al. (2007), for *A. tamarense* by Hamasaki et al. (2001), for *C. ovata* by Yamaguchi et al. (2010), and for *P. minimum* by Grzebyk et al. (1996). At 30 °C the specific growth rates of *C. ovata* and *A. tamarense* decreased sharply, indicating that metabolism was disrupted at this temperature in those species. Reports by Richmond (1986) and Renaud et al. (2002) indicate that cell death can occur

at elevated temperatures. Among the HABs we have studied, only *P. minimum* is tolerant of high temperatures, sustaining a specific growth rate of 1.03±0.01 d^{-1} at 30 °C. *Prorocentrum minimum* has been described as eurythermal, and it blooms in warm seasons under eutrophic conditions. Rabbani et al. (1990) found that *P. minimum* blooms along the coast of Pakistan at water temperatures of 27–29 °C. Our growth rate responses to temperature followed trends similar to those found in previous studies (e.g. Rabbani et al., 1990; Sayegh and Montagnes, 2011).

One of the commonly observed changes with temperature in phytoplankton is altered levels of FAs (Ackman et al., 1968; Satu and Murata, 1980; Thompson et al., 1992b). We also found that TFA concentrations changed substantially with elevated temperature. Most notable was the strong decline in TFA concentrations with elevated temperature in *A. sanguinea*, *A. tamarense*, and *C. ovata*. No report is available for changes of FA compositional concentrations in dinoflagellates under different temperature conditions that can be compared to, but Teoh et al. (2004) reported that

Chlorella (UMACC 237) and *Klebsormidium* (UMACC 227) produced more MUFAs at high temperatures than at low temperatures. In contrast to the other three species, *P. minimum* showed a distinctly different change in FA profile with temperature (Fig. 2.3.3d). Its MUFA concentrations were much higher above 20 °C than below 20 °C and PUFA concentration also did not decrease with temperature as shown in other HAB species. *Prorocentrum minimum*, a neritic, bloom-forming dinoflagellate and cosmopolitan in temperate, brackish waters to tropical regions, is the cause of harmful blooms in many estuarine and coastal environments. Its blooms appear to have undergone a geographical expansion over the past several decades (Heil et al., 2005). They can be toxic to humans via ingestion of shellfish containing hepatotoxic or neurotoxic substances. The physiological flexibility of *P. minimum* in response to changing environmental parameters (e.g., light, temperature, salinity) is believed to be responsible for its global expansion (Heil et al., 2005). Our results suggest that retaining PUFAs even at increased water temperature may demonstrate such an adaptability to environmental changes for making this species expand its habitat globally.

Varying fatty acid unsaturation is an important adaptation by phytoplankton to their growth environment (James et al., 2013). Lynch and Thompson (1982) found that *Dunaliella salina* showed a considerable decrease in fatty acid unsaturation in response as temperature increased from 12 to 30 °C. This may be linked to a requirement to maintain a sufficient level of membrane fluidity (Los and Murata, 2004). In general, lower temperature reduces the fluidity of cell membranes and cells in response to it compensate by increasing levels of unsaturated fatty acids to increase fluidity (Juneja et al., 2013). Increased levels of unsaturated fatty acids may also protect the photosynthetic machinery from photoinhibition at low temperatures (Nishida and Murata, 1996). In our study, FA unsaturation decreased with increasing temperature in four HAB species as the proportion of PUFAs declines but SAFAs' contribution rises with increasing temperature (Fig. 2.3.3 and Fig. 2.3.5). With the increasing temperature, proportion of PUFAs decreased in *A. tamarense*, *C. ovata*, and *P. minimum,* but it increased in *A. sanguinea* (Fig. 2.3.3). This was because, although the PUFA

concentrations in *A. sanguinea* decreased by more than 70% from 15 °C to 25 °C, the MUFA concentrations declined by more than 90% (Fig. 2.3.2a), so the proportional PUFA contribution to the total fatty acid concentrations increased slightly.

