이학박사학위논문

# Ecological roles of dinoflagellates as symbionts, mixotrophic predators and prey for zooplankton in Korean coastal ecosystems and an effective cultivation of microalgae for heterotrophic dinoflagellates.

한국 연안생태계에서 와편모류의 공생생물,
혼합영양성 포식자 및 동물플랑크톤의 먹이로서의
생태학적 역할과 와편모류 포식자들을 위한
효과적인 미세조류 배양기술

2017 년 02 월

서울대학교 대학원

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# Abstract

## Ecological roles of dinoflagellates as symbionts, mixotrophic predators and prey for zooplankton in Korean coastal ecosystems and an effective cultivation of microalgae for heterotrophic dinoflagellates.

- Ph.D. Thesis, Feb. 2017 -


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Marine dinoflagellates are ubiquitous and observed at diverse habitats such as water column, macroalgae, and marine organisms. They are one of the major components of marine ecosystem and play diverse roles in even Korean. Despite the huge economic losses due to dinoflagellates red tides, multiple approach for the diveres rolse of dinoflagellates are rare. To understand ecological roles of dinoflagellates in Korean coastal ecosystems and their potential impact on marine environments and humans, multiple approach studies based on extensive investigations are needed. Thus, I investigated the three kinds of ecological roles of the dinoflagellates in Korean coastal waters such as symbiotic partners of corals,

mixotrophic predators of red tides species, and prey for zooplankton predators. In addition, as an application, I developed the newly designed effective photobioreactor to cultivate algal preys in high concentration for the massive production of heterotrophic dinoflagellates.

To investigate ecological roles of the symbiotic dinoflagellates *Symbiodinium* spp. in Korean waters, I took samples from the coral *Alveopora japonica* in the coastal waters of Jeju Isalnd on monthly basis from 2012 to 2013 and analyzed the composition of *Symbiodinium* ribotypes using Quantitative PCR. *Symbiodinium* clade F took over > 99% of total *Symbiodinium* abundance inside the *A. japonica* all the sample intervals, while *Symbiodinium* clade B, C, and E were detected. The temporal variation in the abundance and prevalence of the “background” *Symbiodinium* were not affected by temperature, salinity, major nutrients, and chlorophyll-a concentration. Therefore, only main symbiotic dinoflagellate species that occur in high concentration are important for the coral-dinoflagellate mutualisms.

To investigate whether the phototrophic dinoflagellate *Polykrikos hartmannii* is able to feed on any other algal prey or not, its feeding occurrence, identification of prey species, and feeding mechanism were explored. Furthermore, the growth and ingestion rates of *P. hartmannii* on the mixotrophic red tide dinoflagellate *Cochlodinium polykrikoides* that is the major cause species of red tides and economic losses in Korea were also measured. For the first time, *P. hartmannii* is reported to be a mixotrophic dinoflagellate in this study. When diverse algal species were provided as potential prey, *P. hartmannii* fed only on chain-forming toxic mixotrophic dinoflagellates *C. polykrikoides* and *Gymnodinium catenatum*. *P. hartmannii* ingested prey cells by engulfment after anchoring a prey cell using a nematocyst–taeniocyst complex. With increasing mean prey concentration, the ingestion rate of *P. hartmannii* on *C. polykrikoides* increased, to reach saturation at a prey concentration over 945 ng C $ml^{-1}$ (1350 cells $ml^{-1}$). The maximum ingestion

rate of *P. hartmannii* on *C. polykrikoides* was 1.9 ng C predator$^{-1}$ d$^{-1}$ (2.7 cells predator$^{-1}$ d$^{-1}$). The calculated grazing coefficients for *P. hartmannii* on co-occurring *C. polykrikoides* were up to 0.324 d$^{-1}$ (equivalent to 28% of the population of *C. polykrikoides* was removed by *P. hartmannii* populations in a day). The results of the present study showed that *P. hartmannii* might exert considerable and negative influence on the red tide population of *C. polykrikoides*.

To explore the grazing impacts of metazooplankton on red tide dinoflagellates in Korean waters, the spatial and temporal variations in the abundance of metazooplankton were investigated before, during, and after red tides dominated by phototrophic dinoflagellates in South Sea of Korea from May to November 2014. Grazing impacts by dominant metazooplankton taxa on populations of dominant red tide dinoflagellates were also estimated. Simultaneously, some of the important environmental parameters such as water temperature, salinity, chlorophyll-a were analyzed. The calanoid copepods did not exhibit grazing impact high enough to directly control the populations of the red tide dinoflagellates *Prorocentrum donghaiense* and *Cochlodinium polykrikoides* (up to 0.029 d$^{-1}$ and 0.018 d$^{-1}$, resprectively) in South Sea of Korea. However, their predation impacts on the population of the heterotrophic dinoflagellate *Gyrodinium* spp. (up to 0.047 d$^{-1}$), the important predator of *P. donghaiense*, were greater than the grazing impact of calanoids on *P. donghaiense* during the blooming stage of *P. donghaiense* red tides. In addition, the grazing impact of calanoids on diatom species like *Skeletonema costatum* and *Chaetoceros* spp., the inhibitors of *C. polykrikoides*, were much greater (up to 0.05 d$^{-1}$ and 0.032d$^{-1}$, respectively) than their predation impacts on *C. polykrikoides* at the very early stage of *C. polykrikoides* red tides. Therefore, the calanoid copepods may support the rapid population growth of the red tide dinoflagellate species by suppressing the population growths of the heterotrophic dinoflagellate predator and the diatom inhibitors.

Microalgae such as dinoflagellates and green algae have been envisioned to be valuable raw materials for biofuel. Their potential oil productivity is estimated to be at least four times greater than that by the land plants. Furthermore, some heterotrophic dinoflagellate species are known to produce large amount of unsaturated fatty acids (PUFA). I developed an effective photo bioreactor (PBR) for cultivating algal prey species for the economically reasonable production heterotrophic dinoflagellates. The newly designed PBRs include airlift bubble columns made of polycarbonate (PC) material. A set of PBR consisting of five parts can be assemble together that can be increased the total cultivation volume by assemble more parts. Furthermore, the disassembled PBR units are autoclavable at 121°C. The maximum cell concentrations and growth in the same PBRs may mainly depends on $CO_2$ concentration and pH. The maximum cell concentration and maximum growth rate of *D. tertiolecta* were 7.2 x $10^7$ cells $ml^{-1}$ and 1.7 $d^{-1}$ in the PBR when 5% $CO_2$ was continuously supplied. This maximum cell concentration is far greater than that obtained by any other devise for cultivating *D. tertiolecta* under indoor operation conditions. Therefore, the newly developed PBR should be very useful for the massive production of *D. tertiolecta* biomass, that can be fed into another cultivation system to obtain high biomass of biotechnologically useful heterotrophic dinoflagellate.

The results of this study may lead us to better understand ecological roles of dinoflagellates as symbiotic partners of corals, mixotrophic predators, prey for metazooplankton in marine ecosystems and the associated food web interactions.

**Keywords:** Marine Ecology, Symbiont, Dinoflagellate, Metazooplankton, Red tide, Mixotrophy

**Student Number:** 2011-23268

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# Chapter 1: Introduction

Marine planktonic dinoflagellates are the major component in marine planktonic communities (Smayda 1997; Jeong et al. 2013; Park et al. 2013). They are ubiquitous and observed in diverse habitats such as in the water column, on macroalgae and inside of marine organisms (Lessard 1984; Jeong et al. 2012; Kang et al. 2013). Among the numerous phytoplankton taxa, dinoflagellate is one of the most important primary producers in marine ecosystems with diatoms. They are mainly grazed for the prey by zooplankton predators. Furthermore, their trophic cascade between dinoflagellates-zooplankton is the principal trophic pathway in the transfer biomass and energy sources to higher levels of marine organisms such as fish (Poter et al. 1985; Stoecker and Capozzo 1990; Sanders and Wickham 1993; Godhe et al. 2008). Moreover, the spawning and egg production of fish depends on the food concentration and amount of supplied biomass such as copepods that are the major potential grazers of dinoflagellates (James 1987; Peterson et al. 1992). Thus, the primary production of dinoflagellates and their trophic links with potential predators may be a key determinant of the total amount of economic profit such as fish catches from the marine environment (Sanders and Wickham 1993; Turner 2004, 2006) (Fig. 1.1).

Dinoflagellates have three different types of major trophic modes (i.e. autotrophic, mixotrophic and heterotrophic). They often form red tides that are causing of large-scale mortalities of marine organisms along with the large economic losses of humans (Shumway 1990; Smayda 1990; Glibert et al. 2005; Anderson et al. 2012; Fu et al. 2012). However, some mixotrophic and heterotrophic dinoflagellates often co-occurred and abundant with HABs (harmful algal blooms) of the other dinoflagellate species. The co-occurring mixotrophic and heterotrophic dinoflagellate predators exert considerable grazing impact on the red tides organisms (Kim et al. 2004; Yoo et al. 2010; Jeong et al. 2011; NFRDI 2014).

In other words, sometimes their grazing impact negatively directly influence on the red tides organisms, or the grazing activities of the dinoflagellate predators indirectly positively influence on humans with reduce the economic losses by decrease the red tides.

Coral reefs create various economical values derive from humans coastal activities including fishing, tourism and coastal protection that has been estimated to be 375 billion US dollars per a year (Costanza et al. 1997). Most of the coral species have their reef-builders as symbiosis with mixotrophic dinoflagellates of the genus *Symbiodinium* is called zooxanthellae (Berkelmans and van Oppen 2006; Jeong et al. 2014). The *Symbiodinium* species live in the tissues of host coral species in extremely high concentrations and provide up to 90% of total nutritional requirements of the coral species (Muscatine and Porter 1977). Thus, the corals were considered to the product of mutualistic interactions between dinoflagellates and host species (Rosenberg et al. 2007).

The biotechnological development of microalgae biomass production has been growing rapidly in recent years (Chisti 2007; Hu et al. 2008; Lam and Lee 2012; Wijffels et al. 2013). Diverse microalgae are known to be able potentially to synthesize 30-fold more oil per hectare than terrestrial plants (US Depart. of Energy 2009). They are also currently widely used to synthesize valuable pigment, materials and bioresources (Fuentes-Grünewald et al. 2009). In comparison with the other materials of usable bioresources, marine microalgae have several more advantages such as high growth rate, tolerance to high $CO_2$ concentrations and high efficiency $CO_2$ mitigation (Chang and Yang 2003; Hsueh et al. 2007). Among the various valuable microalgae, dinoflagellates have comparatively much greater biovolume up to 88,000 $\mu m^3$, which are several orders magnitude higher than green algae (Tang 1995; Smayda 1997; Stolte and Graces 2006; Olenina et al. 2006). Furthermore, heterotrophic dinoflagellate are known to have unsaturated fatty acids such as EPA [eicosapentaenoic acid] and DHA [docosahexaenoic acid] which are

not present in the prey species (Klein Breteler et al. 1999; Broglio et al. 2003; Veloza et al. 2006). Moreover, humans even cannot synthesize DHA ourselves. In addition, DHA is known to be helpful for development of brain and eye in infants, accordingly, the market of DHA globally well formed (Kroes et al. 2003; Ward and Singh 2005).

Among the multiple effects of dinoflagellates on humans, some of impacts are difficult to directly feel ourselves because it may indirectly and weakly influence on humans. However, some of negative and positive influences of dinoflagellates on humans such as fish kill by large-scale red tides and development of useable bioresources closely happen around us now. Thus, all or any roles of the dinoflagellate in the marine ecosystems and humans industry may be much or less related with humans life. Thus in order to maximize the positive effects of the dinoflagellates and minimize the negative influences of the dinoflagellate on humans, the intensive studies on the dinoflagellates are essentially should be conducted. Therefore, the overall purpose of the present study is to extensively investigate about the diverse ecological roles of dinoflagellates as symbionts, mixotrophic predators and prey for zooplankton in Korean coastal ecosystems and developing an effective method of culturing microalgae for heterotrophic dinoflagellates.

In Chapter 2, I investigated about the speculation surrounds the importance of ecologically cryptic dinoflagellates *Symbiodinium* spp. that occur at low abundances in symbiotic corals. The extensive sampling, long-term monitoring, and experimental manipulation allow us to deduce the ecology and functional significance of these populations and whether they might contribute to the response of coral-dinoflagellate mutualisms to climate change. Quantitative PCR was used here to diagnose the prevalence, seasonal variation, and abundances of *Symbiodinium* spp. within and between colonies of the coral, *Alveopora japonica*.

In Chapter 3, to understand the ecological effects of the mixotrophic dinoflagellate predator *Polykrikos hartmannii* on the causative species of red tides in Korean coastal waters by grazing activities, the feeding ability, feeding occurrence, prey species and feeding mechanism were explored. Furthermore, the growth and ingestion rates of *P. hartmannii* on the mixotrophic dinoflagellate *Cochlodinium polykrikoides*, the optimal prey, as a function of prey concentration were measured.

In chapter 4, in order to investigate whether the standing stocks, distribution and grazing of the metazooplankton negatively directly effect on the population of red tides species, or positively indirectly influence on the dinoflagellate community by feeding on potential microzooplankton predators of red tides species, water samples were collected from 60 stations in the South Sea of Korea, from May to November 2014. Furthermore, the three-dimensional (3-D) distributions of physicochemical and biological parameters such as water temperature, salinity, chl-a and occurrence patterns of plankton community including phytoplankton-microzooplankton-metazooplankton were analyzed. By combining field data on the spatio-temporal distribution patterns of metazooplankton and the target prey species such as phototrophic dinoflagellates, heterotrophic dinoflagellates and ciliates with the ingestion rates of the predators on the prey obtained from the literature, i estimated the grazing coefficients attributable to the predators on co-occurring phytoplankton and their potential microzooplankton predator.

In Chapter 5, in order to cultivate microalgae in extremely high concentrations, and to bulk the total capacity of the target culture enough to commercially use the effective mass cultivation method must be developed. Furthermore, mass cultivation of heterotrophic dinoflagellates in high concentrations to utilize valuable bioresources such as DHA and EPA from them, preferentially the other algal prey species should be prepared to supply in much more high concentrations than the target heterotrophic species. Thus, I newly

designed the effective photo bioreactor (PBR) to mass cultivate the phototrophic microalgal prey to grow heterotrophic dinoflagellates in high concentrations such as *Oxyrrhis marina* and *Gyrodinium dominans*. They are known to be able to synthesize valuable materials when it feed on *Dunaliella tertiolecta* (Chu et al. 2008a, 2008b). Furthermore, in this study, the overall structure of newly designed PBRs and the cultivation systems, cultivation conditions and maximum grown concentrations were investigated and compared with the prospective studies. In addition, I investigated the different responses of intracellular pigment and extracellular glycerol production according to the supplied CO2 concentrations of *D. tertiolecta* when the species were cultivated in the newly developed PBR in extremely high concentration.

The results of this thesis will provide a basis to intensively understand the diverse ecological roles of dinoflagellates as symbionts, mixotrophic predators and prey for zooplankton in Korean coastal ecosystems, and the development of effective microalgal mass cultivation method.

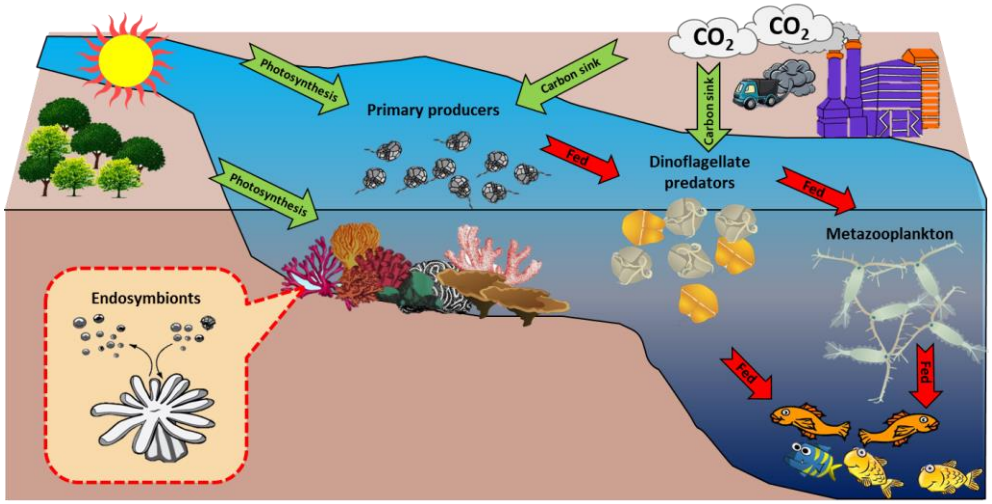



Fig. 1.1 Diagram of the diverse roles of dinoflagellates and their dynamic interactions in the marine ecosystems

# Chapter 2. Temporal variations in the composition of the symbiotic dinoflagellate *Symbiodinium* inside the scleractinian coral *Alveopora japonica* from the coastal water of Jeju island, Korea.

## 2.1 Introduction

In a time of rapid climate change, the persistence of reef corals may depend in large part on the physiological resiliency of their mutualistic associations with endosymbiotic dinoflagellates (genus *Symbiodinium*). Colonies associated with stress–tolerant symbionts may withstand temperatures 1-2 ˚C warmer and thus survive episodes of extreme warming that would normally eliminate corals with more ‘thermally sensitive’ species of symbiont (Glynn et al. 200; Rowan 2004; Berkelmans and van Oppen 2006,; Sampayo et al. 2008; LaJeunesse et al. 2009b). As surface waters warm or undergo wider seasonal oscillations in temperature, the increase in better adapted host-symbiont combinations is one possible mechanism that could maintain viable reef coral communities. However, the capacity for change in these symbioses may be regulated by the fairly high degree of specificity exhibited by host and symbiont.

Patterns of specific associations often reflect long-standing environmental conditions under which colonies have grown (Hennige et al. 2010; LaJeunesse et al. 2010a; Baker et al. 2013) especially among animals whose larvae must acquire symbionts from environmental sources (horizontal transmission). For many symbiotic cnidarians, their specificity is influenced in part by irradiance and temperature (Finney et al. 2010). Thus, many species of host show some flexibility and associate with more than one species of *Symbiodinium* over their entire ecological and geographical distributions (e.g. Ulstrup and van Oppen 2003;

LaJeunesse et al. 2004; 2010a; Baker and Romanski 2007; Bongaerts et al. 2010; Finney et al 2010; Tonk et al. 2014). Important questions remain as to whether community-level changes can occur rapidly enough to keep pace with current and projected rates of warming.

A rapid change in host–symbiont combinations (weeks to months) would hypothetically involve the proliferation of low density resident 'background' *Symbiodinium* species that are host-compatible and physiologically adapted to the changed environment (Berkelmans and van Oppen 2006; Rowan et al. 1997;Jones et al. 2008; LaJeunesse et al. 2009b; Kemp et al. 2014; but see McGinley et al. 2012). This dynamic replacement, often called 'symbiont shuffling,' is an alternative to natural selection (differential mortality), and would minimize the impact on coral populations subjected to near-term environmental change (LaJeunesse et al. 2010a). However, stability is most frequently documented among naturally growing colonies that are monitored over extended time (Goulet and Coffroth 2003, Thornhill et al. 2006ab, 2009; Suwa et al. 2008; Pettay et al. 2011, Baums et al. 2014). By comparison, experimental manipulations, involving acute changes in irradiance or temperature, can induce shifts in the dominant resident population (Berkelmans and van Oppen 2006, Toller et al. 2001, Grottoli et al. 2014). In nature, severe thermal stress may also increase the prevalence and abundance of stress–tolerant, or opportunistic (atypical), species in corals before, during, and after a duration of environmental stress (Toller et al. 2001, Thornhill et al. 2006a, Jones et al. 2008, LaJeunesse et al. 2009b), yet it remains unclear as to the long term impact of these changes since these associations are typically unstable (Thornhill et al. 2006b, LaJeunesse et al. 2009b).

Ecologically cryptic, or 'background,' *Symbiodinium* spp. are occasionally detected in coral colonies at low background densities via quantitative PCR (qPCR) (e.g. Mieog et al. 2007, Correa et al. 2009, LaJeunesse et al. 2009b, Silverstein et al. 2012), and by next generation DNA sequencing (Quigley et al. 2014, Thomas et al.

2014; but see Arif et al. 2014). These observations have fueled unsubstantiated speculation about the future ecological importance of these entities by raising the plausibility for large-scale rapid changes in host-symbiont combinations through 'shuffling' (Mieog et al. 2007). These presumptions are confounded because: 1) despite some flexibility; most hosts appear limited to a small number of *Symbiodinium* spp. in forming functionally integrated mutualisms; and 2) there is a growing awareness that not all *Symbiodinium* are mutualistic. Indeed most evolutionarily divergent *Symbiodinium* 'clades' contain distinct species that exhibit major differences in ecology.

The process of isolating and culturing *Symbiodinium* cells from animal tissues or from the environment (water column and sediment) typically recovers genetic entities (i.e. species) that are not representative of the mutualistic species that occur in nearby hosts at high densities (Santos et al. 2001, LaJeunesse 2002, Jeong et al. 2014, LaJeunesse et al. 2015). Many of these atypical species appear to exist at low densities and are often undetected by conventional genetic techniques. Furthermore, after isolation into culture, they subsequently fail to form stable mutualisms when exposed to aposymbiotic hosts (LaJeunesse 2001, Jeong et al. 2014). This raises questions as to whether these *Symbiodinium* spp. are relevant to a functionally integrated and stable mutualism.

We sought to gauge the ecological importance of 'background' *Symbiodinium* in the temperate symbiotic coral, *Alveopora japonica* (Eguchi), thriving in the coastal waters off Jeju Island, Republic of Korea (Figs 1A, B, Chen et al. 2013). Colonies of this scleractinian are common to coastal waters benthic communities in China, Korea, Taiwan, and Japan (Fig. 1C, Song 1991;, Sugihara et al. 2014). While extensive sampling of this animal over a range of habitats and spatial scales by independent investigators indicates that *A. japonica* exhibits high specificity for an uncharacterized lineage of Clade F (Rodriguez-Lanetty et al. 2003; Chang et al. 2011;de Palmas 2015), a second species, *S. voratum* (= Clade E), was recently

isolated into culture from the tissues of *A. japonica* (Jeong et al. 2014), suggesting that other *Symbiodinium* spp. occur within this animal. Thus, we explored whether additional *Symbiodinium* spp. co-occur with the Clade F *Symbiodinium* sp. in the tissues of *A. japonica*, and if so, to what extent did their prevalence and abundance vary within and between colonies in populations collected each month over a 15-month duration. Individual *A. japonica* polyps were analyzed using quantitative (q) PCR targeting ribosomal DNA to diagnose the presence and absolute abundances of distantly related groups of *Symbiodinium* (i.e. 'clades'). Additional DNA sequencing was employed to identify whether detection of a 'clade' involved a single or multiple species. These findings were combined with previously published work to evaluate the importance of environmentally cryptic, or 'background,' diversity. We then reason why the concept of specificity should encompass the stability and functional integration of host and symbiont partners.

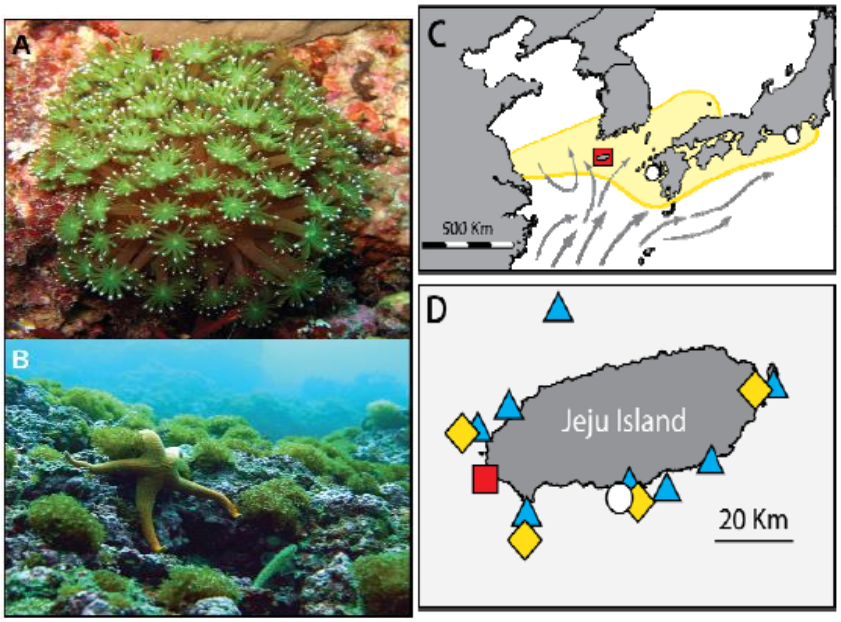



Fig. 2.1 The habitat and geographical distributions of *Alveopora japonica*. (A) *A. japonica* forms small hemispherical colonies that measure 2-10 cm in diameter with fleshy polyps that are extended during the day. (B) This species can occur in large numbers in protected turbid environments at depths of 5-25 (Sugihara et al. 2014). (C) The geographical range of *A. japonica* occurs in temperate latitudes in the northwestern Pacific (light yellow shading). Site of sampling is indicated by the red square on the inset map of Jeju Island. Locations in previously published papers where *A. japonica* has been collected for analyses of *Symbiodinium* identity are indicated by yellow diamonds (Rodriguez-Lanetty 2003), white circles (Chang et al. 2011), and blue triangles (de Palmas et al. 2105). Grey arrows are predominant surface currents originating from lower latitudes.

## 2.2 Materials and methods

### 2.2.1 Environmental measurements, colony collection, transport, and acclimation

We collected 30 colonies of *A. japonica* (4−7 cm in diameter, Fig. 1A) from Sindo (33˚16’37.65” N, 126˚10’5.51” E), Jeju Island, The Republic of Korea at depths of 10−13 meters every month from July 2012 to September 2013 using SCUBA (Fig. 1D). Thirty random colonies were collected from the same *A. japonica* population in a concentric arc around a fixed center point within an area of 40 m2. Colonies were transported to the laboratory at Seoul National University and then individually acclimated in 1-L glass beakers with filtered seawater (FSW) at a temperature range of 18–25℃ under a photon flux density of 50 μmol quanta m-2 s-1 (14L/10D), simulating the corresponding temperature, salinity, and irradiance at the sampling site.

Environmental water temperatures and salinities were measured at the site of sampling using a YSI Professional Plus instrument (YSI Inc., Yellow Springs, OH, USA). Sea surface temperature and chlorophyll a (a proxy for phytoplankton abundance) data were acquired from the Giovanni online data system http://gdata1.sci.gsfc.nasa.gov/daac-bin/G3/gui.cgi?instance_id=mairs_monthly_hres, maintained by the NASA Goddard Earth Sciences (GES) Data and Information Services Center (DISC). MODIS/Aqua monthly Chl a and SST values averaged over a 4 km grid were acquired from reflectance measurements taken in the vicinity of the collection site at Sindo, Jeju Island (an area between 33.109–33.309 N and 125.982–126.144 E). These values were averaged for each month of the study period and graphed. Each month, water samples were obtained for analyzing nutrients and chlorophyll-a concentrations as described by (APHA 1995).

### 2.2.2 Polyp excision, maceration, and DNA extraction

Three polyps (1–1.5 cm in length) were removed from each colony (n=90 polyps per month) and rinsed thoroughly with FSW to minimize contamination by exogenous *Symbiodinium* cells. Each polyp was gently blotted using Kimwipes (Kimberly-Clark Co., US), placed in a 1.5-mL tube, and wet weight determined. To each tube, 1 ml of FSW was then added and the polyp homogenized using a micropestle, and the contents vortexed. To confirm the total cell density of *Symbiodinium* in *A. japonica*, one hundred microliters were transferred from one sample of each colony to 10-ml vial containing 4.9ml FSW, fixed with 5% Lugol's solution. Cells were counted on 1-ml Sedgwick-Rafter cambers (SRCs) and light microscope. The remaining 900- L aliquot was centrifuged at 13,000 rpm for 1 min at room temperature. The supernatant was discarded and the pellet was resuspended with 200 μL of phosphate-buffered saline (PBS) (Boineer Corp., Korea). The mixture was immediately subjected to total DNA extraction using the AccuPrep Genomic DNA extraction kit (Bioneer Corp., Korea) for qPCR and other genetic analyses.

### 2.2.3 PCR amplification, sequencing, and phylogenetic analyses

Amplifications of ITS1-5.8S-ITS2 rDNA and the LSU region D1–D3 was performed using the primer set and conditions developed by (Litaker et al. 2003, Kang et al. 2011). The process of rDNA sequencing and alignment was conducted using the methods of (Kang et al. 2011). Sequences of other *Symbiodinium* spp. were retrieved from GenBank and aligned. Maximum Parsimony was assessed using the software PAUP* (Swofford 2002). Bayesian analyses were conducted using MrBayes v.3.1 (Ronquist and Huelsenbeck 2003) using a default GTR + G + I model. For each alignment, 4 independent Markov Chain Monte Carlo (MCMC) analyses were performed. MP bootstrap values were determined using 1,000 replicates.