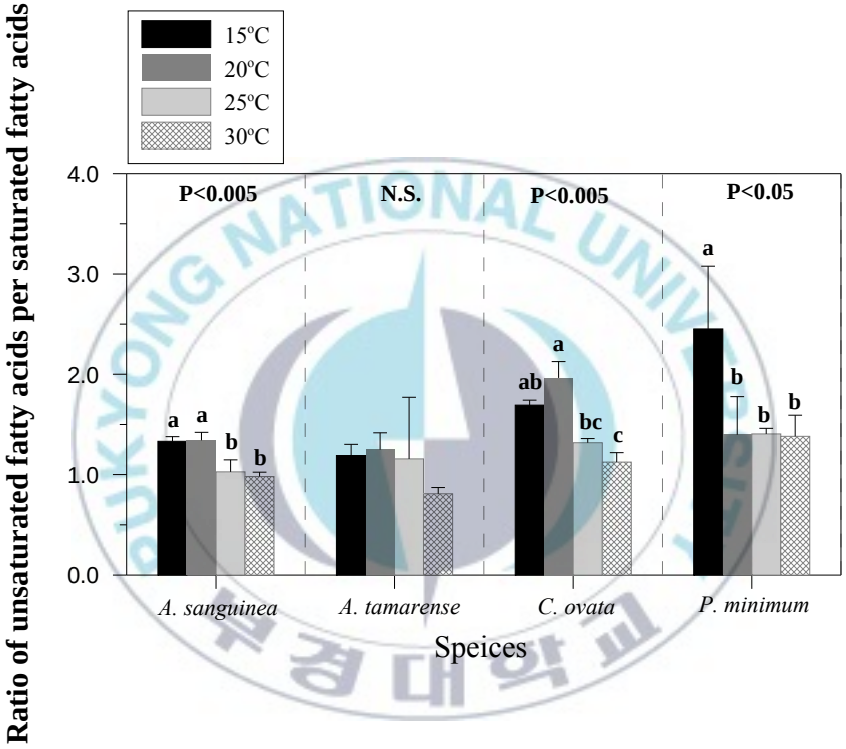


Fig. 2.3.5. The degree of unsaturation of fatty acids in (a) *Akashiwo sanguinea*, (b) *Alexandrium tamarense*, (c) *Chattonella ovata*, and (d) *Prorocentrum minimum* at four temperatures (N.S. = not significant).

PUFAs and some free fatty acids play a key role in the ichthyotoxicity from HABs (Cho et al., 2001). Recent studies showed that high concentration of PUFAs can be toxic for phytoplankton (Uchida et al., 1988; Fu et al., 2004), sea urchin (Sellum et al., 2000), and fish (Yasumoto et al., 1990). Suzuki and Matsuyama (1995) reported when PUFAs are actively released by cell damage, they could be lethal to fish. Among the PUFAs, EPA and DHA are among the most harmful (Arzul et al., 1995; Fu et al., 2004). Its exhibits lethal toxicity to mice when injected intraperitoneally, it can cause diarrhea (Sajiki et al., 1993), it is haemolytic, and it is also highly repressive for the bioluminescence of *Vibrio fischeri* (Fu et al., 2004). Especially, predominance of EPA in *C. ovata* is known to be the most toxic fatty acid to scallop (*Pecten maximus*) embryos (Arzul et al., 1998). The DHA has also been reported as inhibiting the growth of the bacteria and microalgae, but also having hemolytic activity (Ikawa, 2004). In general, raphidophyte species sustain relatively high EPA concentrations (Marshall et al., 2002), whereas most dinoflagellate species contain high DHA concentrations and in some cases high EPA concentrations as well (e.g. Evjemo et al., 2008).

These fatty acids are often major contributors to the total fatty acid concentrations (Mansour et al., 1999). Our data agree with those from previous studies that DHA was abundant in the dinoflagellates *A. sanguinea* *A. tamarense* and *P. minimum*, and that the raphidophyte *C. ovata* contained predominantly the EPA (Table 2.3.1 and 2.3.2 at 15 ^{o}C). Other unsaturated fatty acids are also important for aquatic toxicity. Highly unsaturated fatty acid 18:4 was instrumental in causing finfish mortalities (Okaichi, 1989). In our study, this was detected in *A. sanguinea* (Table 2.3.1).

In conclusion, the reduction in concentration and proportion of PUFA to total fatty acids with rising water temperature could lead to less fluid cell membranes, which would affect HAB cell's viability. Given HABs-derived PUFAs association with hemolytic effects, it is considered that ocean temperature rise might lower the toxicity of HABs although better cell growth and/or ruptured cells which was considered more toxic than intact cells could compensate for the reduced per cell toxicity. Our results will require further confirmation, but may suggest that rising ocean temperature

could be affects on growth and fatty acid concentration and composition in HABs, and might thus be impacts on the marine ecosystem and aquaculture industry.

2.3.5 References

Ackman, R,G,, C.S. Tocher and J. McLachlan. 1968. Marine phytoplankter fatty acids. J. Fish. Res. Board. Can. 25: 1603~1620.

Arzul, G., P. Gentien, G. Bodennec, F. Toularastel, A. Youenou and M.P. Crassous. 1995. Comparison of toxic effects of *Gyrodinium cf. nagasakiense* polyunsaturated fatty acids. 395~400 pp. In Lassus, P., Arzul, G., Eradr, E., Gentien, P., Marcaillou, C. (Eds), Harmful Marine Algal Blooms. Lavoisier.

Asakawas, M., K. Miyazawa and T. Nouguchi. 1993. Studies on paralytic shellfish poison (PSP) toxification of bivalves, in association with appearance of *Alexandrium tamarense*, in Hiroshima bay, Hiroshima prefecture. J. Food Hyg. Soc. Jpn. 34: 50~54.

Becker, E.W. 2007. Microalgae as a source of protein. Biotechnol. Advanc. 25: 207~210.

Bligh, E.G. and W.J. Dyer. 1959. A Rapid Method of Total Lipid Extraction and Purification. Can. J. Biochem. Physiol. 37: 911~917.

Chang, M. and W.S. Kim. 1997. Prologue and epilogue to the first international symposium on plankton blooms. Ocean Res. 19: 135~137.

Cho, E.S., B.D. Choi, Y.C. Cho, T.J. Kim and H.G. Kim. 2001. Discrimination of three highly toxic *Alexandrium tamarense* (Dinophyceae) isolates using FITC-conjugated lectin probes. J. Plankton Res. 23(1): 89~95.

Deng, X., Y. Li and X. Fei. 2009. Microalgae: a promising feedstock for biodiesel. African J. Microbiol. Res. 3(13): 1008~1014.

Dorantes-Aranda, J.J., P.D. Nichols, T.D. Waite and G.M. Hallegraeff. 2013. Strain variability in fatty acid composition of Chattonella marina (Raphidophyceae) and its relation to differing ichthyotoxicity toward rainbow trout gill cells. J. Phycol. 49: 427~438.

Evjemo, J.O., N. Tokle, O. Vadstein and Y. Olsen. 2008. Effect of essential dietary fatty acids on egg production and hatching success of the marine copepod *Temora longicornis*. J. Exp. Mar. Biol. Ecol. 365: 31~37.

Fu, M., A. Koulman, M. Van Rijssel, A. Lützen, M.K. de Boer, M.R. Tyl and G. Liebezeit. 2004. Chemical characterisation of three haemolytic

compounds from the microalgal species *Fibrocapsa japonica* (Raphidophyceae). Toxicon. 43: 355~363.

Grzebyk, D. And B. Berland. 1996. Influences of temperature, salinity and irradiance on growth of *Prorocentrum minimum* (Dinophyceae) from the Mediterranean Sea. J. Plankton Res. 18: 1837~1849.

Guillard, R.R.L. 1973. Division rates. In Stein, J. R. (ed.), Handbook of Phycological Methods-Culture Methods and Growth Measurements. Cambridge University Press, Cambridge. 289~311 pp.

Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. *In* Smith, W.L. and Chanley, M.H. (Eds.) Culture of Marine Invertebrate Animals. Plenum Press, New York, USA. 29~60 pp.

Hallegraeff, G.M. 2010. Ocean climate change, phytoplankton community responses and harmful algal blooms: A formidable predictive challenge. J. Phycol. 46(2): 220~235.

Hamasaki, K., M. Horie, S. Tokimitsu, T. Toda and S. Taguchi. 2001. Variability in toxicity of the dinoflagellate *Alexandrium tamarense*

isolated from Hiroshima Bay, western Japan, as a reflection of changing environmental conditions. J. Plankton Res. 23: 271~278.

Heil, C.A., P.M. Glibert and C. Fan. 2005. *Prorocentrum minimum* (Pavillard) Schiller A review of a harmful algal bloom species of growing worldwide importance. Harmful algae. 4: 449~470.

Hiroishi, S., H. Okada, I. Imai and T. Yoshida. 2005. High toxicity of the novel bloom forming species of *Chattonella ovata* (Raphidophyceae) to cultured fish. Harmful Algae. 4: 783~787.

Ikawa, M. 2004. Algal polyunsaturated fatty acids and effects on plankton ecology and other organisms. UNH Center for Freshwater Biology Research. 6(2): 17~44.

James, G.O., C.H. Hocart, W. Hillier, G.D. Price and M.A. Djordjevic. 2013. Temperature modulation of fatty acid profiles for biofuel production in nitrogen deprived *Chlamydomonas reinhardtii*. Bioresour. Technol. 127: 441~447.

Jiang, Y. and C. Feng. 2000. Effects of temperature and temperature shift on docosahexaenoic acid production by the marine microalga

Crypthecodinium cohnii. JAOCS. 77: 613~617.

Juneja, A., R.M. Ceballos and G.S. Murthy. 2013. Effects of environmental factors and nutrient availability on the biochemical composition of algae from biofuel production: a review. Energies. 6: 4607~4638.

Jüttner, F. 2005. Evidence that polyunsaturated aldehydes of diatoms are repellents for pelagic crustacean grazers. Aquat. Ecol. 39: 271~282.

Kim, D.I., Y. Matsuyama, S. Nagasoe, M. Yamaguchi, Y.H. Yoon, Y. Oshima, N. Imada and T. Honjo. 2004. Effects of temperature, salinity and irradiance on the growth of the harmful red tide dinoflagellate *Cochlodinium polykrikoides* Margalef (Dinophyceae). J. Plnakton Res. 26(1): 61~66.

Laabir, M., C. Jauzein, B. Genovesi, E. Masseret, D. Grzebyk, P. Cecchi, A. Vaquer, Y. Perrin and Y. Collos. 2011. Influence of temperature, salinity and irradiance on the growth and cell yield of the harmful red tide dinoflagellate *Alexandrium catenella* colonizing Mediterranean waters. J. Plankton Res. 33(10): 1550~1563.

Los, D.A. and N. Murata. 2004. Membrane fluidity and its roles in the

perception of environmental signals. Biochim Biophys Acta (Biomembranes). 1666: 142~157.

Lynch, D.V. and G.A.Jr. Thompson. 1982. Low temperature-induced alterations in the chloroplast and microsomal membranes of *Dunaliella salina*. Plant Physiol. 47: 541~568.

Mansour, M.P., J.K. Volkman, A.E. Jackson and S.I. Blackburn. 1999. The fatty acid and sterol composition of five marine dinoflagellates. J. Phycol. 35: 710~720.

Marshall, J.A. and G.M. Hallegraeff. 1998. Comparative ecophysiology of the harmful alga Chattonella marina (Raphidophyceae) from South Australian and Japanese waters. J. Plankton Res. 21: 1809~1822.

Marshall, J.A., P.D. Nichols and G.M. Hallegraeff. 2002. Chemotaxonomic survey of sterols and fatty acids in six marine raphidophyte algae. J. Appl. Phycol. 14: 255~265.