### 2.2.4 Preparation of specific dual-labeled BHQplus probes and primer sets for qPCR

Ribosomal DNA sequences (ITS1, 5.8S, ITS2, LSU) were used to construct clade-specific primers and probes (Table 1) to discriminate a particular clade (lineage) of interest from those of other *Symbiodinium* clades (Table 2). These were subsequently compared to published sequences using BLAST homology searches on GenBank. The primers and probes were dual-labeled with the fluorescent dyes FAM and BHQplus (Biosearch Technologies Inc., Novato, CA, USA) at the 5′ and 3′ ends.

The specificity of each of the 4 *Symbiodinium* clade-specific primers and probe sets (for clades B, C, E, and F) were also tested using concentrated rDNA extracted from 100,000 cells of each of the 19 *Symbiodinium* strains representing approximately 10 species (Table 2). The DNA extraction method was similar to the extractions conducted on host tissues as previously described.

Table 2.1. Sequences of *Symbiodinium* clade-specific primers and probes used for qPCR analysis in this study.

| Type | rDNA region | Primer / Probe | Sequence (5'-3') |
|---|---|---|---|
| B | ITS1-5.8S | Forward | AGCGCAAGCTTTCTGGAAAG |
| | | Reverse | CACGGAGTTCTGCAATTCACA |
| | | Probe | FAM-TGGTCCAAAACTACAACTT-BHQPlus |
| C | ITS1 | Forward | TGGTGCGAGTGTCGCTCT |
| | | Reverse | TGTGGAAGTTGGAACAATGCC |
| | | Probe | FAM-TTTAGTGACAACTGTCTGG-BHQPlus |
| E | ITS2 | Forward | CGCTGCTGCATCAGAATTTGC |
| | | Reverse | GGCAGGCTGGCATGATTG |
| | | Probe | FAM-CGGCGCGCTGAAC-BHQPlus |
| F | LSU | Forward | GTAATGGCGAATGAACAGGG |
| | | Reverse | GAAACGCAGGATTCTCACCC |
| | | Probe | FAM-GTAAGACTCTTGGAAT-BHQPlus |

Table 2.2. *Symbiodinium* cultured strains of species used to develop and test the specificity of the primers and probes for *Symbiodinium* in Clades B, C, E, and F.

| Species | Clade | Strain No. | Location of origin |
|---|---|---|---|
| *Symbiodinium* sp. | A | CCMP 828 | Florida |
| *Symbiodinium tridacnidorum* | | CCMP 832 | Great Barrier Reef |
| *Symbiodinium* sp. type A4 | | CCMP 2456 | Bermuda |
| *Symbiodinium microadriaticum* | | CCMP 2458 | Gulf of Aqaba |
| *Symbiodinium pilosum* | | CCMP 2461 | Jamaica |
| *Symbiodinium microadriaticum* | | CCMP 2464 | Florida |
| *Symbiodinium microadriaticum* | | CCMP 2467 | Gulf of Aqaba |
| *Symbiodinium necroappetens* | | CCMP 2469 | Jamaica |
| *Symbiodinium minutum* | B | CCMP 830 | Bermuda |
| *Symbiodinium* sp. | | CCMP 1633 | Hawaii |
| *Symbiodinium psygmophilum* | | CCMP 2459 | Bermuda |
| *Symbiodinium* sp. type B3 | | CCMP 2462 | Bahamas |
| *Symbiodinium goreauii* | C | CCMP 2466 | Jamaica |
| *Symbiodinium trenchii* | D | CCMP 2556 | Florida |
| *Symbiodinium voratum* | E | CCMP 421 | New Zealand |
| *Symbiodinium voratum* | | SVIC3 | Jeju island |
| *Symbiodinium voratum* | | SVFL1 | Jeju Island |
| *Symbiodinium* sp. type F2 | F | CCMP 2455 | Jamaica |
| *Symbiodinium kawagutii* | | CCMP 2468 | Hawaii |

### 2.2.5 Real-time PCR assay conditions and standard curve

qPCR reactions were performed using 1- L template DNA combined with 0.2 M forward and reverse primers, 0.15 M probe (final concentration), 5 L HiFast Probe Hi-Rox (Genepole, Gwangmyung, Korea), and PCR-grade water (total final volume = 10 L). The thermal cycling conditions consisted of 3 min at 95℃, followed by 40 cycles of 10 s at 95℃, 20 s at 60℃, and 20 s at 72℃. The fluorescence of each reaction tube was quantified in each cycle, and the threshold for a positive reaction was automatically determined using the qPCR instrument (Rotor Gene 6000, Qiagen GmBH, Germany).

Standard curves for TaqMan-based qPCR assays were obtained using the DNA extracted from a known number of cells from cultured strains CCMP 2459 (*Symbiodinium* psygmophilum representing Clade B), CCMP 2466 (*S. goreaui* representing Clade C), SNU IC 3 (*S. voratum* representing Clade E), CCMP 2468 (*S. kawagutii* representing Clade F) to construct standard curves representative of different clades. The cell density of each *Symbiodinium* clade in each *A. japonica* polyp was calculated by comparing Ct values and our qPCR standard curves determined for each clade.

### 2.2.6 Statistical analyses

The correlation coefficients between the each ‘background’ *Symbiodinium* were calculated using Pearson’s correlations (Conover 1980, Zar 1999).

## 2.3 Results

The average wet weight of each polyp was 0.006 grams (±0.003 g). The mean number of *Symbiodinium* cells per polyp was approximated at 8 million (± 3,359,398 SD) based on direct cell counts. (0.07 $x10^9$ to 7.9 x $10^9$ cells/g Ww). The *Symbiodinium* sp. in Clade F occurred as the dominant symbiont in all 1260 polyps from 420 colonies of *Alveopora japonica* examined during the course of this study (Fig. 2A). This species on average accounted for ~99.9% of the total resident *Symbiodinium* population based on qPCR standard curves generated using direct cell counts of cultured isolates. Members of Clades B, C, and E were detected at background densities several orders of magnitude lower (Figs 2A, B). *Symbiodinium* 'Clade B' was detected at every sampling interval, but not always in each polyp nor colony examined (Fig. 2B), and reached densities approximating 0.013% of the total population. *Symbiodinium voratum* (equivalent here to 'Clade E') was most prevalent during Fall and Winter months reaching densities of about 0.023%, but was rarely detected and often absent from colonies during Spring collections (Fig. 2B). 'Clade C' was the least prevalent of the 'background' *Symbiodinium* and was never detected at densities greater than 0.0003%, and only from a small proportion of colonies and polyps, and usually at extremely low densities (Fig. 2B).

The presence of one 'background' *Symbiodinium* did not appear to be significantly affected by the presence and abundance of other *Symbiodinium* ($p>0.1$, linear regression ANOVA for each pair of Clade B, C and E).

During the study period, the water temperature measured at Sindo, Jeju Island, ranged between 10.1 and 27.5℃ (Fig. 2C) and the salinity oscillated between 30.3–34.7‰ (data not graphed). Chlorophyll a contents in surface waters at the study sight ranged between 0.22 to 2.34 mg/m3 and were consistent with values measured from satellite images, which ranged between 1.0 to 0.3 mg/m3 (Fig. 2C). High

primary productivity at the collection site occurred in the late Summer and Spring (Fig. 2C). Nutrient concentrations for ammonium (NH4), nitrate (N03), phosphate (PO4), and silica dioxide (SiO2) were graphed and compared against Chlorophyll a concentration, the and the prevalence of ‘Clade B’ and ‘Clade E’ (Fig. 3).

Sequence analyses of ITS1-5.8S-ITS2 and large subunit (LSU) rDNA resolve the specific identities for Clades B, E, and F. The phylogenetic analyses of the LSU sequences indicate that the Clade F entity is a *Symbiodinium* that is distinct among the known breadth of Clade F diversity described from foraminifera around the world (Fig. 4) and is referred to from this point forward as ‘FAjap’. The ITS2 sequence for the Clade B *Symbiodinium* was matched with that of Symbiodinium psygmophilum (= type B2) a cold-water adapted Clade B species known from the north Atlantic and Mediterranean Sea, but the ITS1 differs by a nucleotide substitution (data not shown). The ITS2 for the Clade E matched that of *S. voratum* from the western Pacific (Jeong et al. 2014).

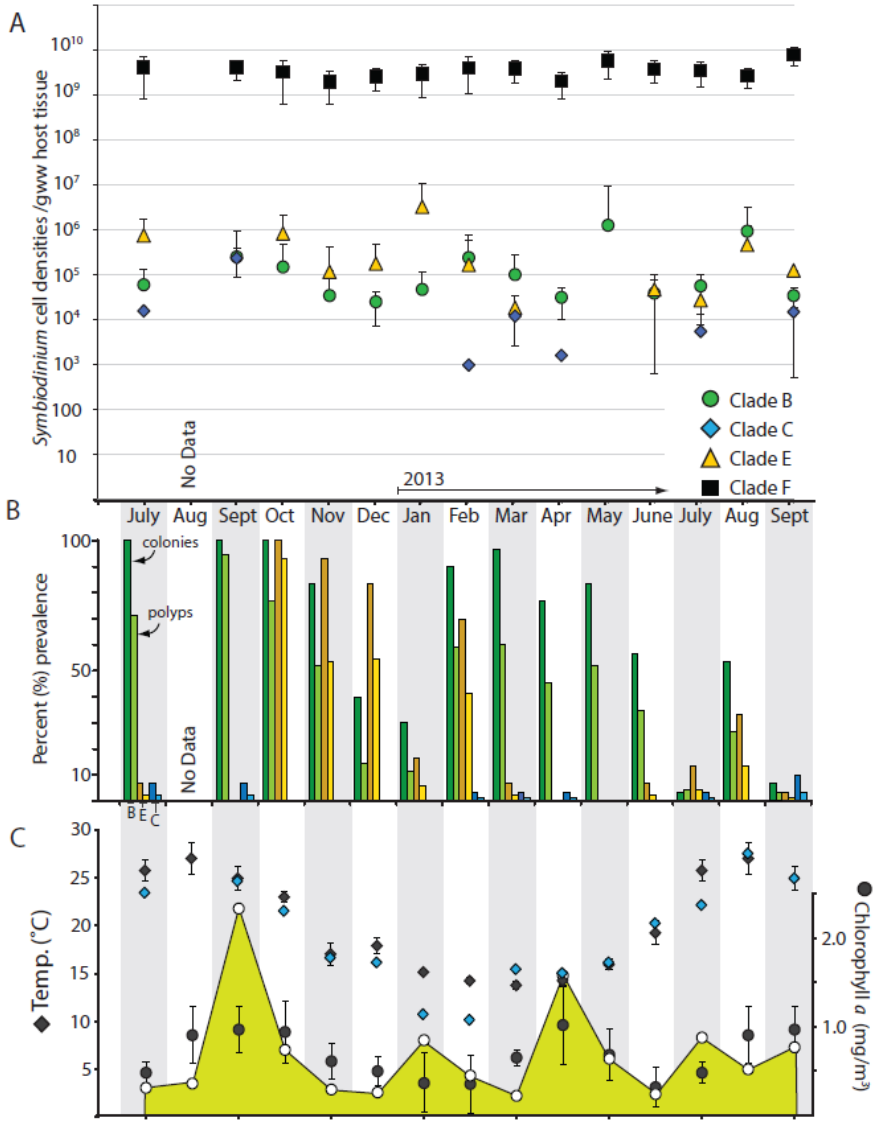



Fig. 2.2 Temporal stability, variation and mean abundances of *Symbiodinium* spp. in a population of *A. japonica* sampled every month for 15 months between 2012 and 2013. (A) Quantitative PCR analysis of the abundances of Clades F, E, B and C in colonies (n=30) and polyps (n=90, 3 per colony) examined each month. Mean cell densities for each *Symbiodinium* clade are based only on sample number in which the clade was detected each month. *Symbiodinium* FAjap was dominant in all 1240

samples. (B) The monthly presence/absences (prevalence) of low abundance background populations of Clades B, C, and E detected by qPCR determined for each colony and polyp. (C) The direct measurements of temperature and chlorophyll a content recorded for each month in surface waters at the monitored site (coloured symbols) and 5-year means (July 2008 to October 2013) based on remote sensing data on waters adjacent to the sampling location. Error bars were drawn to indicate standard deviations (±SD).

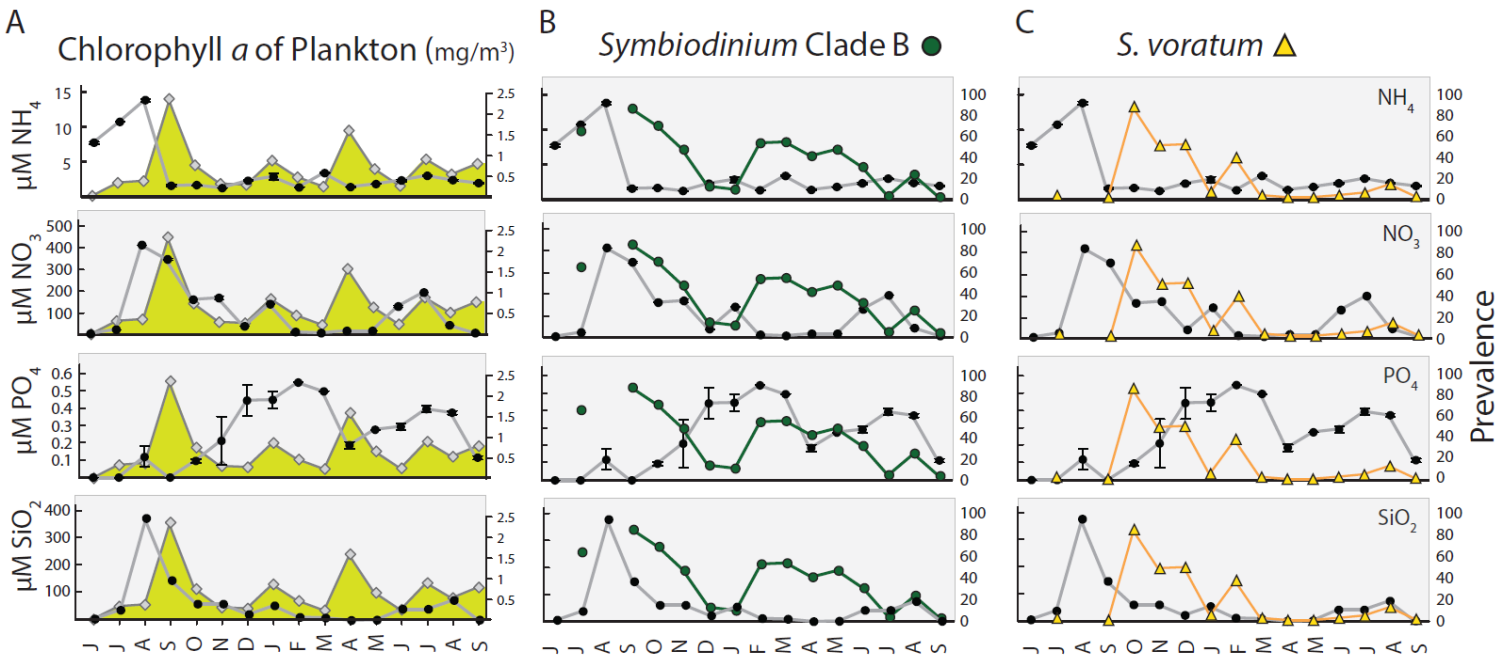



Fig. 2.3 Concentration of ammonium (NH4), nitrate (N03), phosphate (PO4), and silica dioxide (SiO2) in surface waters at Jeju Island, Korea every month over the 15-month survey period. These values are graphed against (A) measures of Chlorophyll a content, as well as the prevalence (percent) of (B) *Symbiodinium* sp. Clade B and (C) S. voratum (Clade E) detected in the polyps (n = 90) of Alveopora japonica using qPCR.

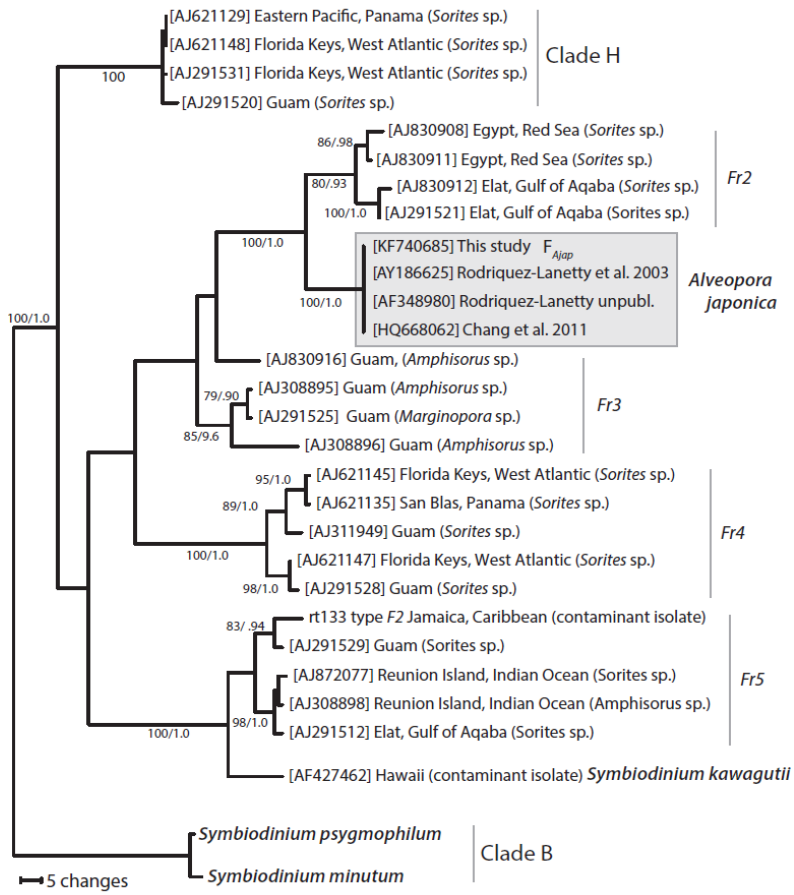



Fig. 2.4 Ribosomal large sub-unit (LSU) DNA phylogeny identifies the phylogenetic position and uniqueness of the *Symbiodinium* FAjap mutualistic with Alveopora japonica. Most Clade F species associate with soritid foraminifera in tropical regions around the world (Pochon et al. 2001, 2007, Garcia-Cuestos et al. 2005), whereas others, with enigmatic ecologies, emerge periodically as contaminants when culturing cells from cnidarian host tissues, such as *S. kawagutii* from Montipora capitata. Numbers under each branch-length refer to bootstrap values $\geq$ 75%, based on 1000 iterations (left), and posterior probabilities $\geq$ 0.9 (right). LSU sequences of representatives from Clades B and H were used as out-groups.

## 2.4 Discussion

### 2.4.1 Minimized roles of symbiotic *Symbiodinium* spp.

This work is original in that it investigated the dynamics of low abundance populations of *Symbiodinium* at monthly intervals. Analysis of environmental samples have identified types of *Symbiodinium* that exist at low abundances in various marine habitats, but what of their ecological function and importance to coral-dinoflagellate mutualisms? The observations presented and discussed below help to understand the ecological relevance of these *Symbiodinium* and should provide the framework for future investigations and guide the reinterpretation of past findings.

Distinct *Symbiodinium* occurred among colonies of *A. japonica* at low abundances (>0.05%), a finding that is entirely consistent with studies conducted on tropical coral species (Mieog et al. 2007, Silverstein et al. 2012). Shifts in the abundances and prevalence of these entities occurred throughout the 15-month sampling period (Fig. 2A). However, while our qPCR targeted rapidly evolving rDNA, the technique resolves only distantly related groups (i.e. clades, Table 1). Thus the power of interpreting ecological function (niche) based on these qPCR data alone was limited. We were then able to resolve species identity with the use of additional genetic markers and methods. Just as our detection of Clade F corresponds explicitly to FAjap (Fig. 5), we found that our detection of Clade E and Clade B corresponded to the presence of a particular species from each group.

The detection of Clade E with qPCR was diagnostic of a single species, *Symbiodinium voratum* (Jeong et al. 2014). Unlike other ‘background’ species, its prevalence (but not abundance) sharply increased a few months after a large pulse of inorganic nitrogen (NH4, NO3) and silica (SiO2) into the environment (Fig. 3). *Symbiodinium voratum* appears to be primarily free-living and less mutualistic

because it has yet to be detected in corals from this region at densities similar to mutualistic *Symbiodinium*, and it does not successfully establish symbioses during controlled experiments with aposymbiotic hosts (Jeong et al. 2014). It is common in the temperate northwest where it is cultivable from water samples, the surfaces of macro-algae, and *A. japonica*, and may actually undergo occasional planktonic blooms and gain additional nutrients by feeding on bacteria and a wide range of micro-algal prey species (reviewed in Jeong et al. 2012). Its osculation in prevalence transitory presence in our samples could merely reflect its relative abundance (and that of its prey) in the environment at the time of sampling. Thus, the presence/absence of S. voratum in these animals may relate to external biotic or abiotic factors that govern the abundance of cells in the external environment (Figs. 2A, 3).

Perhaps most unexpected was the discovery of '*Symbiodinium* Clade B' found in background over the course of the study and, present in 100 percent of colonies for several months during the survey (Fig. 2B). There are few Clade B *Symbiodinium* spp. that occur in the Indo-Pacific and they are only known from specific host species collected off the coasts of Australia, usually at temperate latitudes (Rodriguez-Lanetty et al. 2001, Goulet et al. 2008, Silverstein et al. 2011), or wherever the anemone Exaiptasia (=Aiptasia) pulculata has been introduced (Thornhill et al. 2013). Our sequencing of ITS2 generated a sequence similar to that of S. psygmophilum (= type B2), a cold water adapted species common to animals in the temperate North Atlantic Ocean and Mediterranean Sea (LaJeunesse et al. 2012). The Pacific population of this lineage may also exist predominantly at high latitudes, but has yet to be detected in Cnidaria from the western Pacific at abundances that would support the nutritional requirements of a host (Muscatine 1990). This finding exemplifies how sensitive detection measures can discover rare *Symbiodinium* whose ecological niche is unknown, but appears to differ from mutualistic species (LaJeunesse et al. 2015).

Finally the identity of the *Symbiodinium* Clade C, which was detected rarely and always at the lowest abundances, remains enigmatic (Fig. 2B). No DNA sequence data were obtained to provide insight as to whether we were observing a unique entity or one of several Clade C *Symbiodinium* spp. that dominate other symbiotic cnidarians co-occuring in the same habitats as *A. japonica* on Jeju Island (Chang et al. 2001, de Palmas et al. 2105). Several phylogenetically distinct lineages in Clade C (i.e. species) exhibit high host specificity at these latitudes (Lien et al. 2012, de Palmas et al. 2015). There is no evidence for a free-living or non-mutualistic species of *Symbiodinium* in Clade C, and therefore the low prevalence and extremely low abundances of Clade C in *A. japonica* may reflect temporary fluctuations in environmental concentrations of Clade C spp., which may ultimately relate to the normal discharge of excess cells from other nearby symbiotic Cnidaria (Stimson and Kinzie 1991, Hoegh-Guldberg et al. 1987, Jones and Yellowlees 1997, Baghdasarian and Muscatine 2000).

Finally, stress experiments designed to induce an increase in 'background' *Symbiodinium* to densities similar to FAjap failed when colonies of *A. japonica* died as temperatures were slowly increased by only a few degrees above the mean summer high (~27 ˚C) at Jeju Island (M. J. Lee unpubl. data). Therefore this particular coral, like many others, may not be open to hosting a second species of *Symbiodinium*, even under conditions of ecological opportunity created by stress (LaJeunesse et al. 2009).

### 2.4.2 Role of major symbiotic *Symbiodinium* sp.

The dominance of *Symbiodinium* FAjap in *A. japonica* throughout the duration of the study [>99% of the resident symbiont population] is consistent with previous findings of stability in the identity of the dominant *Symbiodinium* in colonies monitored for a year or more (Goulet and Coffroth 2003, Goulet 2006, Thornhill et al. 2006ab, Suwa et al. 2008, Thornhill et al. 2009, Pettay et al. 2011,

Baums et al. 2014). Seasonal oscillations in irradiance and temperature can affect symbiont cell densities (Fitt 2000), but appear to have little effect on the species composition of a symbiont population within a coral (Thornhill et al. 2006ab). Even so, many reef building corals exhibit flexibility with different *Symbiodinium* spp. over environmental gradients related to changes in water depth, reef habitat, geographical location and historical factors. However, despite the large diversity of *Symbiodinium* spp. present in many of these environments, each coral species ultimately depends on a narrow subset of symbionts for their survival and growth (Thornhill et al. 2014). Exceptions to this generality involve the co-existence of only a few symbiont species within individual colonies (Rowan et al. 1997, Ulstrup and van Oppen 2003, Chen et al. 2005). Collectively, these observations underscore the significance of stability and specificity in coral-dinoflagellate symbioses.

Stability is important for many reasons in the maintenance of a mutualism (Douglas 2008). Processes that govern these symbioses have probably evolved to limit intra and inter- specific competition, thus minimizing the negative effects of cheating and competition (Douglas 2008). Using conventional genetic approaches, Symbiodinium FAjap has been the only *Symbiodinium* sp. found in *A. japonica* at high abundances throughout the coral's distribution (Figs 1C, D). Its high abundance, relative to 'background' *Symbiodinium* (measured by orders of magnitude, Fig. 2A), means that it provides the photosynthate necessary for sustaining host metabolism. The in hospite abundance, temporal stability, and ecological prevalence of this particular Symbiodinium are attributes critical to the physiological performance of a sustained mutualism, and should be considered when defining host-symbiont specificity (Schoenberg and Trench 1980).

### 2.4.3 Functional and ecological significance of background *Symbiodinium*

Microbes of many different kinds associate in various ways with reef corals (Knowlton and Rohwer 2003, Amend et al. 2012, Kirk et al. 2013). Moreover,

corals are heterotrophic and consistently consume a variety of small particles including eukaryotic microalgae (Muscatine 1973, Houlbreque and Ferrier-Pagès 2009). The combined microbial diversity of commensals and prey particles is thus high. The use of qPCR primers specific for certain groups of microalgae can sporadically detect the presence of diatoms, haptophytes, cryptophytes, non-*Symbiodinium* dinoflagellates, etc…, depending on their environmental availablity (Leal et al. 2013). Yet the detection of these microalgae would not be interpreted as entities viable to an animal's symbiosis (nor would their presence be used to argue against the existence of host-symbiont specificity). The realization that *Symbiodinium* belong to different functional groups, some of which are not mutualistic, combined with the recognition that corals are continually cycling live particles into (and out of) of their gastrovascular systems, while possessing a mucosal layer that contains a rich diversity of microbes (Knowlton and Rohwer 2003), questions the validity of extrapolating a significance to 'background' *Symbiodinium* without additional confirmatory evidence.

The existence of a particular *Symbiodinium* sp. at trace densities is potentially important if it has the capability to achieve high densities in a host under circumstances influenced by biotic (i.e. host-compatible) and abiotic (e.g. high light/low light-adapted) factors. One notable example involved increases in the abundance of *S. trenchii* before, during and after the 2005 mass bleaching event in the eastern Caribbean (LaJeunesse 2009). While found only in low abundances in many reef coral taxa several months before the height of this event (Smith 2008), it was subsequently found at high densities in many unbleached colonies of several important reef building species, which appeared unaffected by the severe stress. As temperatures increased, populations of *S. trenchii* proliferated from low abundance background levels to dominate many colonies preventing tissues from whitening. The ecological nature of this *Symbiodinium* is thus potentially significant to the symbiosis ecology of corals when exposed to increasingly stressful environmental

conditions. However, the physiological and ecological importance of *S. trenchii* (Clade D) occurring in background may be a rare exception.

We now recognize that there are *Symbiodinium* spp. in close association with animals whose ecological niche differs from habitually mutualistic species (Jeong et al. 2014, LaJeunesse et al. 2015). They occur in many different habitats and environments including the sediment, water column, and on the surfaces of macro-algae and other organisms (Hirose et al. 2008, Porto et al. 2008, Littman et al. 2008, Takabayashi et al. 2012, Sweet 2013, Kohli 2014). These *Symbiodinium* spp. (e.g. S. voratum, S. necroappetens, S. pilosum) occur in tropical, sub-tropical, and temperate environments where they exhibit diverse ecological roles and may contribute in various ways to marine food webs (Jeong et al. 2012). Therefore, while it is one thing to detect populations at low abundances in hospite, or in the environment, inferring significance to their functionality for dinoflagellate-animal symbioses is another matter entirely.

*Symbiodinium* Clade A contains species that illustrate this range of ecological diversity (LaJeunesse et al. 2009a). In addition to the mutualistic species S. microadriaticum and S. tridacnidorum (Lee et al. 2015), S. pilosum is a species cultured from cnidarians, but has yet to be identified in field collected host tissues. Given its inability to infect aposymbiotic experimental animals, it probably exists as a free-living non-mutualistic species similar to *S. voratum* (Clade E) and *S. kawagutii* (Clade F). Another species in Clade A, S. necroappetens, attains densities that are detected as the dominant *Symbiodinium* in coral tissues that are diseased or severely bleached (LaJeunesse et al. 2015). It has been cultured from healthy symbiotic cnidarians in separate Caribbean locations, suggesting that cells of this species normally exist at low abundances on an animal's surface, in the gastrovascular system, or are possibly intracellular. The detection of 'Clade A' (in a sample) through the application of qPCR could thus be interpreted several ways and involve one of several species with distinct ecological attributes, some of which

have no known benefit to the animal and may exist transiently because of one of several unrelated reasons.

A summary diagram of our conclusions is illustrated and discussed in Figure 5 and is intended to promote contemplation on the basic ecological interactions of these important symbioses. With the increasing use of qPCR screening or next generation DNA sequencing, our knowledge on the diversity and distribution of ecologically rare or cryptic *Symbiodinium* spp. will continue to grow. Rational speculation, predicated on the detection of low abundances entities, about host–symbiont specificity and how background *Symbiodinium* can help corals respond to climate change requires more in-depth evidence and reflection.

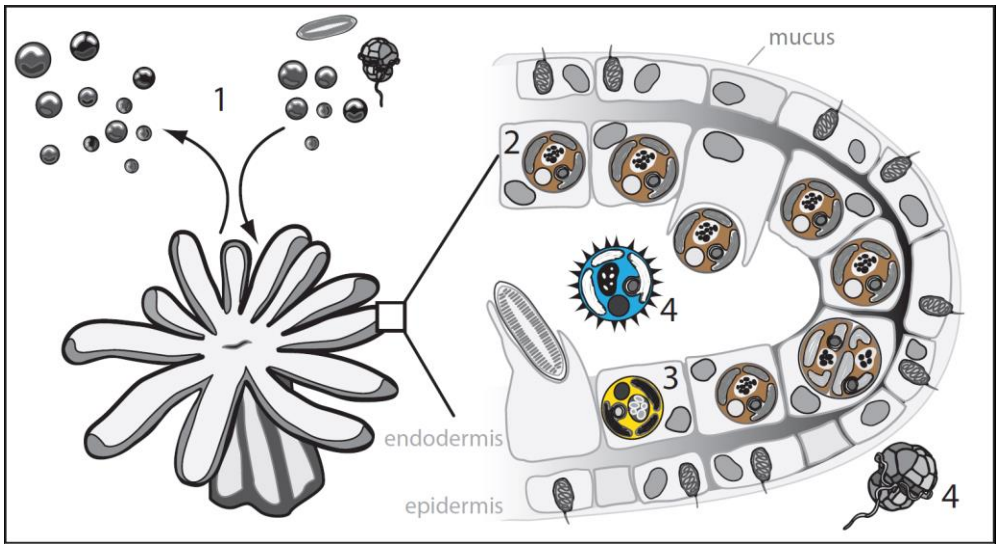



Fig. 2.5. Stable and transitory populations of *Symbiodinium* in coral-dinoflagellate mutualisms. (1) Symbiotic Cnidaria circulate large volumes of water (and mucus) through their gastro-vascular system for respiration, waste removal, and heterotrophic feeding, a process that introduces numerous small organic particulates as well as bacterial and eukaryotic microbes, which probably includes the cells of *Symbiodinium* spp. from the environment. Over the course of a normal day, a colony may expel millions of *Symbiodinium* cells (both viable and necrotic) in the maintenance of the mutualism. At any given time, there may be several distinct *Symbiodinium* sp. found in the micro-biome of the animal. These include temporally stable intracellular symbionts that dominate and are important to the growth of the animal (2), low 'background' and potentially non-mutualistic species (3), and free-living non-symbiotic species (4).

# Chapter 3: Feeding by the phototrophic dinoflagellate *Polykrikos hartmannii* on the red tide species *Cochlodinium polykrikoides.*

## 3.1 Introduction

Phototrophic dinoflagellates are a major component in marine planktonic communities (Smayda, 1997, Jeong et al., 2013, Park et al., 2013). They live in diverse habitats, such as in the water column, on macroalgae, and inside organisms (Johnson, 2011, Jeong et al., 2012, 2013, Kang et al., 2013, Lee et al., 2013, Lim et al., 2013). In the last two decades, several phototrophic dinoflagellates have been revealed to be mixotrophic (Jacobsen and Anderson, 1986, Hansen and Nielsen, 1997, Stoecker, 1999, Jeong et al., 2005a, 2010b, 2012, Turner, 2006, Burkholder et al., 2008, Kang et al., 2011). These mixotrophic dinoflagellates feed on diverse prey, including heterotrophic bacteria, cyanobacteria, small flagellates, other mixotrophic dinoflagellates, and ciliates (Stoecker et al., 1997, Jeong et al., 1999b, 2005a, 2005b, 2005c, 2010a, 2012, Li et al., 2000, Park et al., 2006, Seong et al., 2006, Berge et al., 2008, Glibert et al., 2009, Yoo et al., 2009, Kang et al., 2011, Lee KH et al., 2014). However, the number of phototrophic dinoflagellates of known mixotrophy is only ca. 50 species among ca. 1,200 reported phototrophic dinoflagellates (4–5%) (Jeong et al., 2010b, Gomez, 2012). Furthermore, there are less than 30 species of phototrophic dinoflagellates with quantified growth and ingestion rates. Therefore, to understand the eco-physiology of phototrophic dinoflagellates and their roles in planktonic food webs, the optimal prey species, growth and ingestion rates, and grazing impact should be fully understood.

*Polykrikos hartmannii* is a phototrophic dinoflagellate with chloroplasts (Hoppenrath et al., 2010). There has been a debate on the taxonomy of *P. hartmannii*, Zimmermann first established this species in 1930 (Zimmermann 1930),

however, it was transferred to the genus *Pheopolykrikos* by Matsuoka and Fukuyo in 1986 because it has *Pheopolykrikos* spp. characteristics including the same number of nuclei as the zooids, it can be dissociated to a single cell, and is also photosynthetic (Matsuoka and Fukuyo in 1986). However, Hoppenrath et al. (2010) suggested that *Pheopolykrikos* hartmannii should be transferred to P. *hartmannii* again because this species is clustered with the other *Polykrikos* spp. in the phylogenetic analyses of SSU and LSU rDNA (Hoppenrath and Leander, 2007a, b, Kim et al., 2008). Moreover, the presence of a nematocyst-taeniocyst complex revealed in an ultrastructural study confirmed that this species belonged to the genus *Polykrikos* (Hoppenrath et al., 2010).

*Polykrikos hartmannii* has been reported in the waters of many countries including USA (Hulburt, 1957, Steidinger and Williams, 1970, Badylak and Phlips, 2004), China (Huang and Dong, 2001), Mexico (Garate-Lizarraga et al., 2008), India (Godhe et al., 2000), Japan (Matsuoka and Fukuyo, 1986), and Korea (Kim et al., 2008). This dinoflagellate is usually present in low concentrations (Steidinger and Williams, 1970, Godhe et al., 2000, Garate-Lizarraga et al., 2008), but is sometimes abundant enough to be seen as blooms (Huang and Dong, 2001, FMRI, 2002, IOC, 2002, Badylak and Philps, 2004, Tang et al., 2013). Recently, this dinoflagellate was found to cause mass mortality in fish, even though the presence of toxins, and/or mechanisms of the fish mortality have not been revealed (Tang et al., 2013), more than 60% of the juvenile sheepshead minnows (Cyprinodon variegates) died at *P. hartmannii* concentrations of ≥1,370 cell ml-1. Therefore, the ecophysiology of this species is important for scientists, officials, and the aquaculture industry.

*Polykrikos* spp. are evolutionally important because they are between unicellular species and multicellular species like hydra. Some species in this genus live in single cells, while others form a pseudo-colony consisting of 2–8 cells. Additionally, some species are autotrophic, while others are mixotrophic or

heterotrophic. Therefore, the trophic mode of *Polykrikos* spp. is important to understand the evolution in the genus, and from unicellular to multicellular organisms. *P. hartmannii* is a phototrophic dinoflagellate with chloroplasts that swims as a single cell or forms 2-celled pseudo-colonies. Therefore, mixotrophy in *P. hartmannii* is fundamental for understanding *Polykrikos* evolution. However, the feeding ability of P. *hartmannii* (whether this species is exclusively autotrophic or mixotrophic), has not been clearly revealed, whereas other *Polykrikos* spp. have been found to be heterotrophic, *P. kofoidii* and *P. schwartzii*, and phototrophic, *P. lebourae*, and *P. tanti* (Jeong et al., 2001, Reñé et al., 2014, Kim et al., 2015). Additionally, heterotrophic *Polykrikos* spp. are known to have considerable grazing impact on populations of red tide species in marine ecosystems (Jeong et al., 2001, Tillman, 2004). Therefore, it is necessary to explore the feeding ability, prey type, and growth of *P. hartmannii*, and its grazing impact on red tide species.

A clonal culture of *Polykrikos hartmannii* isolated from coastal waters of the East Sea of Korea in 2013 at the declining stage of a *Cochlodinium polykrikoides* red tide was established. Using high-resolution video-microscopy, its feeding behavior was observed to explore feeding mechanisms and prey species when diverse algal species were provided. Furthermore, the growth and ingestion rates of *P. hartmannii* on the optimal algal prey species, *C. polykrikoides*, as a function of prey concentration were measured. Additionally, the grazing coefficients attributable to *P. hartmannii* on *C. polykrikoides* were established using the ingestion rate data obtained from laboratory experiments, and predator and prey abundance in the field. The results of the present study provide a basis for understanding mixotrophic ability, ecological roles of *P. hartmannii* in marine planktonic food webs, and evolution in the genus *Polykrikos*.

## 3.2 Materials and methods

### 3.2.1. Preparation of experimental organisms

The mixotrophic dinoflagellates *Cochlodinium polykrikoides* and *Lingulodinium polyedrum* were grown at 20 ℃ in enriched f/2 seawater media (Guillard and Ryther, 1962) under continuous illumination of 50 E $m^{-2}s^{-1}$ cool white fluorescent light, while the other phytoplankton species were grown under an illumination of 20 μE $m^{-2}s^{-1}$ cool white fluorescent light in a 14:10 h light–dark (LD) cycle (Table 1). *C. polykrikoides* and *L. polyedrum* did not grow well under illumination in a LD cycle. The mean equivalent spherical diameter (ESD) ± standard deviation was measured by an electronic particle counter (Coulter Multisizer II, Coulter Corporation, Miami, Florida, USA).

*Polykrikos hartmannii* was isolated from plankton samples collected in Uljin, East Sea of Korea (37° 2' N, 129° 26' E) during October 2013, when the water temperature and salinity were 27 ℃ and 33.9, respectively. The samples were placed in 6-well tissue culture plates. A clonal culture of *P. hartmannii* was established following two serial single-cell isolations. When *P. hartmannii* concentration increased, cells were transferred to 50-, 250-, and 500-ml BD Falcon cell culture flasks containing f/2 seawater media. The flasks were capped, and placed on a shelf (wide side down) at 20 ℃ and illuminated with 100 E $m^{-2}$ $s^{-1}$ cool-white fluorescent light in a 14:10 h LD cycle. Once dense cultures of *P. hartmannii* were obtained, they were transferred every 2 months to a 500-ml culture flask containing f/2 seawater media.

The carbon contents of *Cochlodinium polykrikoides* (0.7 ng C per cell) and *Gymnodinium catenatum* (1.9 ng C per cell) were obtained from our previous studies (Jeong et al., 2001, 2010a).

### 3.2.2. Prey species

Experiment 1 was designed to investigate whether *Polykrikos hartmannii* was able to feed on each target algal species when unialgal diets of diverse algal species were provided (Table 1). The initial concentrations of each algal species offered were similar, in terms of carbon biomass. To confirm that *P. hartmannii* did not ingestion some algal species, additional higher prey concentrations were provided.

A dense culture (ca. 1,500 cells $ml^{-1}$) of *Polykrikos hartmannii* growing photosynthetically in f/2 media, under a 14:10 h LD cycle of cool white fluorescent light at 100 µE $m^{-2}$ $s^{-1}$ was transferred to a 250-ml BD Falcon cell culture flask containing f/2 medium. This culture was maintained in f/2 media for 2 d under a 14:10 h LD cycle at 100 µE $m^{-2}$ $s^{-1}$. Under these conditions, >80% of *P. hartmannii* cells were 2-celled pseudo-colonies. Three 1-ml aliquots were then removed from the flask and examined using a compound microscope to determine the *P. hartmannii* concentration. In this enumeration, 2-celled pseudo-colonies were enumerated as 2 cells.

In this experiment, the initial concentrations of *Polykrikos hartmannii* and each target algal species were established using an autopipette to deliver a predetermined volume of culture with a known cell density to wells of a 6-well plate chamber. Triplicate wells of a 6-well plate chamber with mixtures of *P. hartmannii* and the target prey, and triplicate predator control wells containing *P. hartmannii* only were set up for each target algal species. The plate chambers were filled to 5 ml with freshly filtered seawater and then placed on a shelf and incubated at 20 ℃ under a 14:10 h LD cycle of cool white fluorescent light at 100 µE $m^{-2}$ $s^{-1}$. After 12 h, 24 h, and 48 h, > 100 *P. hartmannii* cells were monitored under a dissecting microscope (10–60 X magnification). Additionally, the plate chamber was placed on an inverted microscope (Zeiss-Axiovert 200M, Carl Zeiss Ltd., Göttingen, Germany) and the feeding occurrence of *P. hartmannii* was examined

(100–400 X magnification). Furthermore, two 0.2 ml aliquots were placed on slides and cover-glasses added. The protoplasms of ~50 *P. hartmannii* cells were carefully examined using epifluorescence microscopy (Zeiss-Axiovert 200M, Carl Zeiss Ltd., Göttingen, Germany) (100–630 X magnification) to determine if *P. hartmannii* was able to feed on the target prey species.

### 3.2.3. Feeding mechanisms

Experiment 2 was designed to investigate the feeding mechanisms of *Polykrikos hartmannii* when a unialgal diet of *Cochlodinium polykrikoides* and *Gymnodinium catenatum* was provided as prey. Feeding by *P. hartmannii* on these prey species was revealed in Experiment 1. The initial concentrations of predators and prey were the same as above.

In this experiment, the initial concentrations of *Polykrikos hartmannii* and each target algal species were established using an autopipette to deliver a predetermined volume of culture with a known cell density to wells of a 6-well plate chamber. Triplicate wells of a 6-well plate chamber with mixtures of *P. hartmannii* and the target prey, and triplicate predator control wells containing *P. hartmannii* only were set up for each target algal species. The plate chambers were filled to 5 ml with freshly filtered seawater and placed on a shelf and incubated at 20 ℃ under a 14:10 h LD cycle of cool white fluorescent light at 100 μE $m^{-2}$ $s^{-1}$. After 12 h, 24 h, and 48 h, the plate chamber was placed on an inverted microscope and the feeding behavior of > 100 unfed *P. hartmannii* cells for each target prey species were observed using compound microscopy and/or inverted microscopy (50–400 X magnification). A series of pictures showing the feeding process for a *P. hartmannii* cells was taken using a video analyzing system (Sony DXC-C33, Sony Co., Tokyo, Japan) mounted on an inverted microscope (50-400 X magnification).

### 3.2.4. Growth and ingestion rates.

Experiment 3 was designed to investigate the growth and ingestion rates of *Polykrikos hartmannii* on *Cochlodinium polykrikoides* as a function of prey concentration. Additionally, Experiment 4 was designed to investigate the growth and ingestion rates of *P. hartmannii* when feeding on *Gymnodinium catenatum* at two high prey concentrations because this prey did not support positive *P. hartmannii* growth in preliminary tests.

A dense culture (ca. 1,500 cells $ml^{-1}$) of *Polykrikos hartmannii* growing photosynthetically in f/2 media and under a 14:10 h LD cycle of cool white fluorescent light at 100 µE $m^{-2}$ $s^{-1}$ was transferred to a 250-mL culture flask containing f/2 medium. This culture was maintained and the *P. hartmannii* concentration was determined as described above.

The initial concentrations of *Polykrikos hartmannii* and *Cochlodinium polykrikoides* (or *Gymnodinium catenatum*) were established as described above. Triplicate 50-ml BD Falcon cell culture flasks containing mixtures of predators and prey, triplicate prey control flasks containing prey only, and triplicate predator control flasks containing predators only, were set up for each predator-prey combination. To obtain similar water conditions, the water of a predator culture was filtered through a 0.7-µm GF/F filter and then added to the prey control flasks in the same amount as the volume of the predator culture that was added to the experiment flasks for each predator–prey combination. All the flasks were filled to 26 ml with freshly filtered seawater. To determine the predator and prey densities at the beginning of the experiment, a 6-ml aliquot was removed from each flask, fixed with 5% Lugol's solution, and the cells in three 1-ml Sedgwick-Rafter chambers (SRCs) were enumerated by light microscopy. All flasks were with filled with 2 ml of f/2 medium to the final volume of 22 ml. To determine the initial predator and prey densities (cells $ml^{-1}$) at the beginning of the experiment (*P. hartmannii* and *C.*

*polykrikoides* = 15/30, 26/69, 40/147, 121/477, 140/767, 195/1452, 228/2696, 381/4042, *P. hartmannii* and *G. catenatum* = 67/402 and 121/1120) and after 4 d incubation, 6-ml aliquots were removed from each flask and fixed with 5% Lugol's solution, and all *P. hartmannii* cells and all or >300 prey cells in three 1-ml SRCs were enumerated. Prior to taking the subsamples, the condition of *P. hartmannii* and its prey was assessed under a dissecting microscope. The flasks were filled again to 22 ml with f/2 medium, capped, placed on a shelf (wide side down), and incubated at 20 ℃ under a 14:10 h LD cycle of 100 μE $m^{-2}$ $s^{-1}$ of cool white fluorescent light.

The specific growth rate of *Polykrikos hartmannii*, μ ($d^{-1}$), was calculated as follows:

$$\mu = \frac{\mathrm{Ln}\,(P_t / P_0)}{t} \qquad (1)$$

where $P_o$ is the initial concentration of *P. hartmannii* and $P_t$ is the final concentration after time t. The time period was 2 d.

Data for *Polykrikos hartmannii* growth rate were fitted to the following equation:

$$\mu = \frac{\mu_{max} \quad (x - x')}{K_{GR} + (x - x')} \qquad (2)$$

where $\mu_{max}$ = the maximum growth rate ($d^{-1}$), x = prey concentration (cells $ml^{-1}$ or ng C $ml^{-1}$), x' = threshold prey concentration (the prey concentration where μ = 0), and $K_{GR}$ = the prey concentration sustaining ½ $\mu_{max}$. Data were iteratively fitted to the model using DeltaGraph® (SPSS Inc., Chicago, IL, USA).

Ingestion and clearance rates for 4-d were also calculated using the equations of Frost (1972) and Heinbokel (1978). The incubation times for calculating the ingestion and clearance rates were the same as for estimating the growth rate.

Ingestion rate data were fitted to a Michaelis-Menten equation:

$$IR = \frac{I_{max}(x)}{K_{IR} + (x)} \quad (3)$$

where $I_{max}$ = the maximum ingestion rate (cells predator$^{-1}$d$^{-1}$ or ng C predator$^{-1}$d$^{-1}$), x = prey concentration (cells ml$^{-1}$ or ng C ml$^{-1}$), and $K_{IR}$ = the prey concentration sustaining ½ $I_{max}$.

### 3.2.5. Cell volume of *Polykrikos hartmannii*

After the 4-d incubation, the cell length and maximum width of each single cell and pseudo-colony of *Polykrikos hartmannii*, preserved in 5% acid Lugol's solution (n = 6–30 for each prey concentration), were measured using an image analysis system on images collected with an inverted microscope (AxioVision 4.5, Carl Zeiss Ltd., Göttingen, Germany). The shape of a single *P. hartmannii* cell was oval, while the 2-celled pseudo-colony was a rod. The following equations were used to calculate the cell volume of the preserved single *P. hartmannii* cell and the pseudo-colony: volume = 4/3 π [(cell length + cell width)/4]$^3$, and volume = π (cell width/2)$^2$ × cell length, respectively. The carbon content of *P. hartmannii* was also estimated from cell volume according to Menden-Deuer and Lessard (2000).

### 3.2.6. Swimming speed

A dense culture (ca. 1,500 cells ml$^{-1}$) of *Polykrikos hartmannii* growing photosynthetically as described above was transferred to a 250-mL BD Falcon cell

culture flask containing f/2 medium and was maintained in f/2 media for 2 d under 14:10 h LD cycle at 100 μE $m^{-2}$ $s^{-1}$. Aliquots from the flask were added to a 50-ml cell culture flask and allowed to acclimate for 30 min. The video camera focused on one field seen as one circle in a cell culture flask under a dissecting microscope at 20 ℃ and swimming of single *P. hartmannii* cells and colonies was then recorded (10-30 X magnification) using a video analyzing system (Samsung, SV-C660, Seoul, Korea) and taken using a CCD camera (Hitachi, KP-D20BU, Tokyo, Japan) as in Jeong (1994). The mean and maximum swimming velocities were analyzed for all swimming cells seen for the first 10 min. The average swimming speed was calculated based on the linear displacement of cells in 1 sec. during single-frame playback. The swimming speeds of 30 single cells and 30 colonies were measured. The swimming speed of *Gymnodinium catenatum* was measured in the same manner.

### 3.2.7. Potential grazing impact

By combining field data on predator and target prey abundance with the prey ingestion rates in the present study, we estimated the grazing coefficients of *Polykrikos hartmannii* on co-occurring *Cochlodinium polykrikoides.* Abundance data of *P. hartmannii* and *C. polykrikoides* used in this estimate were obtained by analyzing the water samples from South Sea of Korea in June 2014 to October 2014.

The grazing coefficients (g, $d^{-1}$) were calculated as:

$$g = CR \times PC \times 24 \quad (4)$$

where CR (ml $predator^{-1}h^{-1}$) is the clearance rate of *Polykrikos hartmannii* on a target prey at a prey concentration and PC is a predator concentration (cells $ml^{-1}$). The CR values were calculated as:

$$CR = IR/x \quad (5)$$

where IR (cells eaten predator$^{-1}$h$^{-1}$) is the ingestion rate of *P. hartmannii* on the target prey and x (cells ml$^{-1}$) is the prey concentration. These CR values were corrected using $Q_{10}$ = 2.8 (Hansen et al., 1997) because the in situ water temperature and the temperature used in the laboratory for this experiment (20 $^{o}$C) were sometimes different.

## 3.3 Results

### 3.3.1. Prey species

Among the algal prey offered, *Polykrikos hartmannii* ingested only chain-forming, naked, mixotrophic dinoflagellates *Cochlodinium polykrikoides* and *Gymnodinium catenatum* (Figs. 1, 2, 3, Table 1). However, it did not feed on the diatom *Skeletonema costatum*, prymnesiophyte *Isochrysis galbana*, cryptophytes (e.g., *Teleaulax* sp. and *Rhodomonas salina*), raphidophytes (*Heterosigma akashiwo* and *Chattonella ovata*), the naked dinoflagellates (*Amphidinium carterae, Akashiwo sanguinea, G. impudicum,* and *G. instriatum*), and thecate dinoflagellates (*Alexandrium tamarense, Heterocapsa triquetra, Lingulodinium polyedrum, Prorocentrum minimum, Pr. micans,* and *Scrippsiella trochoidea*) (Table 1). *P. hartmannii* did not even try to attack these inedible species cells when they were encountered.

### 3.3.2. Feeding mechanisms

*Polykrikos hartmannii* fed on its prey by engulfment after anchoring the prey cell using the nematocyst-taeniocyst complex (Fig. 1). *P. hartmannii* deployed the nematocyst-taeniocyst complex to one cell of *C. polykrikoides* or a *G. catenatum* chain. In most cases, a cell in the top of a prey cell was attacked (Fig. 2), even

though the cell in the middle of a chain was sometimes attached. After the predator had ingested one prey cell in the chain, the remaining cells in *C. polykrikoides* or *G. catenatum* chains escaped (Fig. 2). The predator completely engulfed one cell of a *Cochlodinium* and a *Gymnodinium* chain in ca. 140 and 200 sec, respectively (Figs. 2, 3).

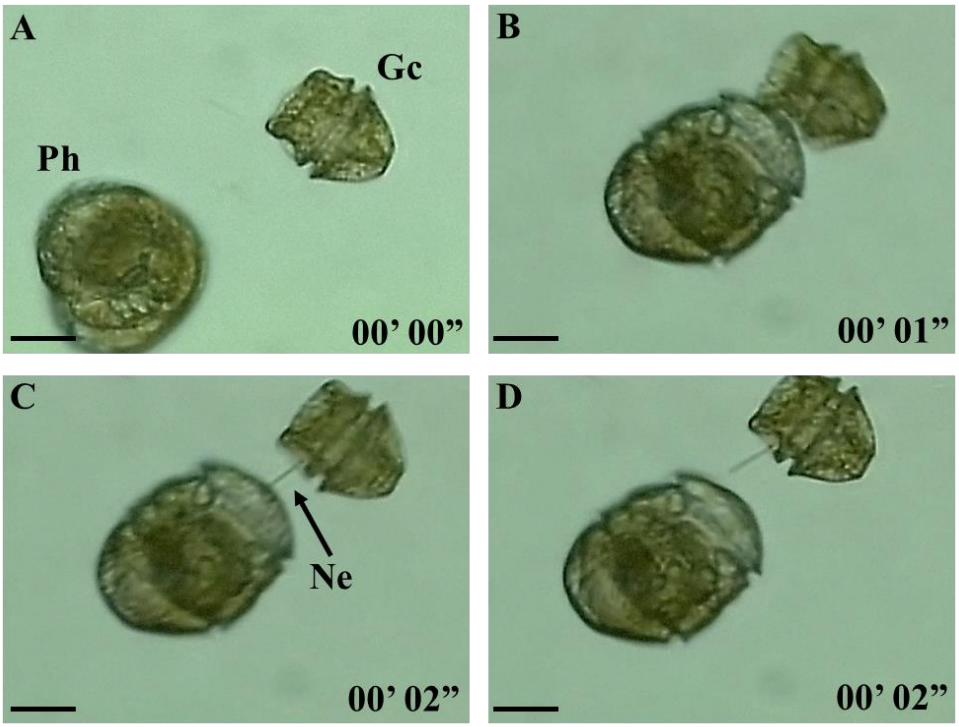



Fig. 3.1 The capture process by *Polykrikos hartmannii* (Ph) on *Gymnodinium catenatum* (Gc). (A) A *P. hartmannii* cell approaching a *G. catenatum* cell. (B) *P. hartmannii* deployed a nematocyst-taeniocyst complex into the *G. catenatum* cell. (C, D) The *P. hartmannii* cell captured the *G. catenatum* cell by using a nematocyst-taeniocyst complex (Ne). The time indicated the elapsed time. Scale bars are 20 μm. Supplementary video 1 for this process is on the website.