Marshall, J.A., B. Munday, Y. Yoshizawa and G.M. Hallegraeff. 2001. Effect of irradiance on superoxide production by *Chattonella marina* (Raphidophyceae) from South Australia and Japan. In Hallegraeff, G.M.,

Balckburn, S.I., Bolch, C.J.S., Lewis, R. (Eds), Harmful Algal Blooms. 2000. IOC UNESCO, Paris. 316~319 pp.

Matsubara, T., S. Nagasoe, Y. Yamasaki, T. Shikata, Y. Shimasaki, Y. Oshima and T. Honjo. 2007. Effects of temperature, salinity, and irradiance on the growth of the dinoflagellate *Akashiwo sanguinea*. J. Exp. Mar. Biol. Ecol. 342: 226~230.

Montagnes, D.J.S. and D.J. Franklin. 2001. Effect of temperature on diatom volume, growth rate, and carbon and nitrogen content: reconsidering some paradigms. Limnol. Oceanogr. 46: 2008~2018.

Nichols, P.D., J.B. Guckert and D.C. White. 1986. Determination of monosaturated fatty acid double-bond position and geometry for microbial monocultures and complex consortia by capillary GC-MS of their dimethyl disulphide adducts. J. Microbiol Meth. 5: 49~55.

Nishida, I. and N. Murata. 1996. Chilling sensitivity in plants and cyanobacteria: The crucial contribution of membrane lipids. Annu. Rev. Plant Biol. 47: 541~568.

Okaichi, T. 1989. Red tide problems in the Seto Inland Sea, Japan. In:

Okaichi, T., Anderson, D.M., Nemoto, T., (Eds), Red Tides, Biology, Environmental Science and Toxicology. 137~142 pp.

Peters, J., J. Dutz and W. Hagen. 2007. Role of essential fatty acids on the reproductive success of the copepod *Temora longicornis* in the North Sea. Mar. Ecol. Prog. Ser. 341: 153~163.

Rabbani, M.M., A.U. Rehman and C.E. Harms. 1990. Mass mortality of fishes caused by dinoflagellate bloom in Gwdar Bay, Southwestern Pakistan. In: Graneli, E., Sundstrom, B., Edler, L., Anderson, D.M., (eds), Toxic Marine Phytoplankton. Elsevier, New York. 209~214 pp.

Renaud, S.M., H.C. Zhou, D.L. Parry, T. Loung-Van and K.C. Woo. 1995. Effects of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae *Isochrysis* sp., *Nitzschia closterium*, *Nitzschia paleacea*, and commercial species *Isochrysis* sp., (clone T.ISO). J. Appl. Phycol. 7: 595~602.

Renaud, S.M., L.V. Thinh, G. Lambrinidis and D.L. Parry. 2002. Effects of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture.

211: 195~214.

Richmond, A. 1986. Cell response to environmental factors. In: Richmond, A. (eds), CRC Handbook of Microalgal Mass Culture. CRC Press, Boca Raton. 69~106 pp.

Roessler, P.G. 1990. Environmental control of glycerolipid metabolism in microalgae; Commercial implications and future research directions. J. Phycol. 26: 393~399.

Sajiki, J., T. Yamanaka, H. Takahashi and Y. Tsuruoka. 1993. Possibility of diarrhea effect by eicosapentaenoic acid (EPA) and autoxidized EPA. Jpn. J. Toxicol. Environ. Health. 39(2): 100~105.

Satu, N. and N. Murata. 1980. Temperature shift-induced responses in lipids in the blue-green alga, *Anabaena variabilis*. The central role of diacylmonoalactosylglycerol in thermo adaption. Biochim Biophys Acta. 619: 353~365.

Sayegh, F.A.Q. and D.J.S. Montagnes. 2011. Temperature shifts induce intraspecific variation in microalgal production and biochemical composition. Bioresour. Technol. 102: 3007~3013.