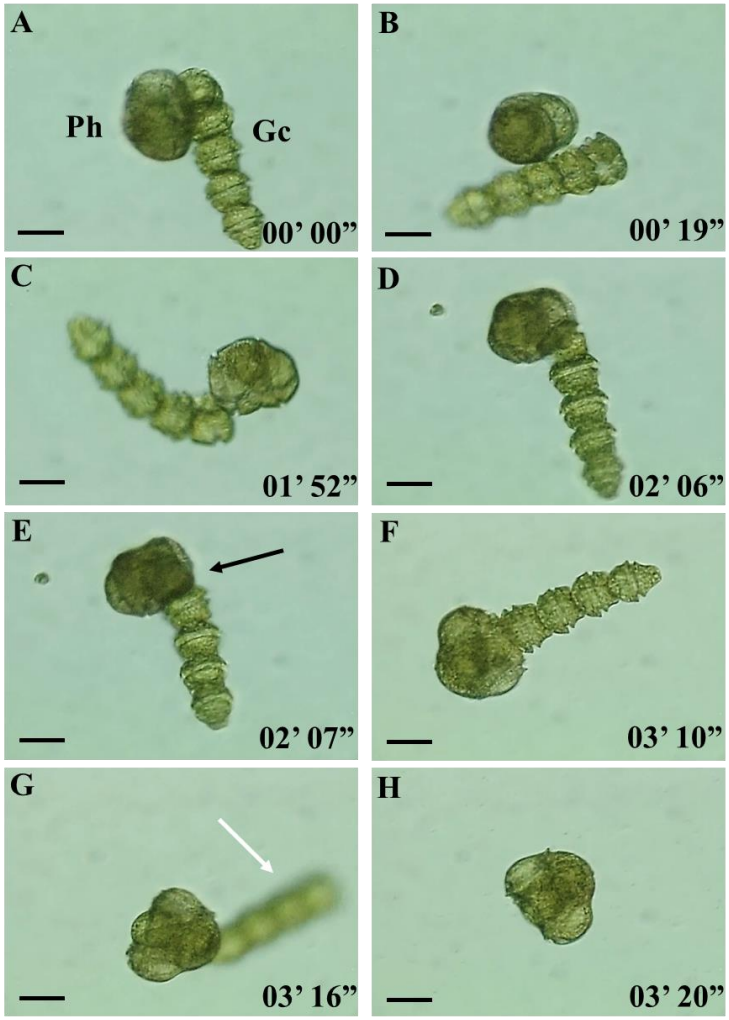



Fig. 3.2 Feeding process of *Polykrikos hartmannii* (Ph) on five-celled chain-forming *Gymnodinium catenatum* (Gc). (A) *P. hartmannii* attacked the cell on the top of the *G. catenatum* chain. (B-F) *P. hartmannii* engulfed the prey cell. (G) The *G. catenatum* chain with 4 unfed cells escaping from *P. hartmannii*. (H) *P. hartmannii* without the escaped 4-celled *Gymnodinium catenatum* chain. The black arrow indicates the almost ingested *G. catenatum* cells. The white arrow indicates the *G. catenatum* chain consisting of 4 unfed cells. The time indicated the elapsed time. Scale bars are 30 μm. Supplementary video 2 for this process is on the website.

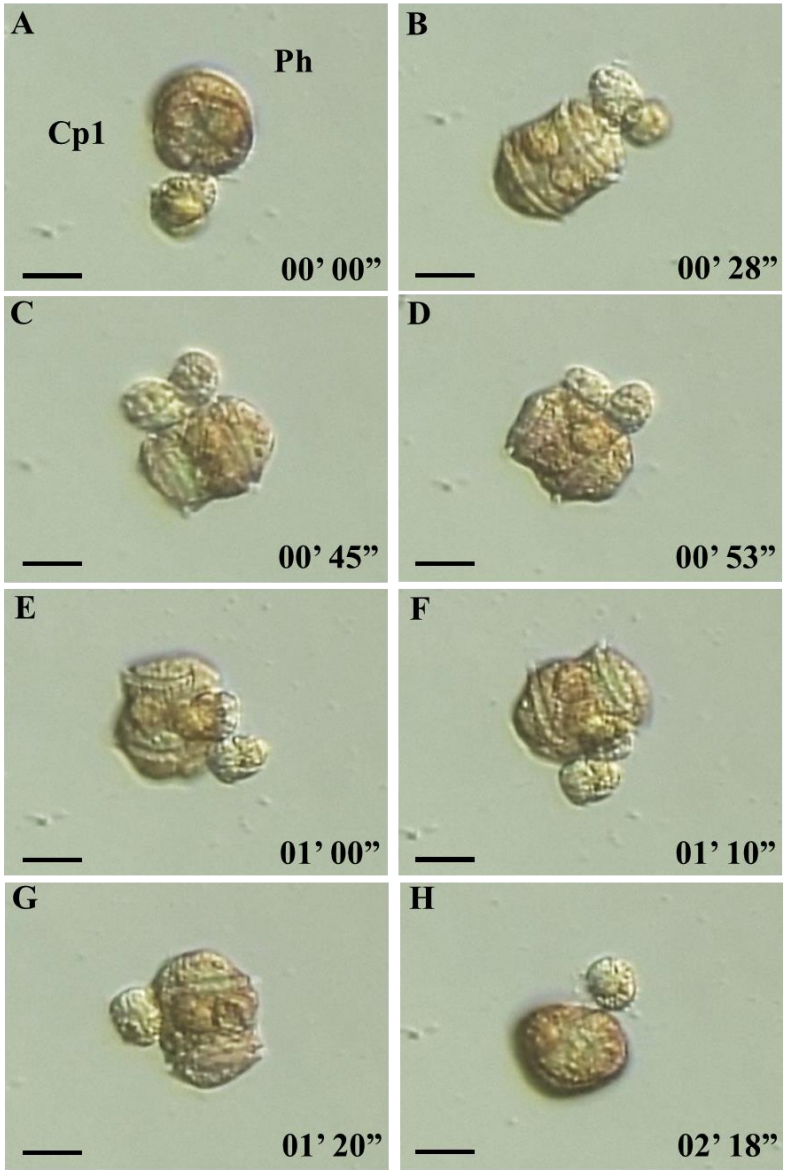



Fig. 3.3 Feeding process of *Polykrikos hartmannii* (Ph) on *Cochlodinium polykrikoides* (Cp). (A-D) The *P. hartmannii* cell attacked the cell on one (Cp 1, black arrow) of a 2-celled *C. polykrikoides* chain. (E, F) Cp 1 was almost engulfed. (G) Cp 1 was completely engulfed. (H) Cp 2 remained with being unfed. Supplementary video 3 for this process is on the website.

Table 3.1

Taxa, sizes, and concentration of prey species offered as food to *Polykrikos hartmannii* in Experiment 1 and feeding occurrence and attack by the predator on the target prey

| Prey species | ESD | Initial prey concentration (cells $ml^{-1}$) | Feeding by *P. hartmannii* | Attack |
|---|---|---|---|---|
| **Diatoms** | | | | |
| *Skeletonema costatum* | 5.9 | 100,000 | N | X |
| **Prymnesiophyceae** | | | | |
| *Isochrysis galbana* | 4.8 | 150,000 | N | X |
| **Cryptophytes** | | | | |
| *Teleaulax* sp. | 5.6 | 100,000 | N | X |
| *Rhodomonas salina* | 8.8 | 100,000 | N | X |
| **Raphidophytes** | | | | |
| *Heterosigma akashiwo* | 11.5 | 80,000 | N | X |
| *Chatonella ovata* | 40.0 | 5,000 | N | X |
| **Mixotrophic dinoflagellate** | | | | |
| *Amphidinium carterae* (NT) | 9.7 | 50,000 | N | X |
| *Prorocentrum minimum* (T) | 12.1 | 20,000 | N | X |
| *Heterocapsa triquetra* (T) | 15.0 | 20,000 | N | X |
| *Gymnodinium impudicum* (NT) | 22.6 | 10,000 | N | N |
| *Scrippsiella trochoidea* (T) | 22.8 | 20,000 | N | X |
| *Cochlodinium polykrikoides* (NT) | 25.9 | 2,000 | Y | O |
| *Prorocentrum micans* (T) | 26.6 | 5,000 | N | X |
| *Akashiwo sanguenia* (NT) | 30.8 | 2,000 | N | X |
| *Alexandrium tamarense* (T) | 32.6 | 2,000 | N | X |
| *Gymnodinium catenatum* (NT) | 33.9 | 2,000 | Y | O |

| Prey species | ESD | Initial prey concentration (cells ml$^{-1}$) | Feeding by *P. hartmannii* | Attack |
|---|---|---|---|---|
| *Gymnodinium instriatum* (T) | 36.5 | 1,000 | N | X |
| *Lingulodinium polyedrum* (T) | 36.6 | 500 | N | X |

ESD, Mean equivalent spherical diameter (μm), NT, Non-thecate, T, Thecate, Y, *P. hartmanii* was observed to feed on a living prey, N, *P. hartmanii* was observed not to feed on a living prey, O, *P. hartmanii* was observed to attack a living prey, X, *P. hartmanii* was observed not to attack a living prey. The abundances of the predator for each target prey were ca. 200 cells ml$^{-1}$.

Table 3.2. Comparison of prey species, the maximum growth (MGR, $d^{-1}$), and ingestion rates (MIR, ng C $predator^{-1}$ $d^{-1}$) of the phylogenetically related heterotrophic and mixotrophic dinoflagellates.

| Prey species | ESD | Ph | Pk | Pl | Ps | Gs |
|---|---|---|---|---|---|---|
| **Diatoms** | | | | | | |
| *Skeletonema costatum* | 5.9 | N | | | N | N |
| **Prymnesiophyceae** | | | | | | |
| *Isochrysis galbana* | 4.8 | **N** | | | Y | Y |
| **Cryptophytes** | | | | | | |
| *Teleaulax* sp. | 5.6 | **N** | | | Y | Y |
| *Chroomonas* sp. 1 | 6.0 | | | Y | | |
| *Rhodomonas* sp. 1 | 7.6 | | | Y | | |
| *Rhodomonas salina* | 8.8 | **N** | | | Y | Y |
| *Chroomonas* sp. 1 | 9.2 | | | Y | | |
| *Rhodomonas* sp. 2 | 10.0 | | | Y | | |
| *Rhodomonas* sp. 3 | 13.3 | | | Y | | |
| **Raphidophytes** | | | | | | |
| *Heterosigma akashiwo* | 11.5 | **N** | | | Y | Y |
| *Chatonella ovata* | 40.0 | N | | | | |
| **Mixotrophic dinoflagellate** | | | | | | |
| *Heterocapsa rotundata* | 5.8 | | | | Y | Y |
| *Amphidinium carterae* | 9.7 | **N** | Y | | Y | Y |
| *Prorocentrum minimum* | 12.1 | N | N | | N | **Y** |
| *Heterocapsa niei* | NA | | Y | | | |
| *Alexandrium lusitanicum* | NA | | N | | | |

| Prey species | ESD | Ph | Pk | Pl | Ps | Gs |
|---|---|---|---|---|---|---|
| *Gymnodinium breve* | NA | | N | | | |
| *Heterocapsa triquetra* | 15.0 | N | N | | N | N |
| *Gymnodinium impudicum* | 17.8 | N | **Y** | | | |
| *Heterocapsa* sp. | 19.1 | N | | **Y** | | |
| *Gymnodinium aureoulum* | 19.4 | | | | | |
| *Gymnodinium agile* | NA | | Y | | | |
| *Scrippsiella faeroense* | NA | | Y | | | |
| *Scrippsiella trochoidea* | 22.8 | N | **Y** | | N | N |
| *Alexandrium fundyense* | NA | | N | | | |
| *Alexandrium monilatum* | NA | | **N** | | | |
| *Cochlodinium polykrikoides* | 25.9 | **Y** | | | N | N |
| *Prorocentrum micans* | 26.6 | N | **Y** | | N | N |
| *Amphidinium* sp. | 28.1 | | | Y | | |
| *Alexandrium tamarense* | 28.1 | N | | | N | N |
| *Coolia monotis* | NA | | Y | | | |
| *Thecadinium kofoidii* | 28.6 | | | Y | | |
| *Ceratium furca* | 29.0 | | Y | | | |
| *Alexandrium affine* | 29.7 | | Y | | | |
| *Akashiwo sanguenia* | 30.8 | N | | | N | N |
| *Gonyaulax polygrama* | 32.5 | | | | N | N |
| *Gymnodinium catenatum* | 33.9 | Y | Y | | | |
| *Prorocentrum fukuyoi* | 34.8 | | | Y | | |
| *Gonyaulax spinifera* | 35.0 | | Y | | | |
| *Gymnodinium instriatum* | 36.5 | N | | | | |

| Prey species | ESD | Ph | Pk | Pl | Ps | Gs |
|---|---|---|---|---|---|---|
| *Prorocentrum lima* | NA | | Y | | | |
| *Lingulodinium polyedrum* | 38.2 | N | **Y** | | N | |
| MGR | | 0.049 | 1.12 | 0.17 | 0.94 | 0.97 |
| MIR | | 1.06 | 24.4 | 1.34 | 0.38 | 0.53 |
| Reference | | (1) | (2, 3, 4, 5) | (6) | (7) | (8) |

ESD, Mean equivalent spherical diameter (μm), Ph, *Polykrikos hartmanii*, Pk, *Polykrikos kofoidi*, Pl, *Polykrikos lebourae*, Ps, *Paragymnodinium* shiwhaense, Gs, *Gyrodiniellum shiwhaense***,** Bold indicate different results from the other predators, (1), This study, (2) Jeong et al., 2001, (3), Balech, 1995, (4), Fukuyo et al., 1997, (5), Dodge, 1982, (6), Kim et al., 2015, (7), Yoo et al., 2010, (8), Jeong et al., 2011

### 3.3.3. Growth and ingestion rates

*Polykrikos hartmannii* fed actively on *Cochlodinium polykrikoides* or *Gymnodinium catenatum* cells, but it did not grow well. With increasing mean prey concentration, the specific growth rates (mixotrophic growth) of *P. hartmannii* on *C. polykrikoides* at 20 ℃ under a 14:10 h LD cycle of 100 μE $m^{-2}$ $s^{-1}$ increased up to 0.049 $d^{-1}$ (Fig. 4). However, the maximum mixotrophic growth rate of *P. hartmannii* on *C. polykrikoides* was not clearly greater than its growth rate (phototrophic growth) under the same light conditions without added prey 0.041 $d^{-1}$ (n = 24).

With increasing mean prey concentration, *Polykrikos hartmannii* ingestion rates increased rapidly before almost saturating at a *Cochlodinium polykrikoides* concentration of 945 ng C $ml^{-1}$ (1,350 cells $ml^{-1}$) (Fig. 5). When the data were fitted to Eq. (3), the maximum ingestion rate of *P. hartmannii* on *C. polykrikoides* was 1.1 ng C predator$^{-1}$ $d^{-1}$ (1.5 cells predator$^{-1}$ $d^{-1}$) and $K_{IR}$ (the prey concentration sustaining ½ $I_{max}$) was 31.5 ng C $ml^{-1}$ (45 cells $ml^{-1}$). The maximum clearance rate of *P. hartmannii* on *C. polykrikoides* was 1.5 μl predator$^{-1}$ $h^{-1}$.

The highest mixotrophic growth rate of *P. hartmannii* with *G. catenatum* at the given prey concentration (0.013 $d^{-1}$) was slightly lower than that without *G. catenatum* (0.026 $d^{-1}$). Additionally, the highest ingestion rate of *P. hartmannii* on *G. catenatum* was 0.2 ng C $ml^{-1}$ (0.4 cells $ml^{-1}$).

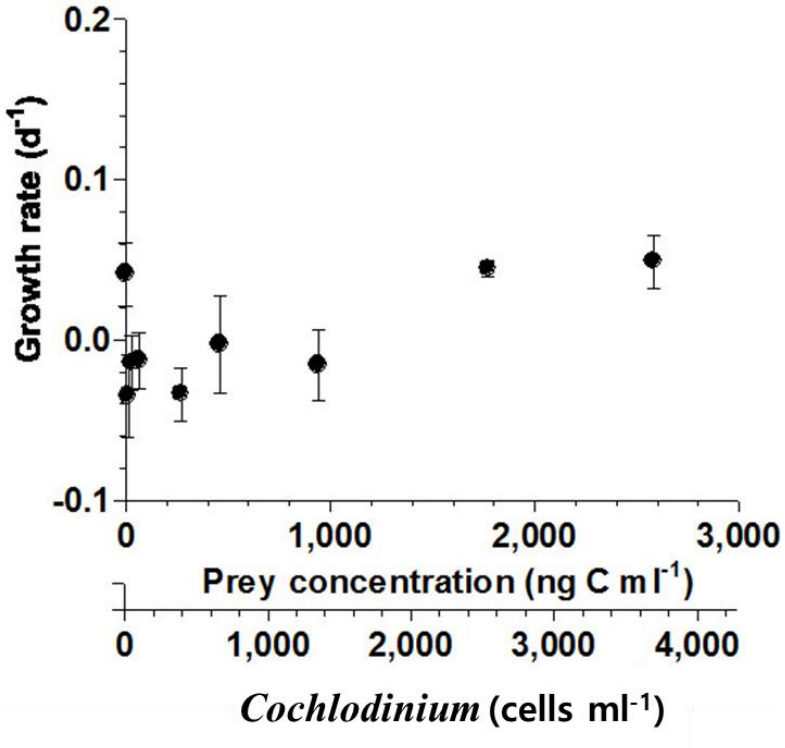



Fig. 3.4. Specific growth rates of *Polykrikos hartmannii* on *Cochlodinium polykrikoides* as a function of mean prey concentration ($x$, ng C $ml^{-1}$). Symbols represent treatment means $\pm$ 1 SE.

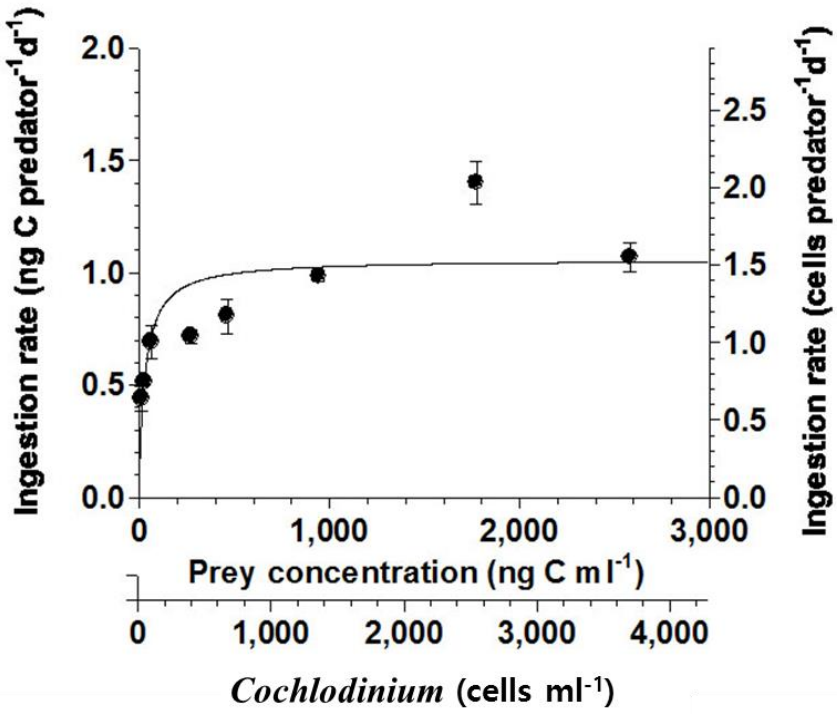



Fig. 3.5. Ingestion rates of *Polykrikos hartmannii* on *Cochlodinium polykrikoides* as a function of mean prey concentration ($x$, ng C $ml^{-1}$). Symbols represent treatment means $\pm$ 1 SE. The curve is fitted by a Michaelis –Menten equation [Eq. (3)] using all treatments in the experiment. (A) Ingestion rate (IR, $d^{-1}$) = 1.1 [($x$)/(31.5+$x$))], $r^2$ = 0.609

### 3.3.4. Cell volume

After a 4-d incubation, the mean cell volumes of single cells of *Polykrikos hartmannii* with *Cochlodinium polykrikoides* at all mean prey concentrations (17,260–24,810 $\mu m^3$) were smaller than those of single cells without *C. polykrikoides* (24,930 $\mu m^3$) (Fig. 6).

After a 4-d incubation, the mean cell volumes of *Polykrikos hartmannii* 2-celled pseudo-colonies with *Cochlodinium polykrikoides* at mean prey concentrations of 12–29 ng C $ml^{-1}$ (72,300–73,040 $\mu m^3$) were slightly higher than those of single cells without *C. polykrikoides* (68,950 $\mu m^3$). However, with increasing mean prey concentrations, the mean cell volumes of *P. hartmannii* 2-celled pseudo-colonies with *C. polykrikoides* after a 4-d incubation, rapidly increased to the mean prey concentrations of < 467 ng C $ml^{-1}$ (i.e., 661 cells $ml^{-1}$), but slowly to the higher mean prey concentrations. The greatest mean cell volume of *P. hartmannii* 2-celled pseudo-colonies was 95,860 $\mu m^3$ at the highest mean prey concentration (Fig. 6).

### 3.3.5. Swimming speed

The average ($\pm$ SE, n = 30) and maximum swimming speeds of single *Polykrikos hartmannii* cells growing photosynthetically were 334 ($\pm$ 6.4) and 456 $\mu m\ s^{-1}$, respectively, while those of 2-celled colonies were 524 ($\pm$ 5.6) and 631 $\mu m\ s^{-1}$, respectively.

The average ($\pm$ SE, n = 10) and maximum swimming speeds of 4-celled chains of *Gymnodinium catenatum* growing photosynthetically were 255 ($\pm$ 17.8) and 350 $\mu m\ s^{-1}$, respectively.

### 3.3.6. Potential grazing impact on red tide species

The grazing coefficients of *Polykrikos hartmannii* on *Cochlodinium polykrikoides* in the water samples taken in the South Sea of Korea in 2014 were 0.01–0.19 $h^{-1}$, when the abundances of *P. hartmannii* and *C. polykrikoides* were 0.2–4.2 cells $ml^{-1}$ and 0.2–33 cells $ml^{-1}$, respectively (Fig. 7).

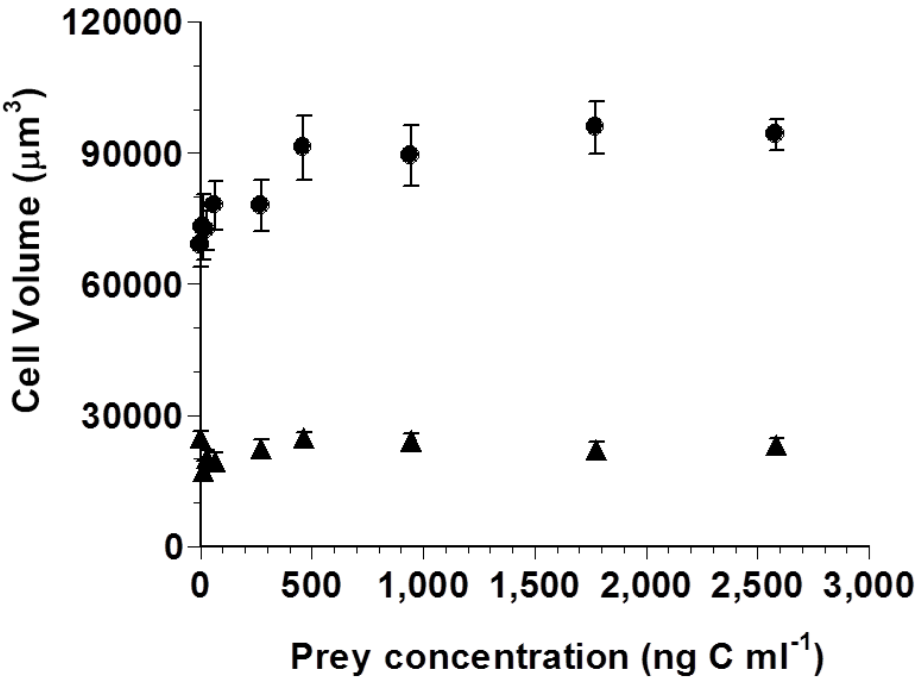



Fig. 3.6. The cell volume ($\mu m^3$) of the *Polykrikos hartmannii* on *Cochlodinium polykrikoides* after 4-d incubation as a function of mean prey concentration. Symbols represent treatment means (±SE)

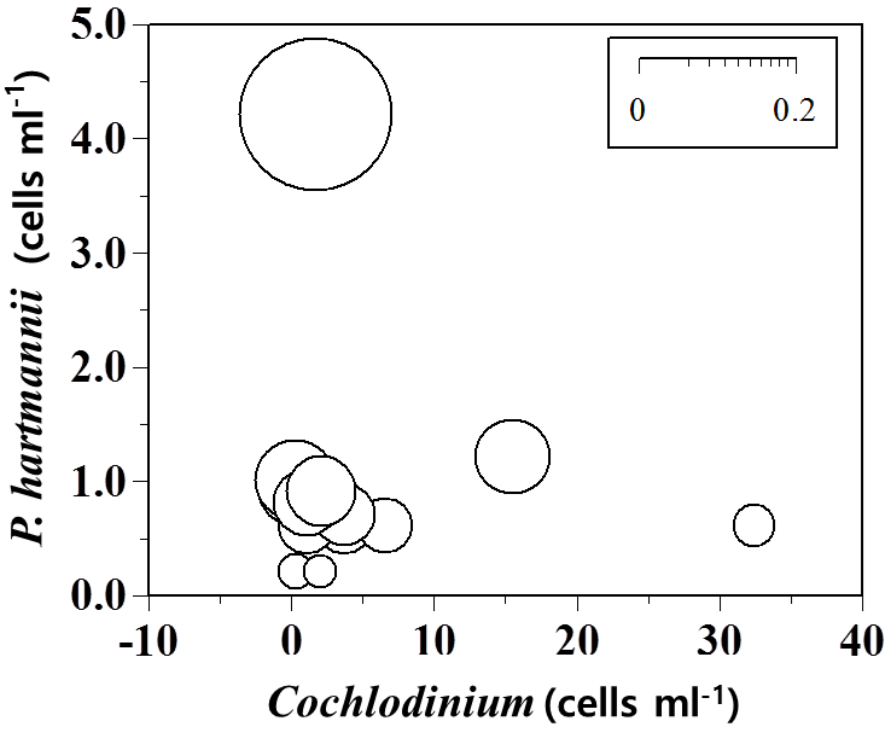



Fig. 3.7. Calculated grazing coefficients (g, $h^{-1}$) attributable to *Polykrikos hartmannii* on populations of *Cochlodinium polykrikoides* (see text for calculation). n = 18

## 3.4 Discussion

### 3.4.1. Feeding mechanisms and prey species

This study reports, for the first time, that *Polykrikos hartmannii* is a mixotrophic dinoflagellate. Prior to the present study, in the genus *Polykrikos*, 4 species (*P. grassei, P. herdmanae, P. kofoidii*, and *P. schwartzii*) were heterotrophic species, 3 species (*P. baranegatenis*, *P. lebourae*, and *P. tanit*) were mixotrophic species, and 2 species (*P. hartmannii* and *P. geminatum*) were exclusively autotrophic species. Now, this study transfers autotrophic *P. hartmannii* to a mixotrophic species. These trophic modes of *Polykrikos* spp. are important in understanding dinoflagellate evolution because acquisition and loss of plastids in this genus have been suggested (see next section).

The mixotrophic dinoflagellate *Polykrikos hartmannii* fed on algal prey by engulfment after anchoring a prey cell using a nematocyst-taeniocyst complex as in *P. kofoidii* (Matsuoka et al., 2000, Tillman and Hoppenrath, 2013). Therefore, this study confirmed that all phagotrophic *Polykrikos* spp. ingest prey cells in the same manner. However, the prey type that *Polykrikos* spp. are able to feed on is different from the other species, we found that *P. hartmannii* is able to feed on only the mixotrophic dinoflagellates, *C. polykrikoides* and *G. catenatum*, among the 18 tested algal prey species including the phototrophic dinoflagellates *A. cartarae, A. tamarense, G. catenatum, G. impudicum, G. instriatum, L. polyedrum, Pr. micans, S. trochoidea*, which *P. kofoidii* is able to feed on, or the cryptophyte *Rhodomonas* sp. and the phototrophic dinoflagellate *Heterocapsa triquetra,* which *P. lebourae* is able to feed on (Table 2). *P. lebourae* is also known to feed on *Amphidinium* sp., *Thecadinium kofoidii,* and *Pr. fukuyoi.* Therefore, the prey type of *P. hartmannii* is less diverse than *P. kofoidii* or *P. lebourae* (Fig. 3. 8).

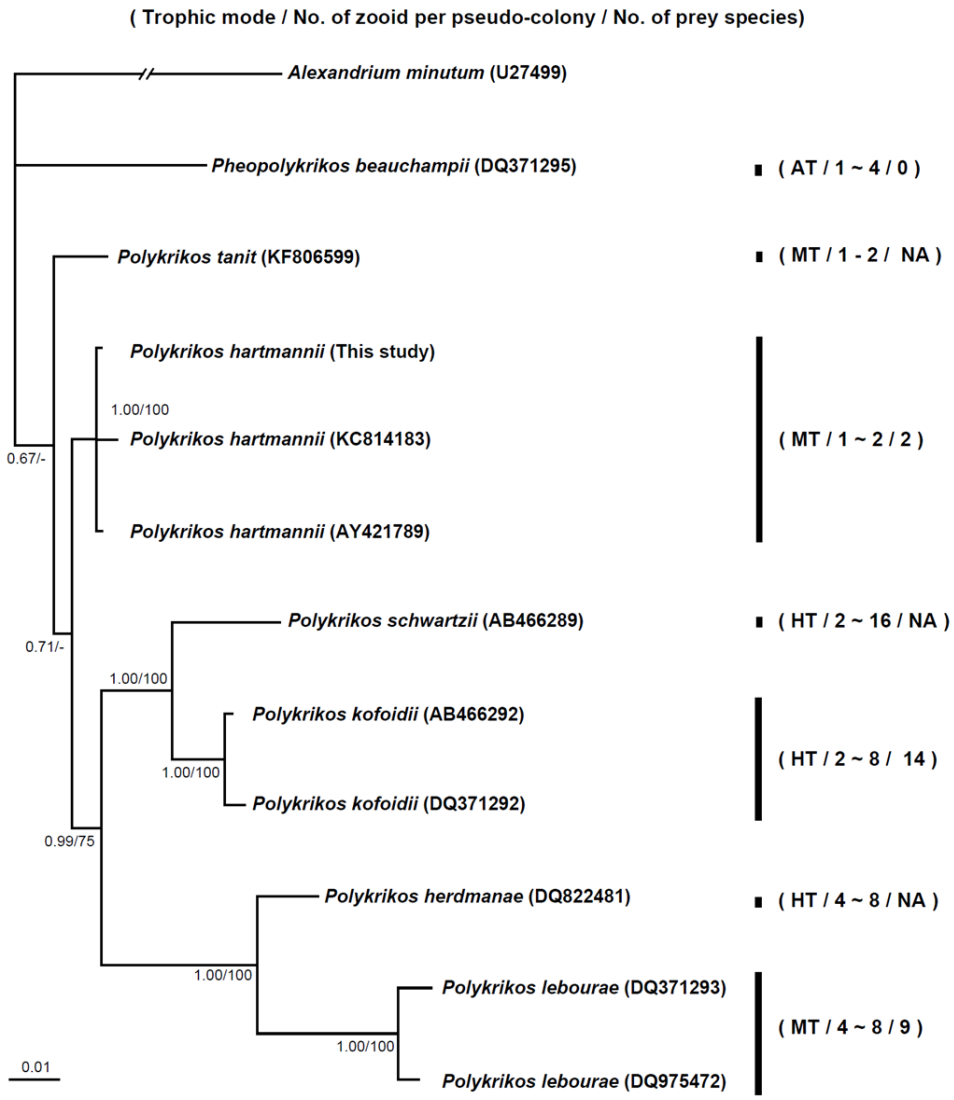



Fig. 3.8. Phylogenetic tree showing the relationships between the kinds of prey and positions of the selected species in the genera *Polykrikos* and *Pheopolykrikos*, based on the small subunit (SSU) rDNA. The maximum likelihood (ML) tree is based on 1716 aligned positions of nuclear partial SSU rDNA, with *Alexandrium tamarense* as outgroup taxa. The numbers above the branches indicate the ML bootstrap values. Trophic mode / number of zooid per pseudocolony / number of prey species so far reported in the parenthesis.

*Cochlodinium polykrikoides* and *Gymnodinium catenatum* are chain-forming dinoflagellates. Deploying the nematocyst-taeniocyst complex on the surface of these dinoflagellates and anchoring these prey cells may be easier than the other prey species. *C. polykrikoides* is one of the fastest swimming mixotrophic dinoflagellates (Jeong et al. 1999b) and its maximum swimming speed (ca. 1,450 μm $s^{-1}$) is much greater than that of *P. hartmannii* (456 μm $s^{-1}$ for single cells and 631 μm $s^{-1}$ for 2-celled pseudo-colonies). Therefore, the nematocyst-taeniocyst complex, which is known to paralyze prey cells, may help in capturing this fast swimming dinoflagellate prey. However, the nematocyst-bearing dinoflagellates *Paragymnodinium shiwhaense* and *Gyrodiniellum shiwhaense* are not able to feed on *C. polykrikoides* and *G. catenatum* (Kang et al., 2010, 2011, Yoo et al., 2010, Jeong et al., 2011). Therefore, the nematocyst-taeniocyst complex may be a more effective tool than the nematocyst to capture *C. polykrikoides* or *G. catenatum* cells.

*Cochlodinium polykrikoides* and *Gymnodinium catenatum* are also toxic dinoflagellates killing fish (Anderson et al., 1989, Hallegraeff and Fraga, 1998, Gobler et al., 2008, Mulholland et al., 2009, Park et al., 2013). Therefore, *P. hartmannii* is likely to have enzymes that detoxify toxins produced by these two prey species. Only a few protistan predators such as the large ciliate *Strombidinopsis* spp. are known to feed on *C. polykrikoides* (Jeong et al. 1999a, 2008). Thus, this study also includes *P. hartmannii* as a protistan predator feeding on *C. polykrikoides*. *P. kofoidii*, in the same genus, is known to grow efficiently feeding on *G. catenatum* (Jeong et al. 2001, 2003). Thus, some *Polykrikos* spp. may be evolved to feed on *G. catenatum*.

### 3.4.2. Growth and ingestion rates and cell volume

*Polykrikos hartmannii* with *Cochlodinium polykrikoides* prey (mixotrophic growth rate) was not markedly greater than that without added prey (autotrophic growth rate), even though it fed actively on the prey. We found that the maximum

ingestion rate of *P. hartmannii* on *C. polykrikoides* is 1.1 ng C predator$^{-1}$ d$^{-1}$, while the carbon content of a *P. hartmannii* cell is 3.2 ng C per cell, indicating that *P. hartmannii* can acquire 33% body carbon from *C. polykrikoides* in a day. The low maximum ingestion rate of *P. hartmannii* may be responsible for this small difference between the maximum mixotrophic and autotrophic growth rates (ca. 0.01 d$^{-1}$), which is much smaller than that of the other mixotrophic dinoflagellates (reviewed by Jeong et al. 2010b, Kang et al., 2011, Lee KH et al., 2014, Lee SK et al., 2014). Therefore, *P. hartmannii* is able to feed actively on prey, but the mixotrophy does not contribute much to growth.

The maximum ingestion rate of *Polykrikos hartmannii* on *Cochlodinium polykrikoides* is much lower than *Polykrikos kofoidii* (24.4 ng C pseudo-colony$^{-1}$d$^{-1}$), but slightly less than *Polykrikos lebourae* (~1.3 ng C pseudo-colony$^{-1}$d$^{-1}$). Even when the maximum ingestion rate of a *P. kofoidii* pseudo-colony is divided by the number of cells in a pseudo-colony (4), the maximum ingestion rate of *P. kofoidii* (6.1 ng C predator$^{-1}$d$^{-1}$) is still much greater than that of *P. hartmannii* or *P. lebourae* (8 cells in a pseudo-colony, ~0.16 ng C predator$^{-1}$d$^{-1}$). In addition, the maximum growth rate of *P. kofoidii* (1.12 d$^{-1}$) is much greater than *P. hartmannii* (0.049 d$^{-1}$), or *P. lebourae* (0.17 d$^{-1}$). Therefore, exclusive hetereotrophy may increase both ingestion and growth rates in *Polykrikos*. Hoppenrath and Leander (2007a) showed that *P. hartmannii* is located in the basal position of *P. kofoidii* or *P. lebourae* in the synthetic phylogeny of polykrikoid dinoflagellates. Therefore, *P. kofoidii* or *P. lebourae* may be evolved from *P. hartmannii* to the direction of having more diverse prey species and higher maximum growth rates than their ancestor.

The maximum mean cell volumes of single cells of *Polykrikos hartmannii* with *Cochlodinium polykrikoides* was similar to that without *C. polykrikoides*, while the maximum mean of the cell volumes of 2-celled pseudo-colonies of *P. hartmannii* with *C. polykrikoides* was 39% greater than that without *C.*

*polykrikoides*. The feeding by single cells of *P. hartmannii* on *C. polykrikoides* has not been observed in many trials, while that of 2-celled pseudo-colonies has frequently been observed. This evidence suggests that compared to the cell volume of *P. hartmannii* starved cells, no increase in cell volume of *P. hartmannii* single cells with *C. polykrikoides* may be due to no or little feeding occurrence, while the considerable increase in cell volume of *P. hartmannii* 2-celled pseudo-colonies with added *C. polykrikoides* may be due to feeding. Feeding by 2-celled pseudo-colonies, but no feeding by single cells was observed in preliminary tests and thus these feeding experiments were conducted when the ratio of 2-celled pseudo-colonies relative to total cells exceeded 80%. Therefore, the formation of pseudo-colonies is likely to increase feeding activities of *P. hartmannii* and may be an important evolutionary direction in the genus *Polykrikos.*

### 3.4.3. Potential grazing impact on red tide species

The grazing coefficients (g) of *Polykrikos hartmannii* on co-occurring *Cochlodinium polykrikoides* obtained in the present study were up to 0.190 $h^{-1}$ (up to 17.3 % of the *C. polykrikoides* populations were removed by a *P. hartmannii* population in 1 d). Therefore, *P. hartmannii* may have a considerable grazing impact on populations of co-occurring *C. polykrikoides*. However, more abundance data on the co-occurring *P. hartmannii* and *C. polykrikoides* are needed to explore grazing impact. *P. hartmannii* has become abundant at the declining stage of *C. polykrikoides* red tides in the South Sea of Korea without its abundance (NFRDI, 2014). Therefore, it is worthwhile to measure the abundances of *P. hartmannii* on co-occurring *C. polykrikoides*.

In conclusion, the present study demonstrated that: (1) *Polykrikos hartmannii* is a mixotrophic dinoflagellate. (2) When diverse algal species were provided as potential prey, *P. hartmannii* was able to feed on only chain-forming toxic mixotrophic dinoflagellates *Cochlodinium polykrikoides* and *Gymnodinium*

*catenatum*. (3) *P. hartmannii* ingested a prey cell by engulfment after anchoring the cell using a nematocyst-taeniocyst complex. (4) The maximum ingestion rate of *P. hartmannii* on *C. polykrikoides* was 1.1 ng C predator$^{-1}$ d$^{-1}$. (5) The mixotrophic growth rate of *P. hartmannii* with added *C. polykrikoides* cells (0.049 d$^{-1}$) was not clearly greater than of those without added prey (0.041 d$^{-1}$). The amount of ingested *C. polykrikoides* cells was not enough to support positive *P. hartmannii* growth. (6) The calculated grazing coefficients for *P. hartmannii* on co-occurring *C. polykrikoides* were up to 0.19 d$^{-1}$. The results of the present study suggest that *P. hartmannii* could have a considerable grazing impact on the population of *C. polykrikoides*. (7) The formation of pseudo-colonies is likely to increase feeding activities of *P. hartmannii* and may be an important evolutionary direction in the genus *Polykrikos.*

# Chapter 4: Multidirectional grazing impacts between the red tide dinoflagellates and their potential planktonic predators in the South Sea of Korea.

## 4.1. Introduction

Diverse metazooplankton taxa (animals > 200μm) such as copepods, cladocerans, chaetognaths, larvae of invertebrates and hydrozoans play important roles in the marine food web and marine ecosystem. Metazooplankton are sometimes play a role of main trophic link between primary production by phytoplankton and the upper trophic levels (Croll et al., 2005, Field et al. 2006). Metazooplankton are the most important grazers of microzooplankton which are able to feed on nanoflagellate (<20 μm) but metazooplankton do not directly utilize them (Poter et al., 1985,, Stoecker and capozzo, 1990). Thus, the feeding activities and grazing interactions between phytoplankton-microzooplankton-metazooplankton are the most important trophic pathway in the transfer biomass and energy from primary production to high levels of organisms such as fish (Sanders and Wickham, 1993). The interactions between the ecophysiological factors and standing stock and species composition of metazooplankton can be a key determinant of fish recruitment in the marine ecosystems. Furthermore, the amount of reproduction and the recruitment rate of fish may cause of direct economic influence on humans. (Mann, 1993, Sanders and Wickham, 1993, Turner, 2004, Turner, 2006, Kim et al., 2013).

The variability of natural marine environmental conditions such as warming, stratification and eutrophication may lead to decrease or increase primary production shift in the phytoplankton community which become causes of red tides (Durbin and Durbin 1981, Colebrook 1984, Rückert and Giani 2008). Red tides is

algal bloom occurred with discoloration of the sea surface in worldwide (Holmes et al., 1967, Eppley and Harrison, 1975, Franks and Anderson, 1992, ECOHAB, 1995, Franks, 1997, Horner et al., 1997, Sordo et al., 2001, Anderson et al., 2002, Alonso-Rodriguez and Ochoa, 2004, Imai et al., 2006, Seong et al., 2006, Jeong et al., 2013). This phenomenon is often causing large-scale fish and shellfish mortalities and great economic losses to the aquaculture and tourist industries (Smayda, 1990, Glibert et al., 2005, Anderson et al., 2012, Fu et al., 2012, Park et al., 2013).

Variety ecophysiological and biological environmental elements can be the factors controlling the development or decline of red tides. Basically the water temperature, salinity, pH and light intensity can control the bloom dynamics (Calbet et al., 2003, Jeong et al., 2015). Furthermore, the inorganic nutrient concentrations directly influence on the growth of the red tides organisms. Thus, depletion of nutrient which essentially required for growth of algal species such as nitrate and phosphate is resulting in the extinction of red tides (Anderson and Lindquist, 1985). In addition, the hydrodynamics such as current and waves which are determined by the topography of the region can not only act as concentrating and aggregating the algal populations but also disperse the red-tides in a short time (Steindinger, 1973, Garces et al., 1999, Vila et al. 2001).

The competition with the co-occurring competitors such as the other phytoplankton species is important to form a red-tides. Their population must grow faster and dying slower than the other phytoplankton to form a red tides and they obtained these advantages through the indirect biological factors and direct biological interactions with other species such as prey-predator relationships in the marine ecosystems (Jeong et al., 2015). Among the diverse biological factors, predation by the predators is the most important direct element for the algal population (Turner and Granéli, 1992, Calbet et al., 2003). However, relatively few studies regarding these multidirectional trophic interactions between phytoplankton-

microzooplankton-metazooplankton were reported (reviewed by Turner and Tester,1997).

The depth of the study area South Sea of Korea is less than 200m. Due to the Tsushima current (KORDI, 1989) which is branched from Kuroshio Current, the water temperature is comparatively higher than the other region of the sea waters in Korea (Kim et al., 1993). Thus, the South Sea of Korea is valuable area by the fish spawning and nursery ground (Kim and Kang, 2000). However, eutrophication has occurred with rapid industrial development from inshore of South Sea of Korea since 1960s (Park et al. 2005). The eutrophication is causative of change of species composition and the phytoplankton populations such as red-tides (Smayda, 1990, Lee and Lee 2000, Glibert et al., 2005, Anderson et al., 2012).

The metazooplankton communities in the South Sea of Korea considered to be well studied (Kim et al., 1993, Kang et al., 1996, Hue et al., 2002, Youn et al., 2010, Moon et al., 2010, Kim et al., 2013, Oh et al., 2013). However, relatively few studies were performed on the variations of metazooplankton community in the South Sea of Korea including wide regions (Moon et al., 2010, Oh et al., 2013). Moreover, most of the studies only focused on the correlations between the populations of metazooplankton and the environmental factors except the biological interactions in the plankton community such as the trophic pathway, trophic types, feeding and predation in the South Sea of Korea.

Some of preceding studies assumed the grazing impact of metazooplankton on red-tides species is significant in the initial phases of the bloom dynamics, either by direct feeding on the algal population or by indirect selective feeding on the predator of red-tides organisms such as microzooplankton (Uye, 1986, Guisande et al., 2002, Johansson and Coasts, 2002). However, whether the predation of metazooplankton exert positively influence on the red tides by indirect grazing on the potential predators of phytoplankton such as the metazooplankton and

microzooplankton, or negatively effect to the red-tides by direct predation on red tides organism is not clear.

Thus, in the preset study, in order to investigate whether the standing stocks, distribution and grazing of the metazooplankton negatively directly effect on the population of red tides species, or positively indirectly influence on the dinoflagellate community through feeding on potential microzooplankton predators of red tides species, water samples were collected from 60 stations in the South Sea of Korea, from May to November 2014. Furthermore, the three-dimensional (3-D) distributions of physicochemical and biological parameters such as water temperature, salinity, chl-a and occurrence patterns of plankton community including phytoplankton-microzooplankton-metazooplankton were analyzed. By combining field data on the spatio-temporal distribution patterns of metazooplankton and the target prey species such as phototrophic dinoflagellates, heterotrophic dinoflagellates and ciliates with the ingestion rates of the predators on the prey obtained from the literature, we estimated the grazing coefficients attributable to the predators on co-occurring phytoplankton and their potential microzooplankton predator. The results of the present study provide a basis for understanding the correlations between the red tides populations and the distributions of metazooplankton community, it will help to verify the structure of trophic pathway and trophic cascade in the marine ecosysstems.

## 4.2. Materials and methods

### 4.2.1. Sampling stations

Samples were collected by 16 times of cruise in 1 or several weeks interval from May to November 2014 at 60 stations where located in the middle of southern coast of the Korean peninsula between Goheung to Tongyoung (approximately 140 km x 40 km , 34.3°E - 34.8°E and 127.3°N -128.4°N) (Fig. 4.1). The water depth

for all sampling stations ranged between 4-53 m. The stations in the western area were shallower than those in the eastern area at the same latitude. The bottom topography of the study area is shown in Figure 4.2.

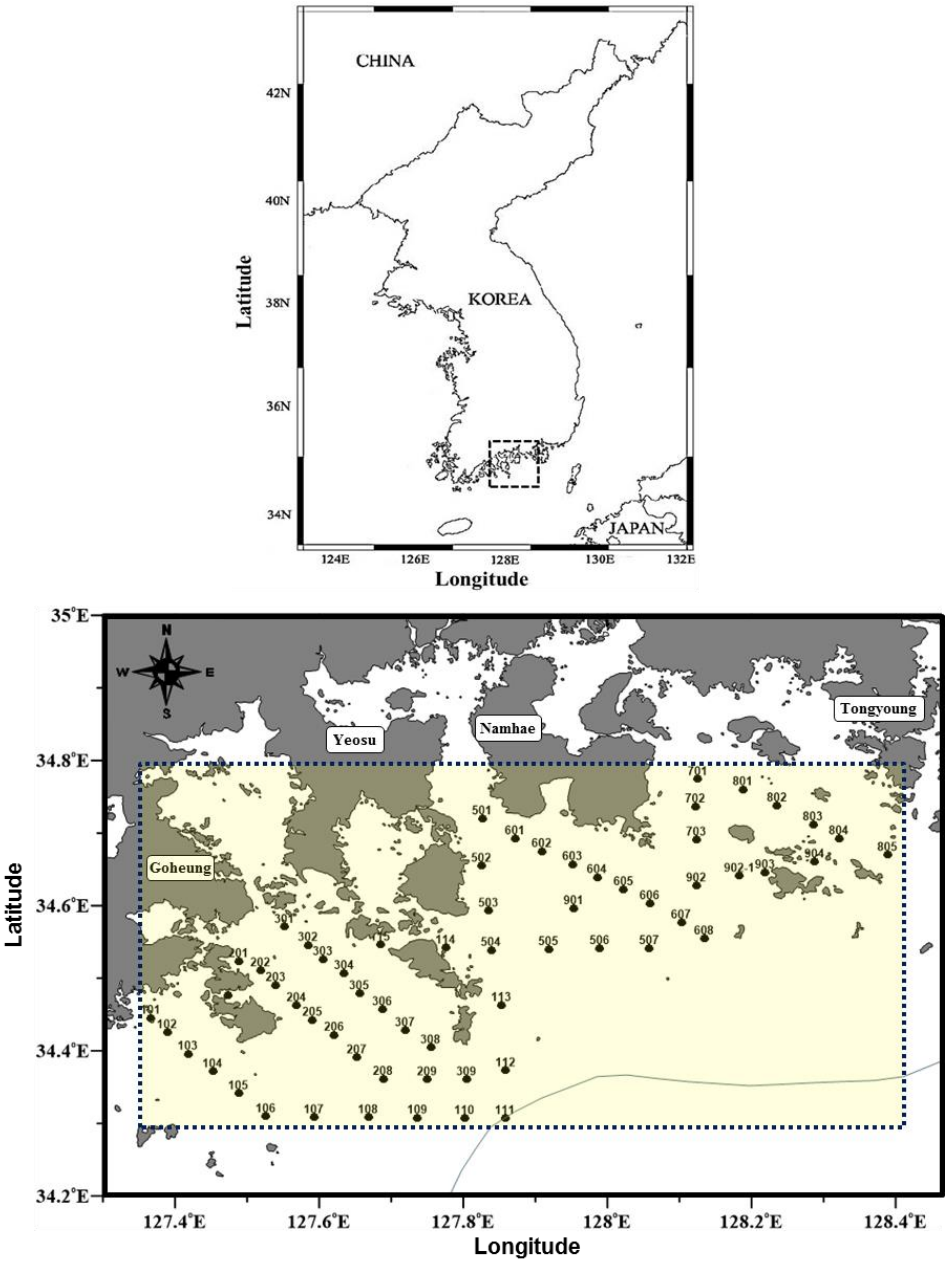



Fig. 4.1 Map of the study area and sampling stations in South Sea of Korea

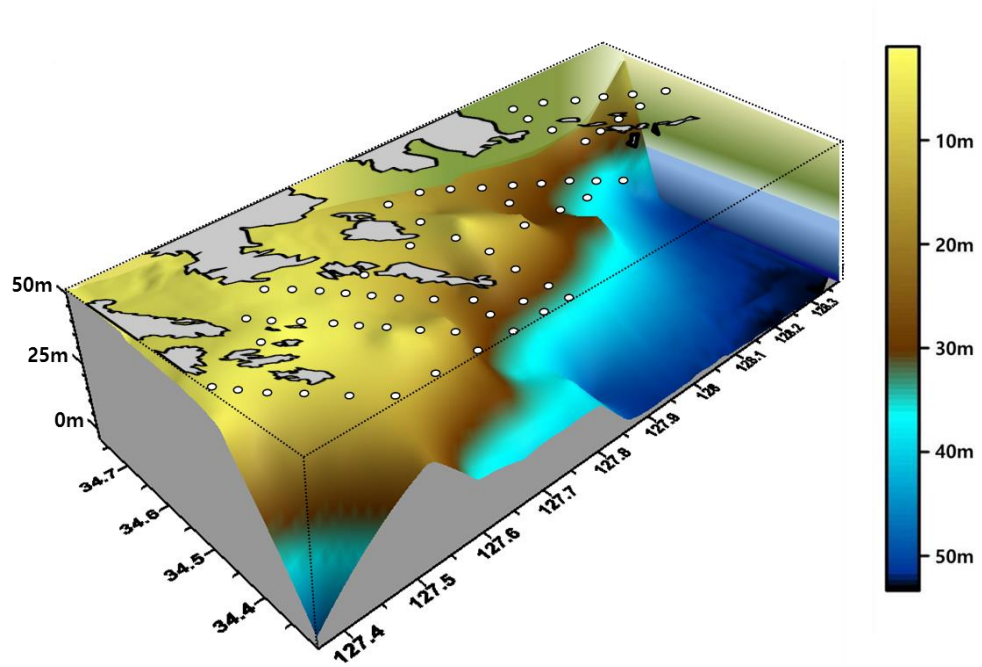



Fig. 4.2 Depth and submarine topography of the study area in the South Sea of Korea

### 4.2.2. Sampling and analyses of physicochemical and biological properties

Sixteen sampling cruises were carried out at 1-2 week intervals between May 7 and November 10, 2014 (Table 4.1). A two-day sampling was carried out by two ships. On the first day, water samples were collected from one ship at stations 101-114, while the other ship collected samples at stations 201-309. On the second day, one ship collected water samples at stations 501-507 and 601-608, while the other ship at stations 701-703, 801-805, and 901-904. The sampling time at the first station in each sampling day was between 07:30-08:00 h and at the last station was between 14:00-15:00 h.

Water temperature and salinity in the water column were measured using two CTDs [YSI6600 (YSI Inc., Yellow Springs, OH, USA) and Ocean seven (Idronaut S.r.l., Milan, Italy)]. The data obtained from the CTDs were calibrated in each cruise. Additionally, the temperature, salinity, pH, and DO for each sampling depth were measured using YSI Professional Plus instrument (YSI Inc., Yellow Springs, OH, USA). The water samples for nutrient concentration analysis were gently filtered through GF/F filters and stored at -20 ℃ until the concentrations of nitrate plus nitrite ($NO_3$+$NO_2$, hereafter $NO_3$) and phosphate ($PO_4$) were measured using a nutrient auto-analyzer system (Quattro, Seal Analytical Gmbh, Norderstedt, Germany). The chlorophyll-a concentration was measured as described in APHA (1995).

Water samples at each station were collected using water samplers, from 2-5 depths depending on the depth of each station. Samples for phytoplankton counting were collected into 500-ml polyethylene bottles and preserved with acidic Lugol's solution. To determine the abundance of each phytoplankton species, the preserved samples were concentrated by 1/5–1/10 using the settling and siphoning method (Welch, 1948). After thorough mixing, all or a minimum of 100 cells of each phytoplankton species in one to ten 1-ml Sedgwick-Rafter counting chambers were counted under a light microscope.

Metazooplankton samples were collected at every stations by towing a 303-m-mesh, 45-cm-diameter, conical plankton net with a flowmeter vertically from the bottom to the surface (or 30m if the bottom depth was more than 30m) every sampling interval from May to November 2014. Each plankton sample was poured into a 500-ml polyethylene bottle and preserved with 4% formalin. Species identification and determination of the abundance of metazooplankton were performed using dissecting and inverted microscopes at magnifications of ×40 and ×200.

### 4.2.3. Data processing

The spatio-temporal (3-D) distributions of T, S, Chl-a and the each taxa of plankton communities were plotted by Voxler ® and Surfer (Golden software). The correlation coefficients between physical, chemical, and biological properties were calculated using the Pearson's correlation (Conover, 1980, Zar, 1999).

By combining field data on the abundances of the predator and the prey species with the ingestion rates of the predator on the prey obtained in the literatures, with some assumptions, we estimated the grazing coefficients.

*Acartia* spp. are known to feed on the various species of dinoflagellates such as *Cochlodinium polykrikoides*, *Prorocentrum donghaiense*, *Gonyaulax polygramma*, *Polykrikos kofoidii*, *Oxyrrhis marina* and the diatoms *Skeletonema costatum*, *Chaetoceros curvisetus* (Jeong et al., 2001, Kim, 2005). It was assumed that the ingestion rate of *Acartia* spp. on dinoflagellates *C. polykrikoides*, *P. donghaiense*, *P. kofoidii*, *O. marina* and the diatoms *S. costatum*, *C. curvisetus* were same as the ingestion rate of the total calanoid copepods on the *C. polykrikoides*, *Prorocentrum* spp., *Alexandrium* spp., *P.* kofoidii and *Gyrodinium* spp., and the diatoms *S. costatum*, *C. curvisetus*, respectively. In addition, Calbet and Saiz (2005) reviewed that the most copepod species feed well on the diverse ciliates and they also reported their ingestion rates.

The grazing coefficients (g, $d^{-1}$) were calculated as:

$$g = CR \times PC \times 24 \qquad (1)$$

where CR (ml $predator^{-1}h^{-1}$) is the clearance rate of the predator on a target prey at a prey concentration and PC is a predator concentration (predator $ml^{-1}$). The CR values were calculated as:

$$CR = IR/X \qquad (2)$$

where IR (cells eaten predator$^{-1}$h$^{-1}$) is the ingestion rate of predator on the target prey and X (cells ml$^{-1}$) is the prey concentration. These CR values were corrected using $Q_{10}$ = 3.2 (Hansen et al., 1997) because the in situ water temperature and the temperature used in the laboratory for this experiment (20-22$^{o}$C) were sometimes different.

## 4.3. Results

### 4.3.1. Abundance of metazooplankton

The mean abundance of metazooplankton was high from June 23 to August 13, while the maximum mean abundance of metazooplankton (1119 ind. m$^{-3}$) was observed on June 23 (Fig. 4.3A). Copepods were the predominant component of metazooplankton in all the sampling times except from July 11 to August 6 (Fig. 4.3) and the maximum mean abundance of copepods (453 ind. m$^{-3}$) was observed on May 21. Cladocerans were the predominant component of the total metazooplankton on July 11, but their maximum mean abundance (274 ind. m$^{-3}$) was highest on June 23 (Fig. 3C). Furthermore, the maximum abundance (178 ind. m$^{-3}$) of benthic invertebrate larvae was observed on June 23 (Fig. 4.3D). Chaetognaths, hydrozoans and the other metazooplankton taxa were present comparatively at high abundance from June 23 to August 13 (Fig. 4.3E-G). The maximum abundance of chaetognatha (87 ind. m$^{-3}$), hydrozoans (210 ind. m$^{-3}$) and the other metazooplankton (178 ind. m$^{-3}$) were observed on September 27, July 22 and June 23.