Sellem, F., D. Pesando, G. Bodennec, A.E. Abed and J.P. Girard. 2000. Toxic effects of *Gymnodinium cf. mikimotoi* unsaturated fatty acids to gametes and embryos of the sea urchin *Paracentrotus lividus*. Water Res. 34: 550~556.

Suzuki, T. and Y. Matsuyama. 1995. Determination of free fatty acids in marine phytoplankton causing red tides by fluorometric high-performance liquid chromatography. JAOCS. 72: 1211~1214.

Teoh, M.L., W.L. Chu, H. Marchant and S.M. Phang. 2004. Influence of culture temperature on the growth, biochemical composition and fatty acid profiles of six Antarctic microalgae. J. Appl. Phycol. 16: 421~430.

Thompson, P.A., M. Guo, P.J. Harrison and J.N.C. Whyte. 1992b. Effects of variation in temperature: II. On the fatty acid composition of eight species of marine phytoplankton. J. Phycol. 28: 488~497.

Uchida, A., A. Shimada and Y. Ishida. 1988. Antibacterial and antialgal substances produced by the dinoflagellate *Peridinium bipes*. Nippon Suisan Gakkaishi. 54: 1941~1945.

Yamaguchi, H., K. Mizushima, S. Sakamoto and M. Yamaguchi. 2010.

Effects of temperature, salinity and irradiance on growth of the novel red tide flagellate *Chattonella ovata* (Raphidophyceae). Harmful algae. 9: 398~401.

Yasumoto, T., B. Inderdal, T. Aune, V. Hormazabal, O.M. Skulberg and Y. Oshima. 1990. Screening for hemolytic and ichthyotoxic components of *Chrysochromulina polyepsis* and *Gyrodinium aureolum* from Norwegian coastal waters. In: Graneli, E., Sundstron, B., Edler, L., Anderson, D.M., (eds.). Toxic Marine Phytoplankton. Elsevier, USA. 436~440 pp.

3. CONCLUSION

This work investigates the response of phytoplankton physiology using the considered the two components of climate changes- CO_2 concentration and temperature. The individual and combined effects of CO_2 and temperature on phytoplankton physiology can be species-specific. Such differences between closely related groups can have large consequences for whole natural community changes. Our mesocosm study showed species-specific difference response, but small phytoplankton and most dominant dinoflagellate species would be better growth under future oceanic condition in compare to under present condition. Its results provide the important messages for the potential possibility on the range extensions of HAB species in the future ocean environments. Indeed, it is generally accepted that HABs are increasing in frequency, intensity, and duration in all aquatic environments on a global scale as a result of climate changes. Therefore, under future ocean environment toxin content produced by HABs recently great concern. In preliminary work, issue on the toxin production by HABs

represents the two perspective point that their toxin production enhanced and/or unpredictable under future ocean condition. For instance, Fu et al. (2010) suggest that toxin produced by *Alexandrium catenella* could be more toxic under acidification conditions. But, another perspective on this issue suggests that cellular toxin concentration produced by *Alexandrium minutum* were high variance between different cells. In this study 3, our results suggest that ocean temperature rise may enhance the cell growth in HABs, but may decrease the PUFAs concentration and composition which was associated with phytoplankton cell viability and toxicity. However, until recently, changes in toxin content under future ocean environments have not been clearly define, since numerous parameters included CO_2 and temperature also influenced the toxin production from HABs. But, indubitably, range extension of HABs will give the greatest problems for human society. Additionally, recent research represents that phytoplankton generally considered as important food sources for copepods, and PUFAs included in EPA and DHA have an important function for egg production and hatching success of copepods (Shin et al., 2003; Evjemo et al., 2008).

Moreover, Kleppel et al. (1991) reported that copepod egg production seems to be more closely correlated with biomass of dinoflagellates than with diatoms. Therefore, PUFA concentration decrease in dinoflagellates will lower the egg production in copepods, and this result closely linked with higher trophic grazers such as fish can significantly impacts on marine food web. Our studies will require further confirmation, but may suggest that rising CO_2 and temperature in the future ocean environment could be changes the growth in phytoplankton species and in their metabolite activity, and might thus be significant impacts on the marine ecosystem and aquaculture industry.