The total mean abundance of metazooplankton at the study area was 297 - 1,119 ind. m$^{-3}$ (mean ± SE = 534 ± 57, n = 16), while the abundance of copepods was 72 - 453 ind. m$^{-3}$ (mean ± SE = 213 ± 26, n = 16) (Table 4.2). The abundance of the cladocerans was 0.1 - 274 ind. m$^{-3}$ (mean ± SE = 54 ± 21, n = 16), while that of the larvae of benthic invertebrates was 23 - 386 ind. m$^{-3}$ (mean ± SE = 84 ± 9, n =

16) (Table 4.2). In addition, the abundance of the chaetognaths was 16 - 87 ind. $m^{-3}$ (mean ± SE = 48 ± 5, n = 16), while that of the hydrozoa was 9 - 210 ind. $m^{-3}$ (mean ± SE = 70 ± 15, n = 16).

Table 4.1 List of metazooplankton species and taxa present in the South Sea of Korea from May to November 2014

| Taxon | May - 07 | May - 21 | Jun - 05 | Jun - 12 | Jun - 23 | Jul - 01 | Jul - 11 | Jul - 22 | Aug - 06 | Aug - 13 | Aug - 22 | Sep - 01 | Sep - 15 | Sep - 27 | Oct - 08 | Nov - 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Copepods** | | | | | | | | | | | | | | | | |
| *Acartia erythrea* | | | | | | | | + | + | + | + | + | + | + | + | + |
| *Acartia omorii* | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *Calanopia thomsoni* | | | | | + | | | | | | | | | | + | |
| *Calanus sinicus* | + | + | + | | + | + | + | + | + | + | + | + | + | + | + | + |
| *Centropages abdominalis* | + | | | | | | | | | | | | | | | |
| *Centropages tenuiremis* | + | | | | | | | | | | | | + | | | |
| *Corycaeus affinis* | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *Euchaeta* sp. | | | | | | | | + | | + | + | | | | | + |
| *Labidocera euchaeta* | | + | + | + | + | + | + | + | + | + | + | + | | + | + | |
| *Labidocera rotunda* | + | + | + | + | + | + | + | | + | + | + | + | + | + | + | |
| *Labidocera* sp. | + | + | | | | + | + | + | + | + | + | + | + | + | + | |
| *Neocalanus* sp. | | | | | | | | | | | | | + | | | |
| *Oithona similis* | | | | | + | | + | | | | + | | + | + | + | + |
| *Oithona* sp. | | | | | | | | | | + | + | | | + | | |
| *Oncaea* sp. | | | | | | | | | | + | + | + | + | + | + | + |
| *Paracalanus parvus* | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *Pseudodiaptomus marinus* | | | | | + | + | + | + | | + | | + | + | + | + | |

(continued)

| Taxon | May - 07 | May - 21 | Jun - 05 | Jun - 12 | Jun - 23 | Jul - 01 | Jul - 11 | Jul - 22 | Aug - 06 | Aug - 13 | Aug - 22 | Sep - 01 | Sep - 15 | Sep - 27 | Oct - 08 | Nov - 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Sinocalanus tennelis* | | | | | | | | | | + | | | | | | |
| *Temora turbinata* | | | | | | | | | | | + | + | + | + | + | |
| *Temora discaudata* | | | | | | + | + | + | + | + | + | + | + | + | + | + |
| *Tortanus forcipatus* | | | + | | + | + | + | + | + | + | + | + | + | + | + | + |
| Harpacticoid | | + | + | | | | | | | | | | | | | + |
| Copepodite | | | + | | + | | | + | + | + | | + | + | + | + | + |
| **Cladocera** | | | | | | | | | | | | | | | | |
| *Evadne nordmanni* | | | + | | | | | | | | | | | | | |
| *Evadne tergestina* | + | + | | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *Penilia avirostris* | | | | + | + | + | + | + | + | + | + | | + | + | + | |
| *Podon polyphemoides* | + | + | + | | | + | + | | | | | | | | | |
| **Larvae** | | | | | | | | | | | | | | | | |
| Barnacle nauplius | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Barnacle crypris | + | + | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| Bivalve larvae | | | + | + | + | + | + | + | + | + | | + | + | + | + | + |
| Decapod megalopa | | + | | + | + | + | + | + | + | + | + | + | + | + | | |
| Decapod zoea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Decapod mysid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Echinodermata larvae | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Gastropoda larvae | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

(continued)

| Taxon | May - 07 | May - 21 | Jun - 05 | Jun - 12 | Jun - 23 | Jul - 01 | Jul - 11 | Jul - 22 | Aug - 06 | Aug - 13 | Aug - 22 | Sep - 01 | Sep - 15 | Sep - 27 | Oct - 08 | Nov - 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Polychaeta larvae | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Fish larvae | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | + |
| **Others** | | | | | | | | | | | | | | | | |
| Chaetognaths | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hydromedusa | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Siphonophora | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Amphipod | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Appendicularia | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Euphausia | + | | | | | | + | + | + | + | + | | + | + | | |
| Doliolida | | | | + | + | + | + | + | + | + | + | + | + | + | | |
| Salpida | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Ostracoda | | | + | + | + | + | + | + | + | + | + | | | + | | + |
| Fish egg | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | + |

Table 4.2 The range of the abundances (inds. $m^{-3}$) of total metazooplankton and each taxon, water temperatures (T, $^{o}C$), salinity and dissolved oxygen (mg $l^{-1}$) where each taxon was found in South Sea of Korea from May to November 2014.

| Taxon | T | Salinity | DO | Ab |
|---|---|---|---|---|
| Total metazooplankton | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 297 – 1,119 |
| Total copepod | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 72 – 453 |
| Total cladocerans | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 0.02 – 274 |
| Larvae of invertebrates | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 23 – 178 |
| Others* | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 28 – 386 |

### 4.3.2. Species composition of metazooplankton

During the study, 23 copepod, 4 cladoceran, 10 invertebrate larvae, and 10 the other metazooplankton species or taxa were found (Table 4.1.). These included the copepods *Acartia erythrea, A. omorii*, *Calanopia thomsoni*, *Calanus sinicus*, *Centropages abdominalis*, *C. tenuiremis*, *Corycaeus affinis*, *Euchaeta* sp., *Labidocera euchaeta*. *L. rounta*, *Labidocera* sp. *Neocalanus* sp. *Oithona similis*, *Oithona* sp., *Oncaea* sp. *Paracalanus parvus*, *Psuedodiaptomus marinus*, *Sinocalanus tennelis*, *Temora turbinata*, *T. discaudata*, *Tortanus forcipatus*, harpacticoida and copepodites, the cladocerans *Evadne nordmannii*, *E. tergestina*, *Penilia avirostris*, and *Podon polyphemoides*. In addition, barnacle larvae, decapod zoea, chaetognaths, siphonophora and appendicularia were observed.

Of these metazooplankters, *Acartia omorii*, *Corycaeus affinis*, barnacle nauplius, decapod zoea, decapod mysid, echinodermata larvae, gastropoda larvae, polychaete larvae, chaetognaths, hydromedusa, siphonophores, amphipod and appendicularia were present in all of the sampling interval (Fig. 4.4, Table 4.1). However, *Centropages abdominalis*, *Neocalanus* sp., *Sinocalanus tennelis* and *Evadne nordmannii* were only present in a single time of sampling at low abundance.

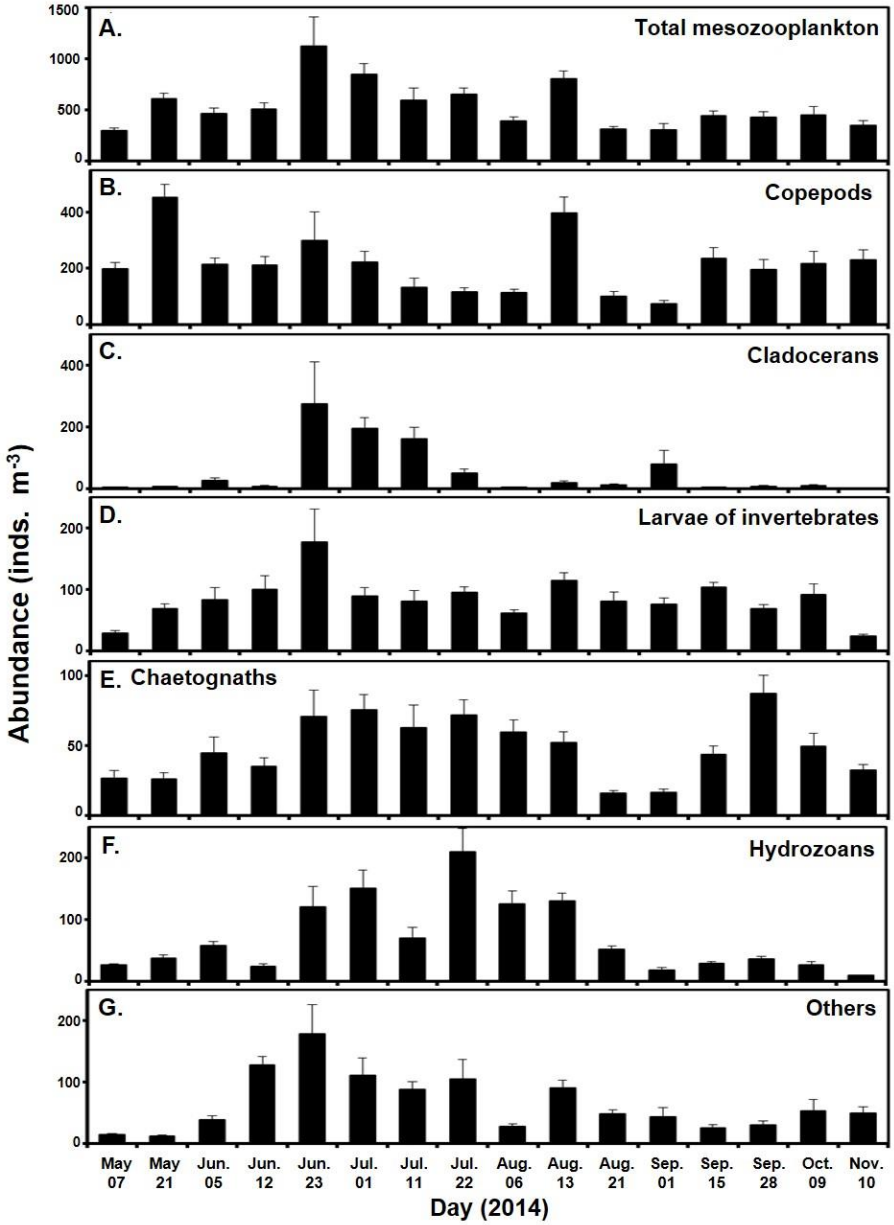



Fig. 4.3 Variations in the abundance (mean+SE, n=62) of total metazooplankton (A), copepods (B), cladocerans (C), larvae of invertebrates (D), chaetognaths (E), hydrozoans (F) and other metazooplankton (G) in the study area from May to November, 2014

Table 4.3 The range of mean abundance of the dominant taxon or species, water temperature, salinity and dissolved oxygen where each taxon was found in South Sea of Korea from May to November 2014.

| Taxon | T | Salinity | DO | Ab |
|---|---|---|---|---|
| *Acartia* spp. | 15.6 – 24.7 | 31.4 – 33.5 | 7.2 – 8.3 | 0.8 – 38 |
| *Calanus sinicus* | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 0 – 68 |
| *Corycaeus affinis* | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 4 – 154 |
| *Paracalanus parvus* | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 15 – 216 |
| *Temora discaudata* | 18.4 – 24.7 | 31.4 – 33.5 | 7.2 – 8.1 | 0 – 85 |
| *Tortanus forcipatus* | 18.4 – 24.7 | 31.4 – 33.9 | 7.2 – 8.1 | 0 – 33 |
| *Evadne tergestina* | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 0 – 273 |
| Barnacle nauplius | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 0.2 – 439 |
| Decapod zoea | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 4 – 109 |
| Chaetognatha | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 16 – 87 |
| Siphonophora | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 7 – 193 |
| Appendicularia | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 1 – 48 |
| Fish egg | 15.6 – 24.7 | 31.4 – 34.1 | 7.2 – 8.3 | 0 – 193 |

T: temperature (℃), S: salinity, DO: dissolved oxygen (mg $l^{-1}$), Ab: abundance (inds. $m^{-3}$)

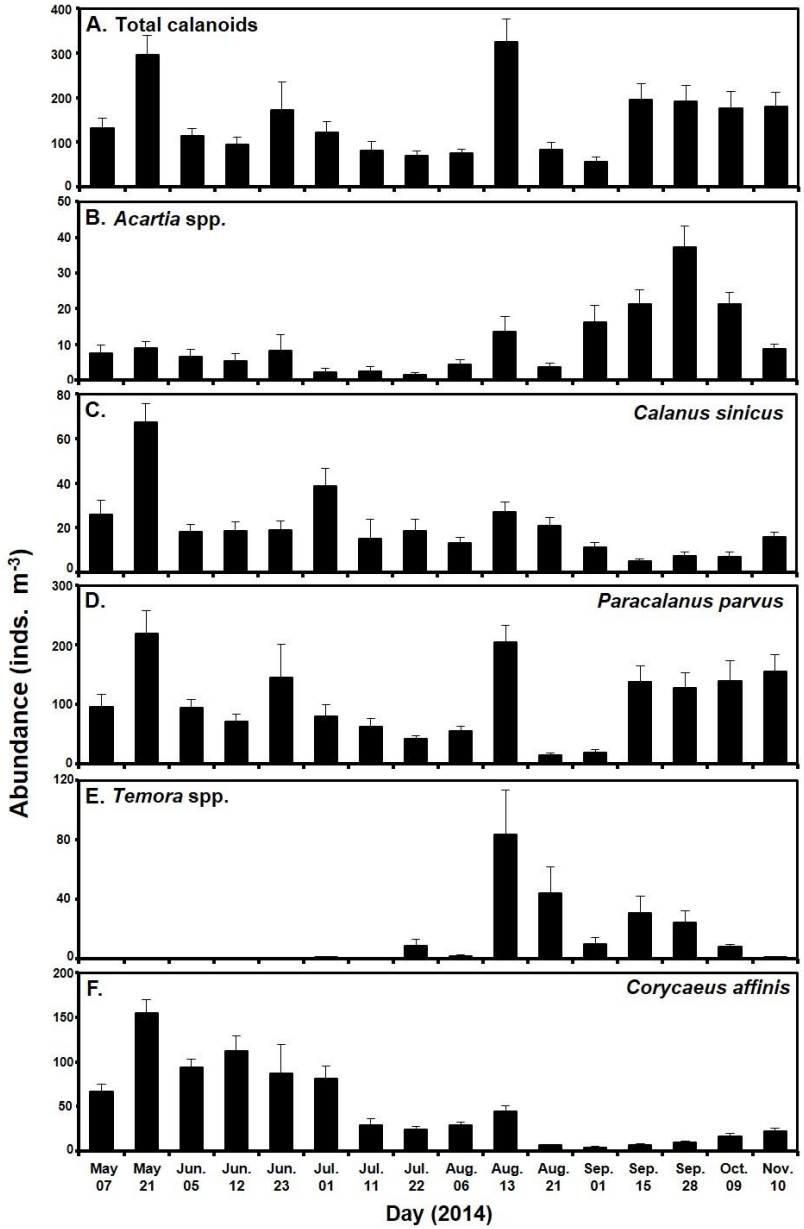



Fig. 4.4 Variations in the abundance (mean+SE, n=62) of dominant copepods total calanoids (A), *Acartia* spp. (B), *Calanus sinicus* (C), *Paracalanus parvus* (D), *Temora* spp. (E) and the predominant cyclopid *Corycaeus affinis* (F) in the study area from May to November, 2014

***Acartia* spp.** Their mean abundance was 0.8 – 38 ind. $m^{-3}$ and they were present when the surface water temperature was 15.6 – 24.7℃ and the salinity was 31.4 – 34.1 (Table 4.3.). These copepods were particularly abundant in the season of spring and fall when the water temperature was less than 24℃ (Fig. 4.4.B).

***Calanus sinicus***. Its mean abundance was 0.3 - 68 ind. $m^{-3}$, and it was present at 15.6 – 24.7℃ and salinity 31.4 – 34.1 (Fig. 4.4.C, Table 4.3.).

***Paracalanus parvus*.** Its mean abundance was 15 - 216 ind. $m^{-3}$, and it was present when the water temperature was 15.6 – 24.7℃ and the salinity was 31.4 – 34.1 (Table 4.3.). This copepod was abundant from May to June 23rd, August 13th and from September 15th to November 10th at 15.6 – 24.1℃ and salinity 32.0 – 34.1 (Fig. 4.4.D).

***Temora discaudata*.** Its mean abundance was 0 - 85 ind. $m^{-3}$, and it was present when the water temperature was 18.4 – 24.7℃ and the salinity was 31.4 – 33.5 (Table 4.3.). This copepod was abundant from August 13th to September 28th at 23.5 – 24.2℃ and salinity 31.4 – 32.4 (Fig. 4.4.E).

***Tortanus forcipatus*.** Its mean abundance was 0 - 33 ind. $m^{-3}$, and it was present when the water temperature was 18.4 – 24.7℃ and the salinity was 31.4 – 33.9 (Table 4.3.). This copepod was abundant in September at 23.8℃ and salinity 32.2 – 32.4 (Fig. 4.4.E).

***Corycaeus affinis*.** Its mean abundance was 4 - 154 ind. $m^{-3}$, and it was present when the water temperature was 15.6 – 24.7℃ and the salinity was 31.4 – 34.1 (Table 4.3.). This copepod was abundant from May to July 1st at the water temperature less than 22℃ and salinity was more than 33 (Fig. 4.4.F).

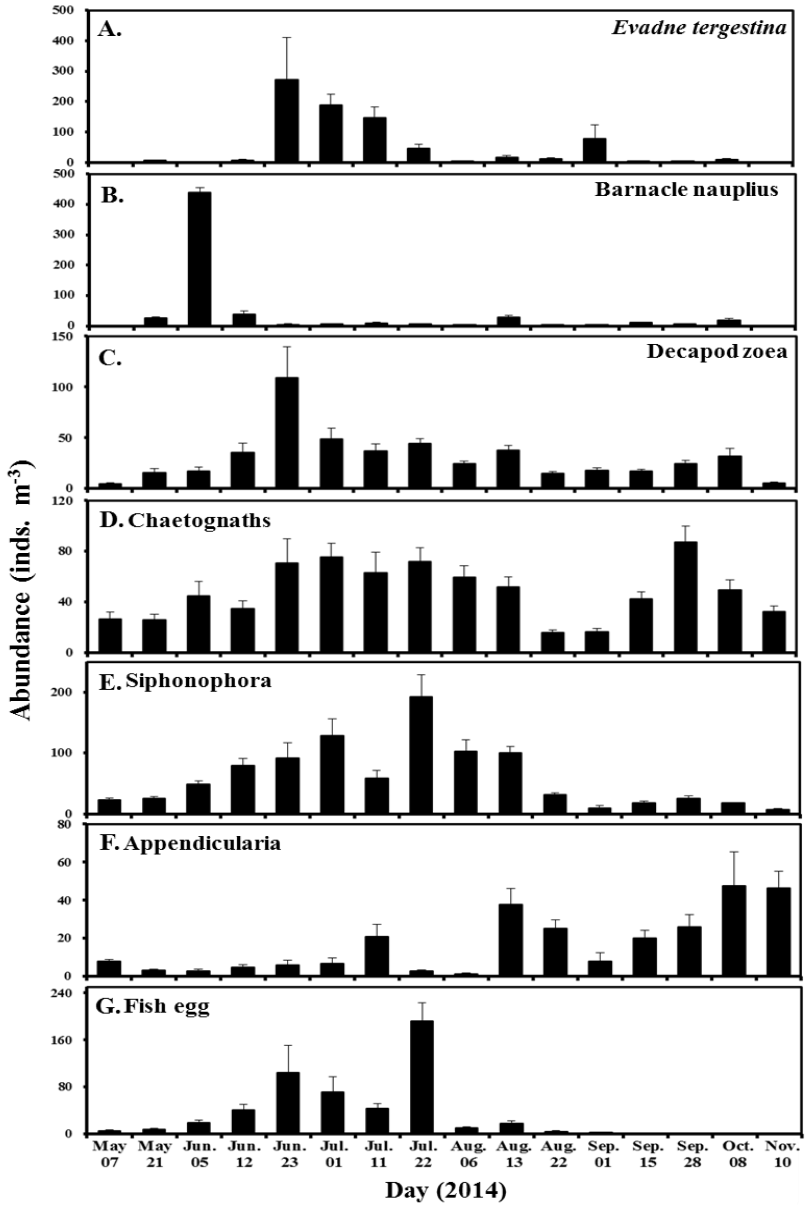



Fig. 4.5 Variations in the abundance (mean+SE, n=62) of dominant metazooplankton taxa Evadne tergestina (A), barnacle nauplius (B), decapod zoea (C), chaetognaths (D), siphonophora (E), appendicularia (F), and fish egg (G), in the study area from May to November, 2014

***Evadne tergestina*.** Its mean abundance was 0 - 33 ind. $m^{-3}$, and it was present at 15.6 - 24.7℃ and salinity 31.4 - 34.1 (Table 4.3). This cladoceran was abundant from June 23rd to July 11st at 21.1 - 22.5℃ and salinity 33.4 – 33.7 (Fig. 4.5A).

**Barnacle nauplius.** Their mean abundance was 0.2 - 439 ind. $m^{-3}$, and they were present at 15.6 - 24.7℃ and salinity 31.4 - 34.1 (Table 4.3). These larvae were only abundant on June 5th at 18.7℃ and salinity 33.8 (Fig. 4.5B).

**Decapod zoea.** Their mean abundance was 4 - 109 ind. $m^{-3}$, and they were present at 15.6 - 24.7℃ and salinity 31.4 - 34.1 (Table 4.3). These larvae were abundant from June 12th to July 22nd at 19.9 – 24.2℃ and salinity 32.8 - 33.7 (Fig. 4.5C).

**Chaetognaths.** Their mean abundance was 16 - 87 ind. $m^{-3}$, and they were present at 15.6 - 24.7℃ and salinity 31.4 - 34.1 (Table 4.3). They were abundant from June 5th to August 13$^{th}$ and from September 15th to October 8th at 18.7 – 24.7℃ and salinity 31.5 – 33.8 (Fig. 4.5D).

**Siphonophora.** Their mean abundance was 7 - 193 ind. $m^{-3}$, and they were present at 15.6 - 24.7℃ and salinity 31.4 - 34.1 (Table 4.3). They were abundant from June 12th to August 13th at 19.9 - 24.7℃ and salinity 31.5 - 33.7 (Fig. 4.5E).

**Appendicularia.** Their mean abundance was 1 - 48 ind. $m^{-3}$, and they were present at 15.6 - 24.7℃ and salinity 31.4 - 34.1 (Table 4.3). They were abundant from August 13th to November 10th at 18.4 - 24.2℃ and salinity 31.4 - 33.5 (Fig. 4.5F).

**Fish eggs.** Their mean abundance was 0 - 193 ind. $m^{-3}$, and they were present at 15.6 - 24.7℃ and salinity 31.4 - 34.1 (Table 4.3). They were abundant from June 12th to July 22nd at 19.9 – 24.2℃ and salinity 32.8 - 33.7 (Fig. 4.5F).

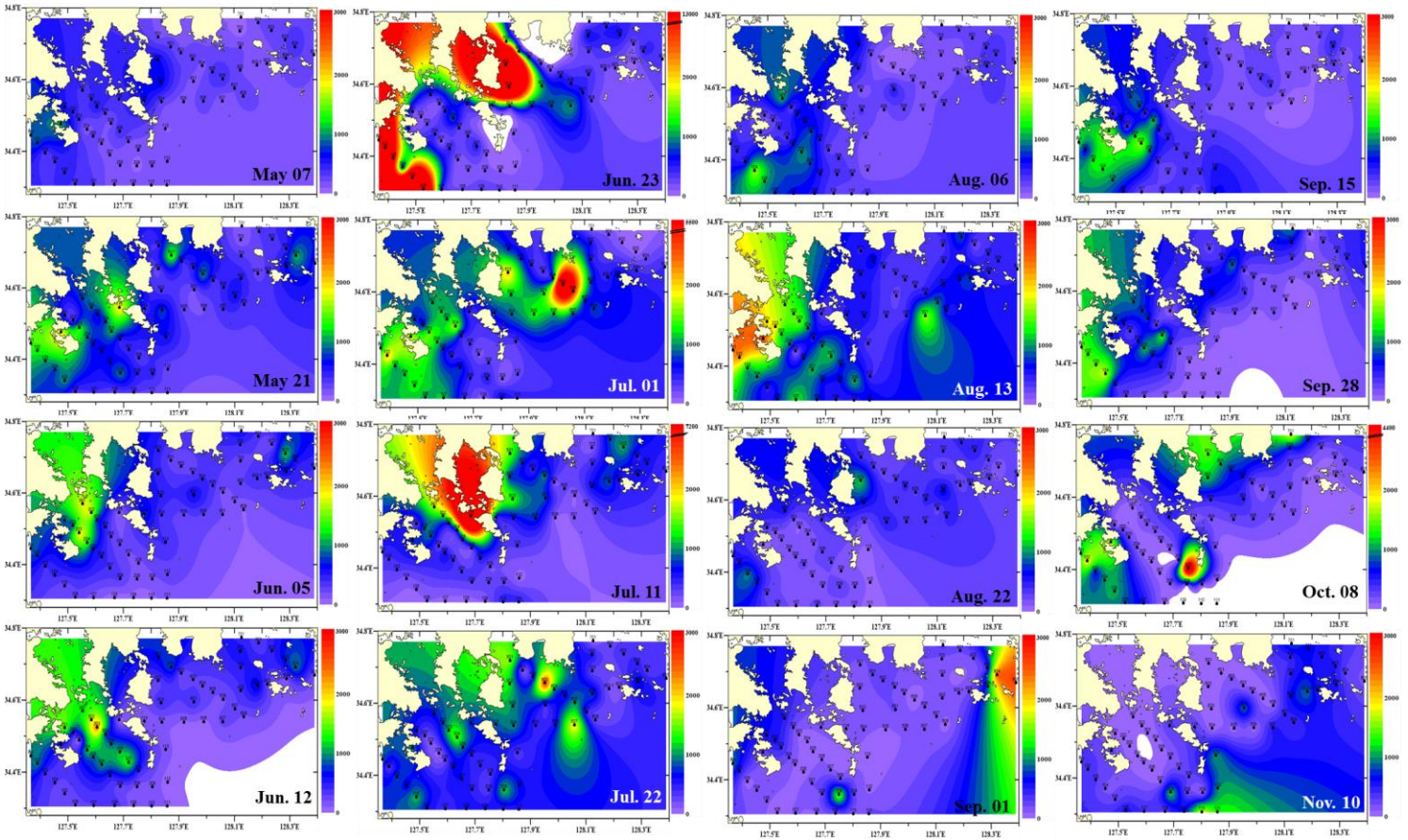



Fig. 4.6 Spatio-temporal distributions of the total metazooplankton (inds. $m^{-3}$) in the study area from May to November, 2014. May to November

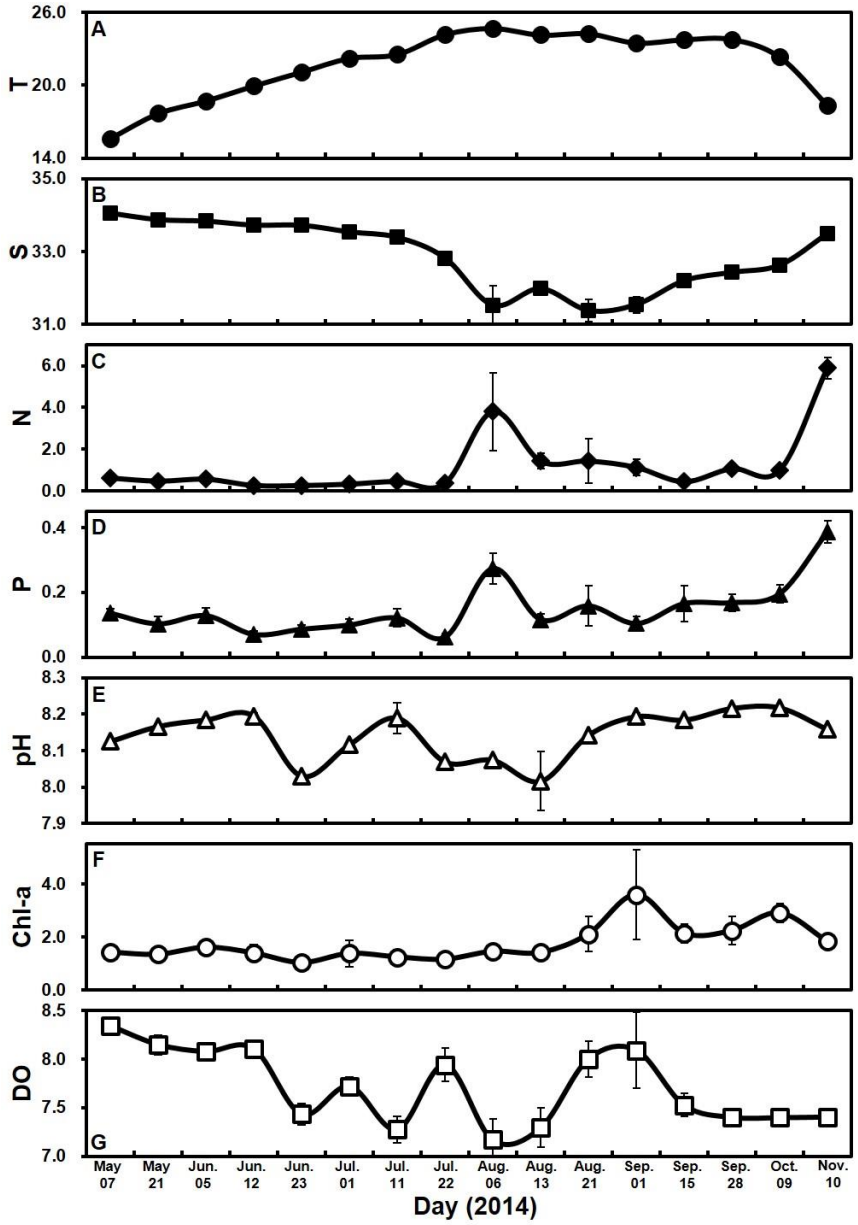



Fig. 4.7 Physical and chemical properties (mean±SE, n=62) of the surface water in the study area during this study. Temperature (A, ℃), salinity (B), $NO_2$+$NO_3$ (C, μM), $PO_4$ (D, μM), pH (E), chlorophyll-a (F, μg/l), dissolved oxygen (G, mg/l)

### 4.3.3. Spatio-temporal distributions of the total metazooplankton

Metazooplankton predominantly inhabited the marginal and shallower regions (< 30m depth) of the study area. From May 7 to August 13, the metazooplankton occurred in high concentrations (< 13,200 inds. $m^{-3}$) near the shallow waters of Goheung and Yeosu (Fig. 4.6). In contrast, on September 1, the metazooplankton regionally concentrated (< 2700 inds. $m^{-3}$) in the shallow water of Tongyoung, and the abundances of the metazooplankton were high (< 1800 inds. $m^{-3}$) in the shallow water of Goheung from September 15 to 27 (Fig. 4.6), respectively. Furthermore, the metazooplankton was distinctively more concentrated (< 1900 inds. $m^{-3}$) in the offshore region (> 30m depth) than the shallow waters in November (Fig. 4.6).

### 4.3.4. Physicochemical properties of the sampling points

From May 7 to November 10 2014, mean surface water temperature at the study area varied from 15.6 to 24.7℃, the highest temperature was in August, while the lowest temperature was in May during the study (Fig. 4.7A). The surface water temperature was higher than 20℃ in all of the study area from July 1 to October 8 (Fig. 4.7A). The thermocline lightly began to be formed first at 10m depth on July 1 and then the thermocline has gradually formed at deeper depth (>20m) from August 6 (Fig. 4.7A, 4.8A). Although the thermocline depth reached to deeper than 30m on September 1, the thermocline was only observed in offshore (Fig. 4.8A). The temperature layers of the study area was entirely mixed and disappeared in October (Fig. 4.7A, 4.8A).

Salinity was observed between 31.4 and 34.1, the highest salinity was in May, while the lowest salinity was in August (Fig. 4.7B) when the daily precipitation amount was up to 200mm $day^{-1}$ (data is not shown) for the past five days in the study area. Nutrient concentrations ($NO_2$+$NO_3$ and $PO_4$) peaked twice in August and November, the concentrations were more than 4 times higher than that at the other season (Fig. 4.7C, D). $NO_2$+$NO_3$ concentration ranged from 0.25 to 5.88

μM, while the $PO_4$ concentration ranged from 0.06 to 0.39 μM (Fig. 4.7C, D). The pH ranged 8.02 – 8.22 during the study, while the chlorophyll-a concentration (chl-a) was 1.0 – 3.6 g $l^{-1}$ peaked on September 1 (Fig. 4.7E, F). In addition, dissolved oxygen (DO) was 7.2 – 8.3 mg $l^{-1}$ (Fig. 4.7G)

### 4.3.5. Spatio-temporal variations in the abundance of phytoplankton and microzooplankton

Refer to the previous study that conducted with the present study (Jeong et al. 2016), the abundances of the total dinoflagellates were generally high in the shallow waters where depth is less than 20 m. The mean abundance of total dinoflagellate peaked (195 cells $ml^{-1}$) on June 12 in the surface water (Fig. 4.8). *Prorocentrum donghaiense, Ceratium furca., Alexandrium fraterculus* and *Cochlodinium polykrikoides* were the typical predominant species and they formed red tides. *P. donghaiense* red tides patches were found at the inner and outer stations of the western area from June 12 to July 1, *C. furca* red tides patches were found at the inner stations of the middle area from July 11 to August 21, *A. fraterculus* red tides patches were found at the inner stations of the middle area on August 21, *C. polykrikoides* red tides patches were found in the western area on August 21, but extended to the middle and eastern areas from September 1. *C. polykrikoides* red tides patches were recorded on October 9, but disappeared on November 10. Thus, *P. donghaiense, C. furca, A. fraterculus*, and *C. polykrikoides* had different temporal and spatial distributions in the study area.

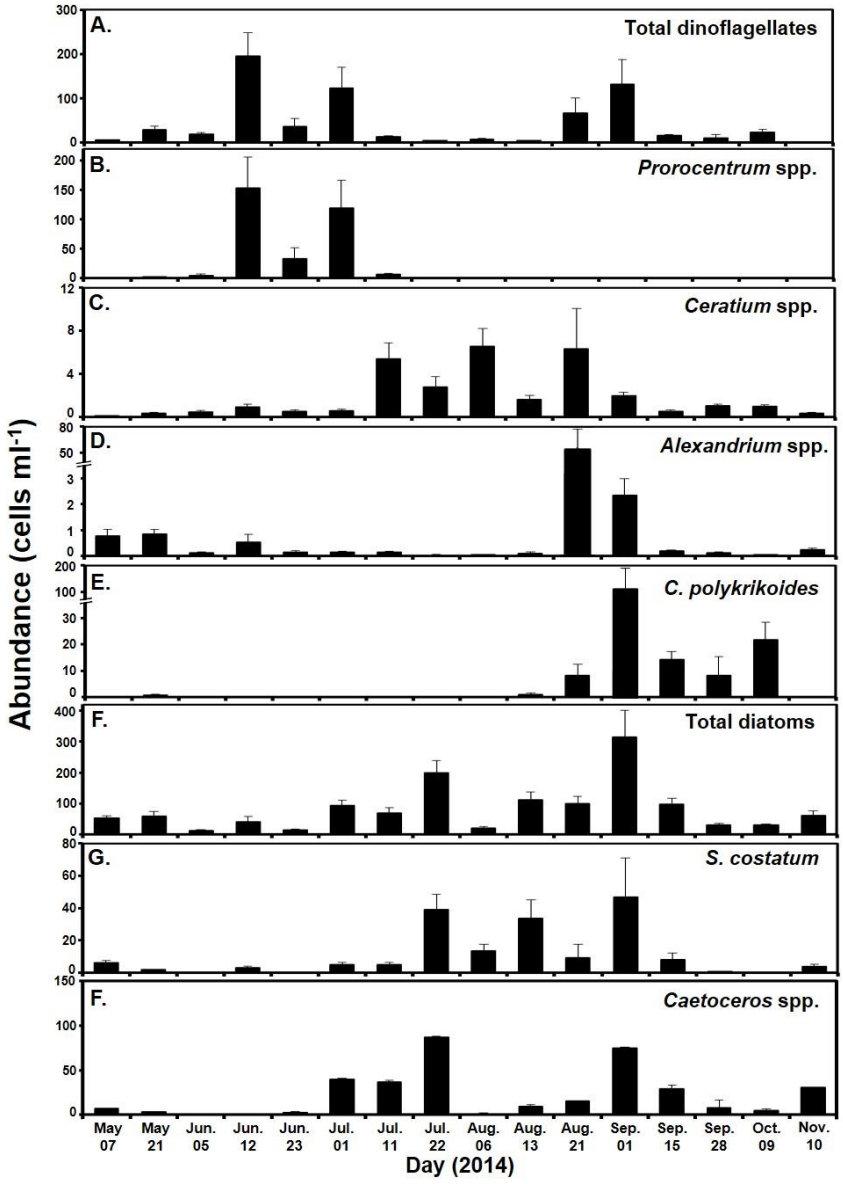



Fig. 4.8 Variations in the abundance (mean+SE, n=62) of total dinoflagellates (A), *Prorocentrum donhaiense* (B), *Ceratium* spp. (C), *Alexandrium* spp. (D), *Cochlodinium polykrikoides* (E), total diatoms (F), *Skeletonema costatum* (G) and *Chaetoceros* spp. (H), in the surface waters of study area from May to November, 2014

The mean abundance of total diatoms ranged from 15 to 315 cells $ml^{-1}$ in the surface water (Fig. 4.8). The diatoms were generally found in high concentration in the nearest stations with the coast of Yeosu - Namhae region where depths were less than 20 m (Jeong et al. 2016). Furthermore, typically *Skeletonema costatum* and *Chaetoceros curvisetus* were the predominant diatom species in the study area, while the proportion of the *S. costatum* and *C. curvisetus* concentration were up to 72% of total mean diatoms during the study. In addition, *S. costatum* and *C. curvisetus* peaked (47 cells $ml^{-1}$ and 87 cells $ml^{-1}$, respectively) on September 1sand July 22, respectively (Fig. 4.8).

**4.3.6. Correlations between the abundances of the major metazooplankton taxa and environmental factors.**

The abundance of total metazooplakton was significantly negatively correlated with pH but positively correlated with the concentration of total dinoflagellate (Table 4.4).

The abundance of copepods was significantly positively correlated with salinity and the concentration of phosphate, but negatively correlated with temperature, pH and DO (Table 4.4A). In addition, the abundances of the cladocerans were significantly positively correlated with DO, but significantly negatively correlated with the concentration of phosphate (Table 4.4A).

The abundance of the invertebrate larvae was significantly positively correlated with temperature and the concentration of silicate, but negatively correlated with salinity and pH (Table 4.4A). However, the abundance of invertebrate larvae significantly positively correlated with the concentrations of chlorophyll-a, abundance of dinoflagellate and tintinnid ciliates (Table 4.4B).

The abundance of the chaetognaths was significantly positively correlated with temperature, concentrations of phosphate and silicate, but negatively correlated

with salinity, pH and DO (Table 4.4A). However, the abundance of chaetognaths significantly positively correlated with the concentrations of chlorophyll-a, abundance of tintinnid ciliates (Table 4B). In addition, the abundance of the hydrozoans was significantly positively correlated with temperature, concentrations of silicate, but negatively correlated with pH (Table 4.4A).

At the species level, the abundance of *Calanus sinicus*, echinodermata larvae and siphonophora significantly positively correlated with the abundance of total diatoms, while the abundance of *Corycaeus affinis*, barnacle nauplius, decapod zoea, echinodermata larvae, fish larvae and siphonophora was significantly positively correlated with the abundance of diatom *Pseudonitzchia* spp. (Table 4.5A).

The abundance of *Corycaeus affinis*, *Labidocera euchaeta*, *L. rotunda*, barnacle nauplius, decapod zoea, fish larvae, hydromedusa and siphonophora was significantly positively correlated with the abundance of total phototrophic dinoflagellate. In addition, the abundance of *Corycaeus affinis*, *Labidocera euchaeta*, *L. rotunda*, *Paraclanus parvus*, *Penilia avirostris*, barnacle nauplius, decapod zoea, fish larvae, chaetognaths, hydromedusa and siphonophora was significantly positively correlated with that of dinoflagellate *Prorocentrum donghaeiense* (Table 4.5B).

The abundance of *Corycaeus affinis*, *Labidocera euchaeta*, *L. rotunda*, *Paraclanus parvus*, copepodite, *Penilia avirostris*, barnacle nauplius, decapod zoea, echinodermata larvae, fish larvae, chaetognaths, hydromedusa and siphonophora was significantly positively correlated with the abundance of ciliate *Tintinnopsis tubulosoides* (Table 4.5C). However, the abundance of sapida was significantly negatively correlated with the abundance of ciliate *Tintinnopsis tubulosoides* (Table 4.5C).

Table 4.4 Correlations between the abundance of metazooplnkton taxon and physical, chemical and biological factors in South Sea of Korea from May to November 2014

**A. Physical and chemical factors**

| Components | T | S | pH | DO | N | P | Si |
|---|---|---|---|---|---|---|---|
| Total Metazooplankton | | | -0.106** | | | | |
| Copepods | -0.123** | 0.083* | -0.102** | -0.082* | | 0.097** | |
| Cladocerans | | | | 0.071* | | -0.069* | |
| Larvae of invertbrates | 0.109** | -0.072* | -0.093** | | | | 0.095** |
| Chaetognaths | 0.117** | -0.081* | -0.112** | -0.109** | | 0.132** | 0.120** |
| Hydrozoans | 0.176** | | -0.118** | | | | 0.208** |
| Others[a] | | 0.063* | | | -0.074* | -0.127** | |

**B. Biological factors**

| Components | Chl-a | DIA | DIN | EUG | CRY | HTD | TCI | NCI |
|---|---|---|---|---|---|---|---|---|
| Total Metazooplankton | | | 0.105** | | | | | |
| Copepods | | | | | | | | |
| Cladocerans | | | | | | | | |
| Larvae of invertbrates | 0.126** | | 0.193** | | | | 0.094** | |
| Chaetognaths | 0.123** | | | | | | 0.107** | |
| Hydrozoans | | | | | | | | |
| Others[a] | -0.070* | | | | | | | |

T: temperature , S: salinity, DO: dissolved oxygen, N: nitrite + nitrate , P: phosphate, Si: silicate, Chl-a: chlorophyll-a, DIA: diatoms, DIN: phototrophic dinoflagellates, EUG: eunglenophytes, CRY: cryptophytes, HTD: heterotrophic dinoflagellates, TCI: tintinnid ciliates, NCI: naked ciliates, [a] See Table 1. * $P < 0.05$. ** $P < 0.01$ (n>960)

Table 4.5. Correlations between the abundance of metazooplnkton and the abundance of the potential prey species in South Sea of Korea from May to November 2014

| **A. Diatom** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Components** | DIA | Caf | Cco | Ccu | Cde | Cso | Ezo | Lbo | Lda | Lme | Lmi | Ppu | Psp | Sco | Tni | Tde |
| *A. erythraea* | | | | | | | | | | | | | | | | |
| *A. omorii* | | | | | | | | | | | | | | | | |
| *C. sinicus* | -0.076* | -0.063* | -0.090** | -0.072* | | | | | | | | | | | | |
| *C. affinis* | | | -0.089** | -0.073* | -0.072* | | 0.108** | -0.063* | | | | | 0.150** | | | |
| *L. euchaeta* | | | | | | | | | | | | | | | | |
| *L. rotunda* | | | | | | | | | | | | | | | | |
| *P. parvus* | | -0.069* | -0.071* | | | | | | | | | | | | | |
| *P. marinus* | | | | | | | | | | | | | | | | |
| *T. turbinate* | | | | | | | | | | | | | | | | |
| *T. discaudata* | | | | | | | | | | | | | | | | |
| *T. forcipatus* | | | | | | | | | | | | | | | | |
| Copepodite | | | | 0.110** | | | | | | | | | | | 0.088** | |
| *E. terestina* | | | | | | | | | | | | | | | | |
| *P. avirostris* | | | | | | | | | | | | | | | | |
| Bar. nauplius | | | | | | | 0.114** | | | | | | 0.256** | | | |
| Decapod zoea | | | | | | | | | | | | | 0.182** | | | |
| Decapod mysid | | | | | | | | | | | | | | | | |
| Echi. larvae | 0.066* | | 0.083* | | | | | | | | 0.114** | 0.072* | 0.091** | | | |
| Fish larvae | | | | | | | | | | | | | 0.086** | | | |
| Appendicularia | | | | | | | | | | | | | | | | |
| Chaetognatha | | | | | | | | | | | | | | | | |
| Salpida | | | | 0.069* | | | | | | | | | | | | |
| Hydromedusa | | | | | | | | | | | | | | | | |
| Siphonophora | 0.065* | | | 0.134** | 0.070* | | | | | | | | 0.136** | | | |
| Fish eggs | | | | | | | | | | | | | | | | |

(continued)

**B. Phototrophic dinoflagellate**

| **Components** | PTD | Asa | Asp | Cpo | Cfu | Cfs | Cko | Ctr | Dca | Gsp | Pba | Pdo | Pmi | Ptr | Str | Sym |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. erythraea* | | | | | | | -0.070* | 0.195** | 0.107** | | | | | | | |
| *A. omorii* | | | | | | | | 0.063* | 0.088* | | | | | | | |
| *C. sinicus* | | | | | | | | -0.077* | | | | | | | | |
| *C. affinis* | 0.148** | | | | | | | -0.076* | | 0.084** | 0.083* | 0.215** | | 0.124** | 0.090** | |
| *L. euchaeta* | 0.067* | | | | | | | | | | | 0.084** | | | | |
| *L. rotunda* | 0.064* | | | | | | | | | | | 0.095** | | | | |
| *P. parvus* | | | | | | | | | | | | 0.072* | | | | |
| *P. marinus* | | | | | | | | | | | | | | | | |
| *T. turbinate* | | | | | | | | | 0.085* | | | | | | | |
| *T. discaudata* | | | | | | | | | | | | | | | | |
| *T. forcipatus* | | | | | | | | | 0.173** | | | | | | | |
| Copepodite | | | | | | | | | | | | | | | | |
| *E. terestina* | | | | 0.080* | | | 0.092** | | | | | | | | | |
| *P. avirostris* | | | | | | 0.136** | 0.169** | | | | | 0.100** | | | | |
| Bar. nauplius | 0.124** | | | | | 0.112** | | | | 0.072* | | 0.164** | | 0.076* | | |
| Decapod zoea | 0.227** | | | | | 0.107** | | | | | | 0.311** | | | | |
| Decapod mysid | | | | | | | | | | | | | | | | |
| Echi. larvae | | | | | | 0.077* | -0.068* | | | | | | | | | |
| Fish larvae | 0.094** | | | | | | 0.074 | | | | | 0.146** | | | | |
| Appendicularia | | | | | | | | | | | | | | | | |
| Chaetognatha | | | | | | | 0.080* | | | | | 0.115** | | | | |
| Salpida | | | | | | | | | | | | | | | | |
| Hydromedusa | 0.093** | 0.118** | | | | | | | | | | 0.118** | | | | |
| Siphonophora | 0.087** | | | | | | | | | | | 0.146** | | | | |
| Fish eggs | | | | | | | 0.066* | | | | | | | | | |

(continued)

**C. Others**

| **Components** | EUG | CRY | HTD | TCI | NCI | Mru | Nsc | Pko | Ppa | Prsp | Tfu | Ttu |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. erythraea* | | | | | | | | 0.140** | | | | |
| *A. omorii* | | | | | | | | | | | | |
| *C. sinicus* | | | | | | | | | | | | |
| *C. affinis* | | | | | | | | | 0.063* | | | 0.204** |
| *L. euchaeta* | | | | | | | | | | | | 0.069* |
| *L. rotunda* | | | | 0.069* | | | | | | | | 0.216** |
| *P. parvus* | | | | | | | | | | | | 0.096** |
| *P. marinus* | | | | | | | | | | | | |
| *T. turbinate* | | | | | | | | | | | | |
| *T. discaudata* | | | | | | | | | | | | |
| *T. forcipatus* | | | | | | | | 0.136** | | | | |
| Copepodite | | | | | | | | | | | | 0.065* |
| *E. terestina* | | | | | | | | | 0.128** | | | |
| *P. avirostris* | 0.230** | | | 0.155** | | | | | | 0.072* | | 0.341** |
| Bar. nauplius | | | | | | | | | | | | 0.175** |
| Decapod zoea | | | | 0.117** | | | | | | | | 0.369** |
| Decapod mysid | | | | | | | | | 0.119** | | | |
| Echi. larvae | | | | | | | | | | | | 0.161** |
| Fish larvae | | | | | | | | | 0.066* | | | 0.132** |
| Appendicularia | | | | | | | | | | | | |
| Chaetognatha | | | | 0.110** | | | | | | | | 0.218** |
| Salpida | | | | | | | | | | | | -0.070* |
| Hydromedusa | | | | | | | | | | 0.080* | | 0.181** |
| Siphonophora | | | | | | | | | | | | 0.173** |
| Fish eggs | | | | | | | | | 0.125** | | | |

Bar.: barnacle, Echi.: echnodermata, DIA: diatom, Caf: *Chaetoceros affinis*, Cco: *Chaetoceros compressus*, Ccu: *Chaetoceros curvisetus*, Cde: *Chaetoceros decipiens*, Cso: *Chaetoceros socialis*, Ezo: *Eucampia zodiacus*, Lbo: *Lauderia borealis*, Lda: *Leptocylindrus danicus*, Lme: *Leptocylindrus mediterraneus*, Lmi: *Leptocylindrus minimus*, Ppu: *Pseudonitzchia pungens*, Psp: *Pseudonitzchia* sp., Sco: *Skeletonema costatum*, Tni: *Thalassionema nitzchioides*, Tde: *Thalssiosira decipiens*, PTD: phototrophic dinoflagellate, Asa: *Akashiwo sanguenea*, Asp: *Alexandrium* sp., Cpo: *Cochlodinium polykrikoides*, Cfu: *Ceratium furca*, Cfs: *Ceratium fusus*, Cko: *Ceratium kofoidii*, Ctr: *Ceratium tripos*, Dca: *Dinophysis caudata*, Gsp: *Gonyaulax* sp., Pba *Prorocentrum balticum*, Pdo: *Prorocentrum dongaiense*, Pmi: *Prorocentrum minimum*, Ptr: *Prorocentrum triestinium*, Str: *Scrippsiella trochoidea*, Sym : *Symbiodinium* sp., EUG: eunglenophytes, CRY: cryptophytes, HTD: heterotrophic dinoflagellate, TCI: tintinnid ciliate, NCI: naked ciliate, Mru: *Mesodinium ruburum*, Nsc: *Noctiluca scintilans*, Pko: *Polykrikos kofoidii*, Ppa: *Protoperidinium parvum*, Prsp: *Protoperidinium* sp., Tfu: *Tiarina fusus*, Ttu: *Tintinnopsis tubulosoide*

### 4.3.7. Grazing impact by the copepods on the phytoplankton and microzooplankton

Calanoid copepods are known to feed on *Prorocentrum donghaiense* (Kim, 2005). When the abundances of *Prorocentrum* spp. and co-occurring total calanoids species were 0.2 – 2725 cells $ml^{-1}$ and 0.9 – 1076 inds. $m^{-3}$, respectively, the calculated grazing coefficients attributable to total calanoids on co-occurring *Prorocentrum* spp. were 0.001 – 0.029 $d^{-1}$ (i.e., up to 2.9% of the population of *Prorocentrum* spp. was removed by calanoid copepods in a day (Fig 4.9, 4.11). Furthermore, when the abundances of *Aalexandrium* spp. and co-occurring total calanoids species were 0.1 – 1280 cells $ml^{-1}$ and 1 – 1080 inds. $m^{-3}$, respectively, the calculated grazing coefficients attributable to calanoid copepods on co-occurring *Aalexandrium* spp. were 0.001 – 0.009 $d^{-1}$ (i.e., up to 0.8% of the population of *Aalexandrium* spp. was removed by calanoids in a day) (Fig 4.9, 4.11).

Calanoids are known to feed on *Cochlodinium polykrikoides* (Kim, 2005). When the abundances of *C. polykrikoides* and co-occurring calanoid copepod were 1 – 2990 cells $ml^{-1}$ and 1 – 2480 inds. $m^{-3}$, respectively, the calculated grazing coefficients attributable to calanoid copepod on co-occurring *C. polykrikoides* were up to 0.018 $d^{-1}$ (i.e., up to 1.8% of the population of *C. polykrikoides* was removed by calanoids in a day) (Fig 4.9, 4.11).

Calanoids are known to feed on the diatom species *Skeletonema costatum* and *Chaetoceros* curvisetus (Kim, 2005). When the abundances of the diatoms species (*S. costatum* and *C. curvisetus*) and co-occuring calanoid copepods were 0.23 – 1390 cells $ml^{-1}$, 1 – 1522cells $ml^{-1}$ and up to 1930 inds. $m^{-3}$, respectively, the calculated grazing coefficients attributable to calanoid copepods on co-occurring *S. costatum* and *C. curvisetus* were 0.005 – 0.05 $d^{-1}$ and 0.004 – 0.032 $d^{-1}$, respectively. (i.e., up to 4.9% and 3.2 % of the population of *S. costatum* and *C. curvistus* were removed by total calanoids in a day, respectively) (Fig 4.9).

Most of calanoid copepods such as *Acartia* spp. are known to feed on microzooplankton including heterotrophic dinoflagellates and ciliates (Calbet and Saiz, 2005, Kim, 2005). When the abundances of *Gyrodinium* spp. and co-occurring calanoid copepods were up to 42 cells $ml^{-1}$ and 2940 inds. $m^{-3}$, respectively. The calculated grazing coefficients attributable to total calanoid copepods on co-occurring *Gyrodinium* spp. were 0.0003 – 0.047 $d^{-1}$ (i.e., up to 4.6% of the population of *Gyrodinium* spp. were removed by total calanoids in a day) (Fig 4.10, 4.11).

When the abundances of *Polykrikos* spp. and co-occurring calanoid copepods were 0.02 – 79 cells $ml^{-1}$ and up to 1035 inds. $m^{-3}$, respectively, the calculated grazing coefficients attributable to total calanoid copepods on co-occurring *Polykrikos* spp. were 0.0001 – 0.008 $d^{-1}$ (i.e., up to 0.8% of the population of *Polykrikos* spp. were removed by total calanoids in a day) (Fig 4.10, 4.11). Furthermore, when the abundances of total ciliates and co-occurring calanoid copepods were up to 273 cells $ml^{-1}$ and 2943 inds. $m^{-3}$, respectively, the calculated grazing coefficients attributable to total calanoid copepods on co-occurring total ciliates were 0.000002 – 0.00001 $d^{-1}$ (i.e., up to 0.001% of the population of total ciliates were removed by total calanoids in a day) (Fig 4.10, 4.11).