Acknowledgments

제가 경남 거제에 온지 어느덧 10년이란 세월이 흘렀습니다. 그동안 좋았던 일과 힘들었던 일도 많았지만 저에게 항상 힘이 되어주셨던 지인분들이 있었기에 이렇게 늦게나마 졸업을 할 수 있었지 않나 생각합니다. 그래서 이렇게 감사의 글로 나마 오랜 시간 동안 항상 저에게 힘이 되어주신 많은 분들께 감사의 말씀을 전합니다.

먼저 부족한 저를 제자로 삼아 주시고, 학위과정을 하고 있는 저를 위해 많은 부분을 배려해주신 지도교수님이신 문창호 교수님께 감사의 인사를 전합니다. 앞으로 더욱 노력해서 부끄럽지 않은 제자가 되도록 하겠습니다. 그리고 바쁘신 와중에도 논문심사위원이 되어주신 오석진 교수님과 멀리서 한걸음에 달려와서 따뜻한 말씀을 전해주신 석사과정 지도교수님이신 신용식교수님께 감사의 인사를 전합니다. 그리고 10년 동안 항상 저를 이해를 해주시고 이끌어 주신 신경순 박사님과 학문적으로 많은 가르침을 주신 최근형 박사님께도 감사를 드립니다.

2005년도부터 한국해양기술원 남해연구소 선박평형수센터에서 저와 함께 동거동락을 했던 팀원 분들께도 감사의 마음을 전합니다. 4남매

아버지로서 직장과 가정에서 모범적인 모습을 보여주는 장민철 박사님, 노무현 전 대통령을 너무나 좋아하는 초보 운전 장풍국 박사님, 매사에 일을 너무나 꼼꼼히 챙기시는 우리팀 살림꾼 이우진 선생님, 결혼도 안 한 총각이 허리문제로 힘들어하는 마음 착한 민호, 선박평형수 시험 설비 작업 반장 영규, 인생은 한방이라며 외치고 다니는 딸 바보 서열, 부산에서 출퇴근하면서 애 둘을 키우는 슈퍼맘 제수씨 미경, 이제 남자친구가 필요한 까칠한 혜지, 항상 늦은 시간까지 일을 하는 형곤, 아직 많은 이야기를 하지 못한 우리팀 막내 주은이에게 고마움을 전하고 싶습니다. 예전에 같은 팀원이었지만 지금은 삼건세기에서 일을 열심히 하고 있는 민재형과 테크로스에서 일을하고 있는 옥명이에게도 고마움을 전합니다. 그리고 매일 연구소 순찰을 돌면서 연구 열심히 안하는 연구원들에게 사랑의 잔소리를 하시는 백승호 박사님, 지금의 아내를 만나게끔 열심히 도와주셨고 항상 많은 부분에서 필요한 조언을 해주시는 정승원 박사님, 77년 뱀띠 동갑내기 우정 친구 현호, 문호, 동욱이에게도 고마움을 전합니다.

마지막으로 사랑하는 나의 가족들에게 고마움을 전합니다. 나의 인생롤 모델이신 자상하신 우리 아버지, 자식들 뒷바라지를 위해 많은 고생

을 하신 우리 어머니, 믿음직한 형, 착한 동생에게 감사의 마음을 전합니다. 그리고 부족한 사위에게 항상 힘이 되어 주시는 장인어른과 장모님, 요즘 부쩍 착해진 처제, 귀염둥이 처남에게도 고마움을 전합니다. 감사해야할 가족들이 너무 많아서 일일이 열거할 수 없지만 제가 항상 고마워하고 사랑한다는 말을 전하고 싶습니다. 마지막으로 제가 열심히 살아가야할 이유인 제 아내와 아들에게 정말 사랑한다는 말을 전하고 싶습니다. 앞으로 더욱 발전하는 봉길이가 되도록 노력하겠습니다.