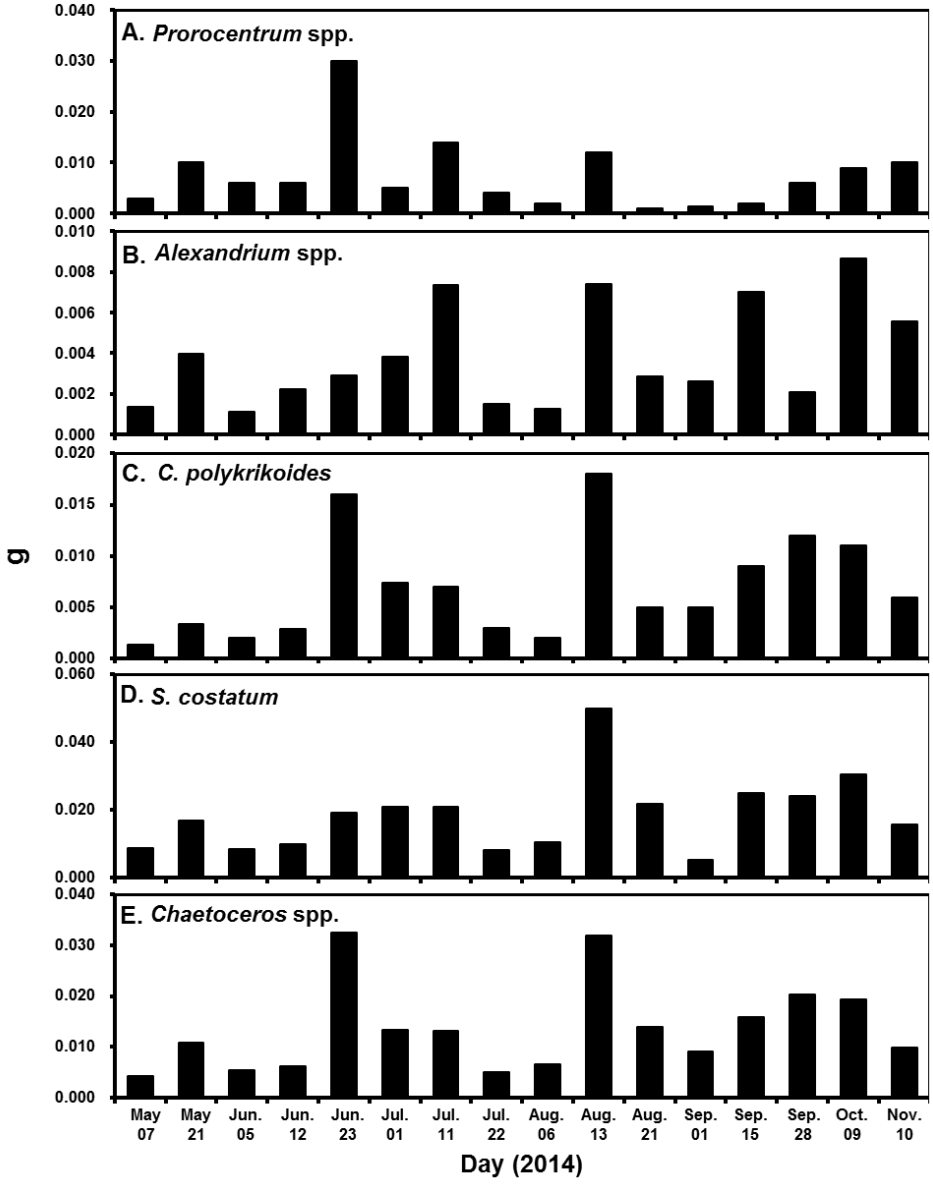



Fig. 4.9 The calculated maximum grazing coefficients (g, d-1) attributable to calanoid copepods on the species of phototrophic dinoflagellate and diatom *Prorocentrum* spp. (A), *Alexandrium* spp. (B), *Cochlodinium polykrikoides* (C), *Skeletonema costatum* (D), and *Chaetoceros* spp. (E) in the study area from May to November, 2014

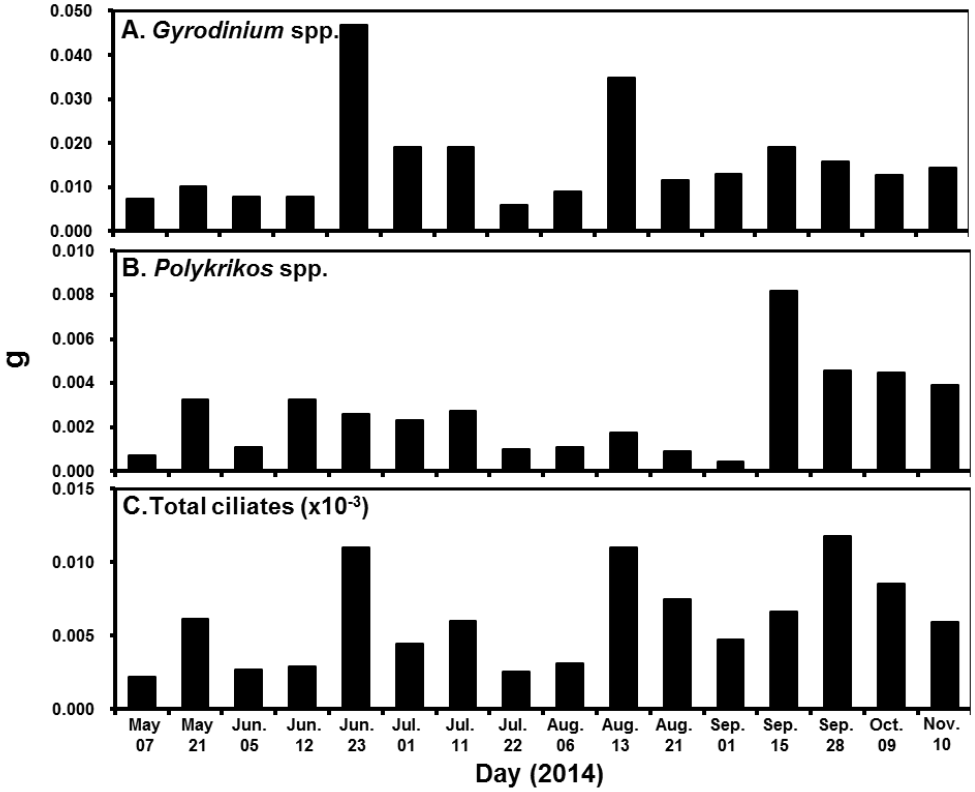



Fig. 4.10 The calculated maximum grazing coefficients (g, d-1) attributable to calanoid copepods on the dominent microzooplankton. *Gyrodinium* spp. (A), *Polykrikos* spp. (B), and total ciliates (C) in the study area from May to November, 2014

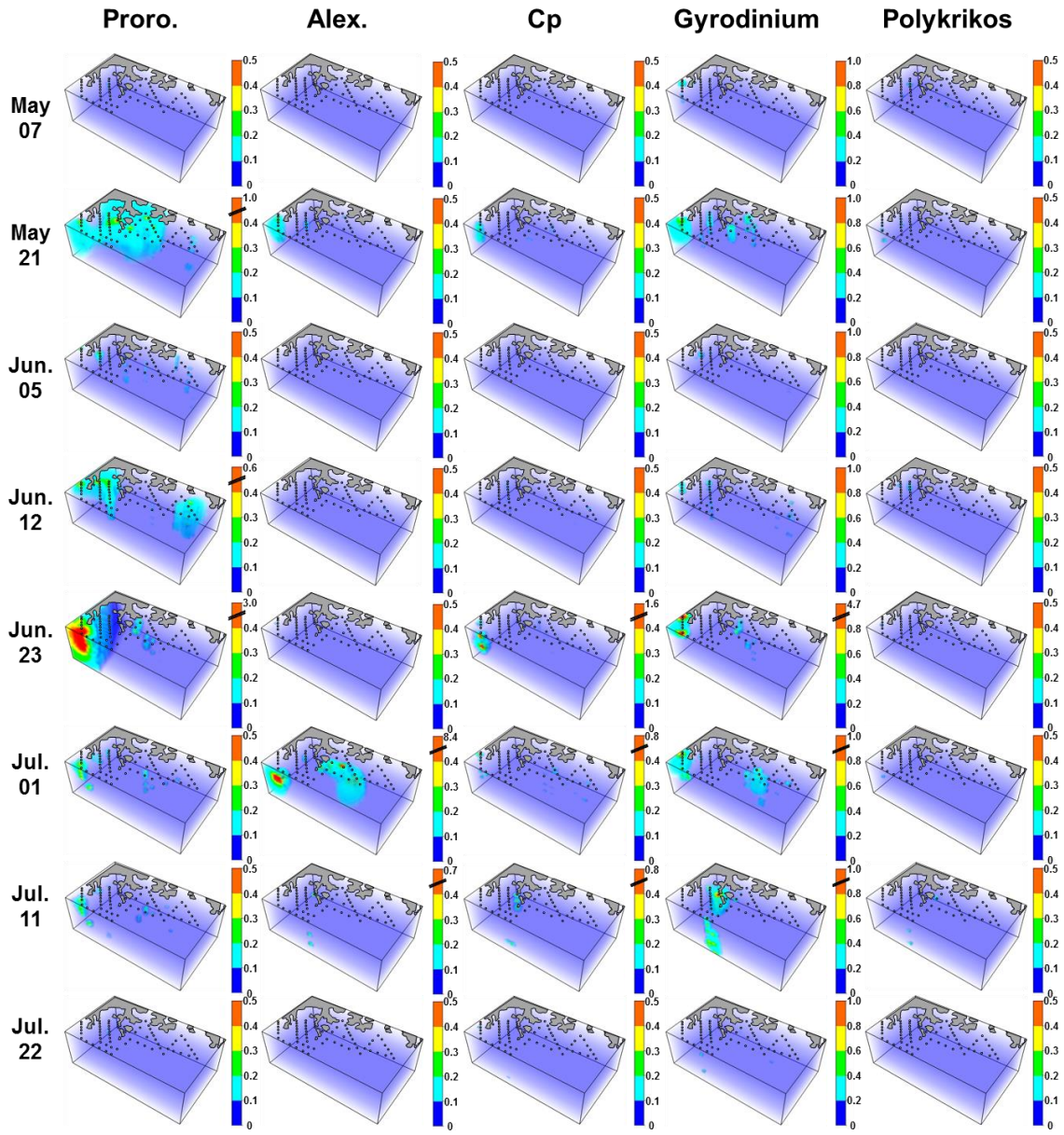
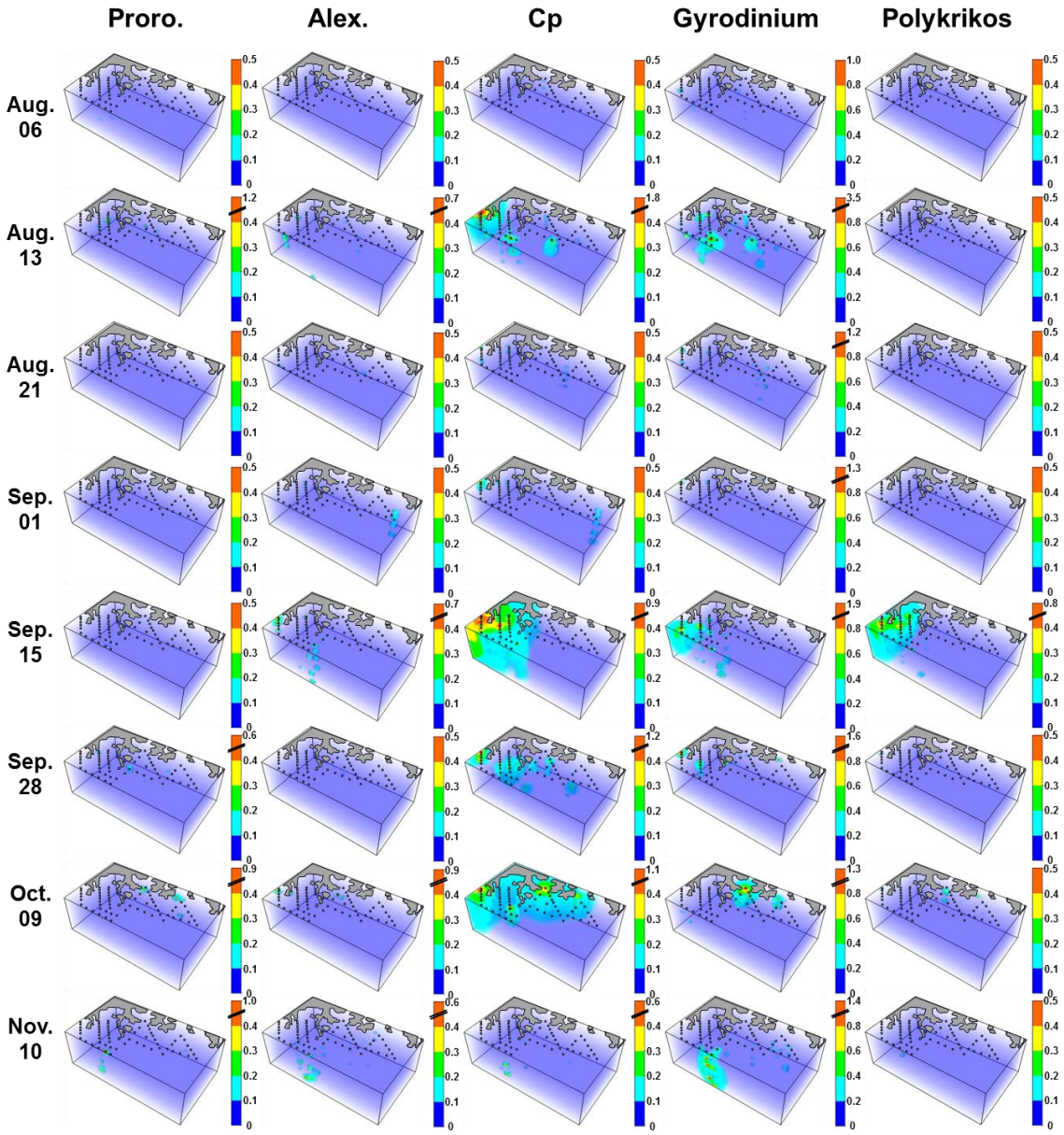



Fig. 4.11 Spatio-temporal distributions of the grazing impact (g $d^{-1}$ x $10^{-2}$) of calanoid copepods on *Prorocentrum* spp., *Alexandrium* spp., *C. polykrikoides*, *Gyrodinium* spp. and *Polykrikos* spp.

(continued)


Proro.
Alex.
Cp
Gyrodinium
Polykrikos
Aug. 06
Aug. 13
Aug. 21
Sep. 01
Sep. 15
Sep. 28
Oct. 09
Nov. 10

## 4.4. Discussion

### 4.4.1. Abundance and distribution of metazooplankton in South Sea of Korea

During the study period (May to November, 2014), the maximum mean abundances of the total metazooplankton and total copepod were observed on June 23 and May 21, respectively ($1.1x10^3$ inds. $m^{-3}$ and $0.4x10^3$ inds. $m^{-3}$, respectively) (Fig 4.3). However, the maximum abundances of the total metazooplankton and copepod were previously reported in November and April, respectively, in the South Sea of Korea ($2.4x10^4$ and $4.8\ x10^3$, respectively) (Moon et al., 2010, Oh et al., 2013, Table 4.6). The maximum abundance of the total metazooplankton in the South Sea of Korea is comparable to that of the reported abundance in the Northwestern Mediterranean (Calbet et al., 2001, Table 6). Furthermore, the maximum abundance of the copepod in the South Sea of Korea is comparable to that of the reported abundance in Balearic Sea, Mallorca in spring (Puelles et al., 2003, Table 4.6). In addition, the maximum mean abundance of the larvae of invertebrate in the South Sea of Korea ($1.8x10^2$ inds. $m^{-3}$) is lower than the reported maximum abundance in Masan Bay of Korea in September (Kim et al., 2013, Table 6).

Recently many studies documented the changes of zooplankton standing stock in worldwide regions (Décima, 2011). Decadal increase of zooplankton and copepod concentration was observed in North Pacific region and Japan and East China seas, in contrary long term, decrease in zooplankton was documented in the California Current System (Roemmich and McGowan, 1995a, 1995b, Rebstock and Kang, 2003, Sheridan and Landry, 2004). In the present study, the mean abundance of the total metazooplankton was little or much lower than the other reported maximum abundances in the South Sea of Korea (Table 4.6). However, the maximum abundances of copepods, cladocerans and larvae of invertebrate were

very similar to that of the maximum concentrations were reported in Jinhae Bay and South Sea of Korea (Table 4.6). Although the abundance and standing stock of copepods, cladocerans and larvae of invertebrates clearly has not changed, the abundance of total metazooplankton has decreased during the past years in South Sea of Korea. However, the result may be caused by the sampling intervals which were limited to between May and November in this study. The previously reported peak abundances of the total metazooplakton were usually found in winter except the Jinhae Bay, Korea and Newport River Estuary, UK (Fulton, 1984, Kang et al., 1996, Table 4.6). Furthermore, the maximum abundances of the copepod were usually reported in winter and spring season in the South Sea of Korea (Kang et al., 1996, Moon et al., 2010, Kim et al., 2013, Oh et al., 2013, Table 4.6). Moreover, the observed abundances of the total metazooplankton and the other taxa were not much different compared with that reported abundances in the other studies from May to November in the South Sea of Korea (Moon et al., 2010, Oh et al., 2013).

Table 4.6. The maximum abundance of the major metazooplankton taxa and the month when the maximum abundance (MA, inds. $m^{-3}$) was observed in temperate waters.

| Taxa | Area | MA | Peak | Ref. |
|---|---|---|---|---|
| Metazooplankton | South Sea, Korea | $1.1x10^3$ | June | This study |
| | | $6.4x10^3$ | April | Oh et al. (2013) |
| | | $2.4x10^4$ | November | Moon et al. (2010) |
| | Jinhae Bay, Korea | $3.7\ x10^3$ | July | Kang et al. (1996) |
| | Masan Bay, Korea | $5.3x10^4$ | January | Kim et al. (2013) |
| | Newport River Estuary, UK | $2.2x10^5$ | July | Fulton (1984) |
| | NW Mediterranean | $7.8x10^4$ | January | Calbet (2001) |
| | Balearic Sea, Mallorca | $9.0x10^3$ | December | Puelles et al. (2003) |
| Copepods | South Sea, Korea | $0.4x10^3$ | May | This study |
| | | $4.8x10^3$ | April | Oh et al. (2013) |
| | | $1.0x10^3$ | February | Moon et al. (2010) |
| | Jinhae Bay, Korea | $0.5\ x10^3$ | April | Kang et al. (1996) |
| | Masan Bay, Korea | $4.9x10^4$ | January | Kim et al. (2013) |
| | Fukuyama Harbor, Japan | $6.4x10^5$ | June | Uye and Liang (1998) |
| | Newport River Estuary, UK | $2.2x10^4$ | July | Fulton (1984) |
| | NE Atlantic ocean | $1.5x10^5$ | July | Gallienne and Robins (2001) |
| | Pearl River Estuary, China | $4.4x10^4$ | July | Tan et al. (2004) |
| | NW Mediterranean | $2.8x10^4$ | February | Calbet (2001) |
| | Balearic Sea, Mallorca | $6.5x10^3$ | Spring | Puelles et al. (2003) |
| Cladocerans | South Sea, Korea | $2.7x10^2$ | June | This study |
| | Jinhae Bay, Korea | $2.1\ x10^2$ | July | Kang et al. (1996) |
| | Masan Bay, Korea | $1.1x10^4$ | February | Kim et al. (2013) |
| | Balearic Sea, Mallorca | $1.0x10^3$ | August | Puelles et al. (2003) |
| | NW Mediterranean | $5.7x10^3$ | September | Calbet (2001) |
| Larvae | South Sea, Korea | $1.8x10^2$ | June | This study |
| | | $1.1x10^2$ | November | Oh et al. (2013) |
| | Masan Bay, Korea | $3.7x10^3$ | September | Kim et al. (2013) |

### 4.4.2. The effects of the physicochemical properties on the metazooplankton community

The world's ocean has been rapidly changing for the past century including global warming and greenhouse effect which can directly lead to increase the water temperature, ocean stratification and ocean acidification (Bograd and Lynn, 2003, Sarmiento et al., 2004, IPCC, 2007, Kim and Miller, 2007, Décima, 2011, Niehoff et al., 2013).

The surface water temperature began to increase more than 18℃ from the shallow water of Goheung in May, and the area where the water temperature was more than 18℃ expanded to entire waters of the South Sea of Korea in July (Fig. 4.14). Along with the increasing of the water temperature was warmer than 18℃, the abundance of the total metazooplankton was began to become dense (> 1000 inds. $m^{-3}$) from the shallow water of Goheung and Yeosu in May, and then the area where the abundances of metazooplankton were greater than 1000 inds. $m^{-3}$ widely expanded in the South Sea of Korea during June to July (Fig 4.6). However, over the spring, the surface water temperature began to be warmer than 23℃ from the shallow water of Goheung and Yeosu, and the hot water covered all of the South Sea of Korea from August to September (Fig 4.14). Along with the increasing of the water temperature more than 23℃, the distributions and abundance of metazooplankton declined from August (Fig. 4.6). Thus, distributions and abundances of the metazooplankton may be seasonally positively or negatively affected by the variations of water temperature in South Sea of Korea.

In the present study, the surface water temperature was significantly negatively correlated with the abundance of the copepod, but positively correlated with the abundance of larvae of invertebrates, chaetognaths and hydrozoans (Table 4.4). Although there were no significant correlations between the water temperature and the abundances of the total metazooplankton during the entire study period, the

result of correlations were seasonally different. While the surface water temperature was significantly positively correlated with the abundance of the every taxa of metazooplankton except copepods before July but variations of the surface water temperature was significantly negatively correlated with the abundance of the total metazooplankton after August (Table 4.7). Tseng et al. (2009) reported that the amount of gut contents of several copepod species is negatively correlated with the water temperature, it means the feeding activity of copepod species became more active with decreasing the water temperature. Such as the result, in contrast with the other metazooplankton taxa, the abundance of copepod was the only taxon that might be significantly negatively correlated with the water temperature during the entire study no matter the season in the South Sea of Korea.

Table 4.7. The differences of the correlations between the water temperature and metazooplankton taxon in the South Sea of Korea.

| Period | TMeso | Cope | Clad | Larv | Chae | Hydr | Othe[a] |
|---|---|---|---|---|---|---|---|
| May to July | 0.146** | -0.119** | 0.124** | 0.132** | 0.202** | 0.269** | 0.196** |
| August to September | -0.162** | -0.087* | -0.185** | | | | -0.183** |

TMeso: total metazooplankton, Cope : copepods, Clad: cladocerans, Larv: larvae, Chae: chaetognatha, Hydr: hydrozoans, Other: Others [a] See Table 1. * $P < 0.05$. ** $P < 0.01$ (n>480)

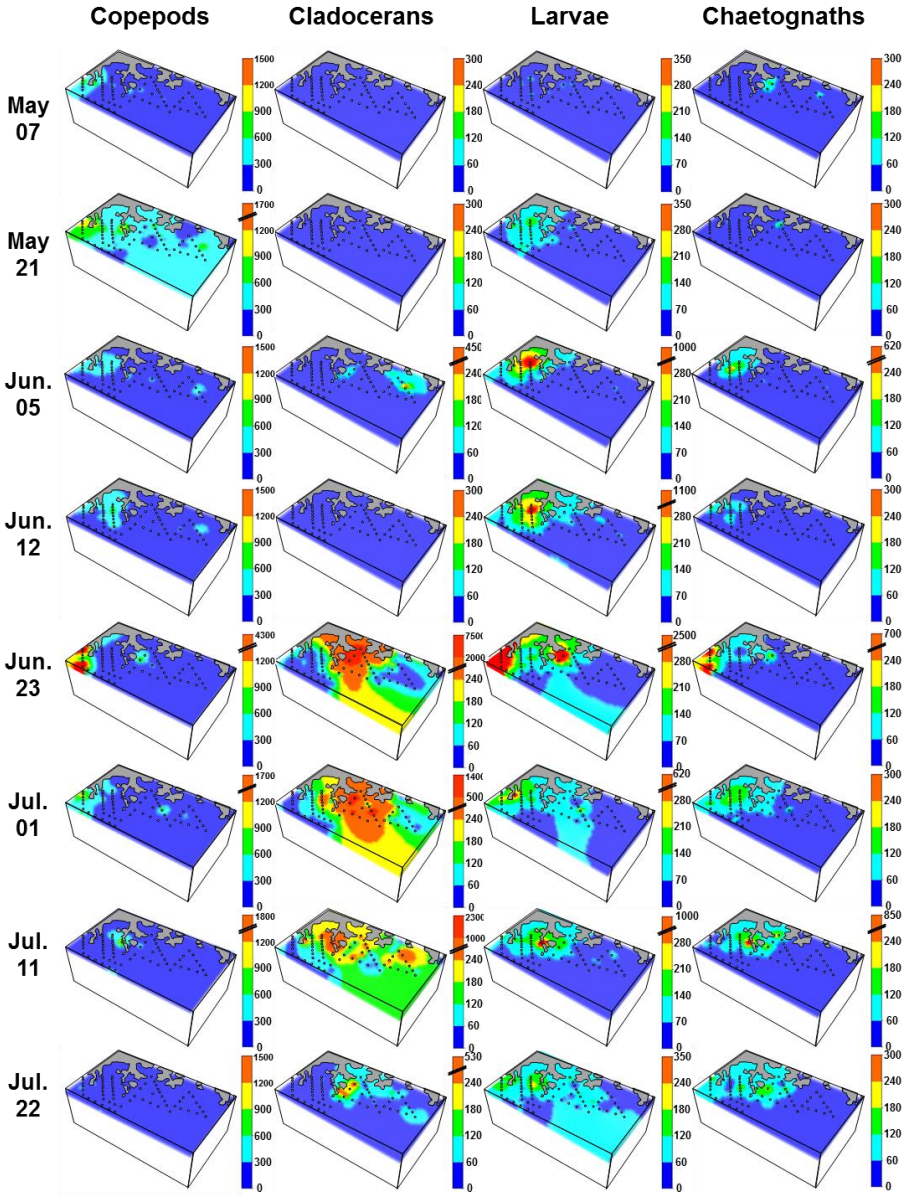
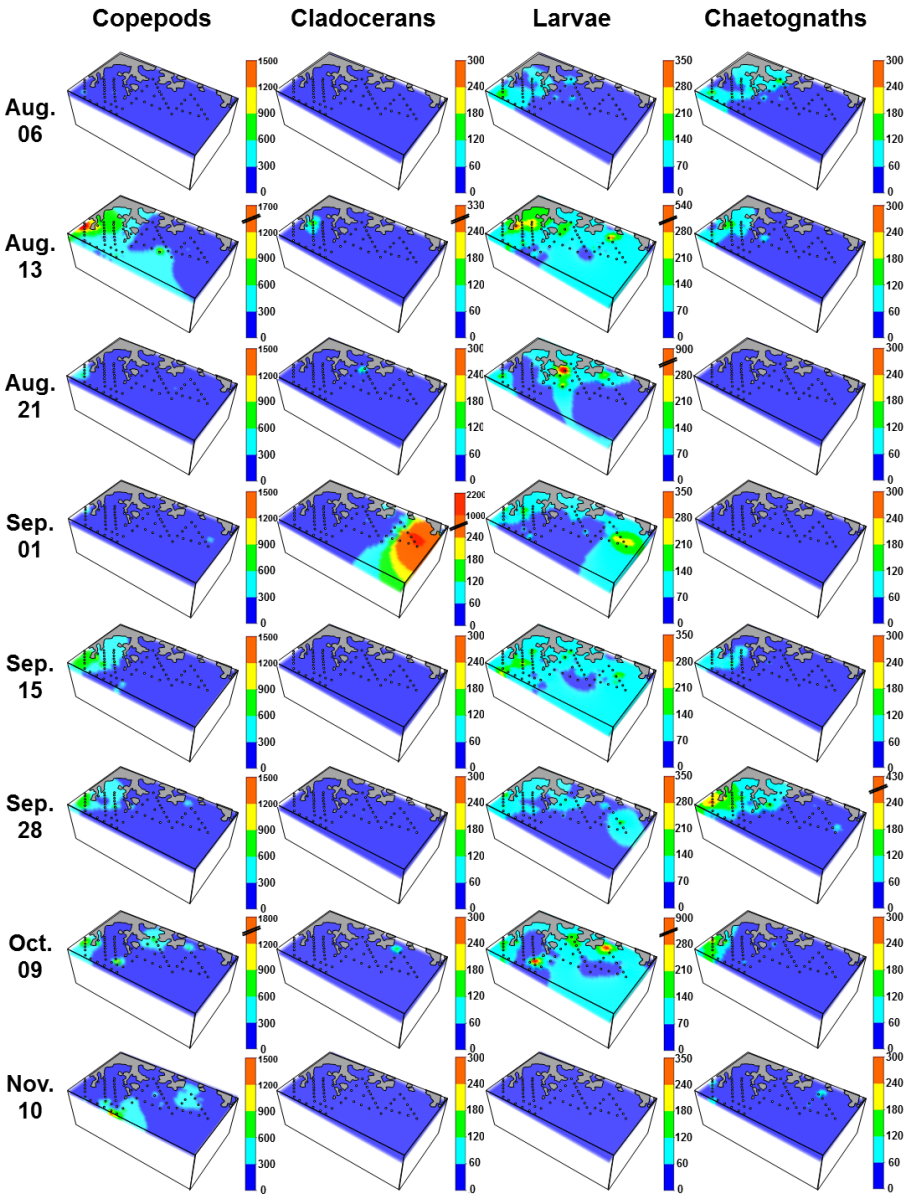



Fig. 4.12 Spatio-temporal distributions of the total copepods (A), cladocerans (B), larvae of invertebrate (C), and chaetognaths (D) concentrations in the study area

(continued)


Copepods
Cladocerans
Larvae
Chaetognaths
Aug. 06
Aug. 13
Aug. 21
Sep. 01
Sep. 15
Sep. 28
Oct. 09
Nov. 10

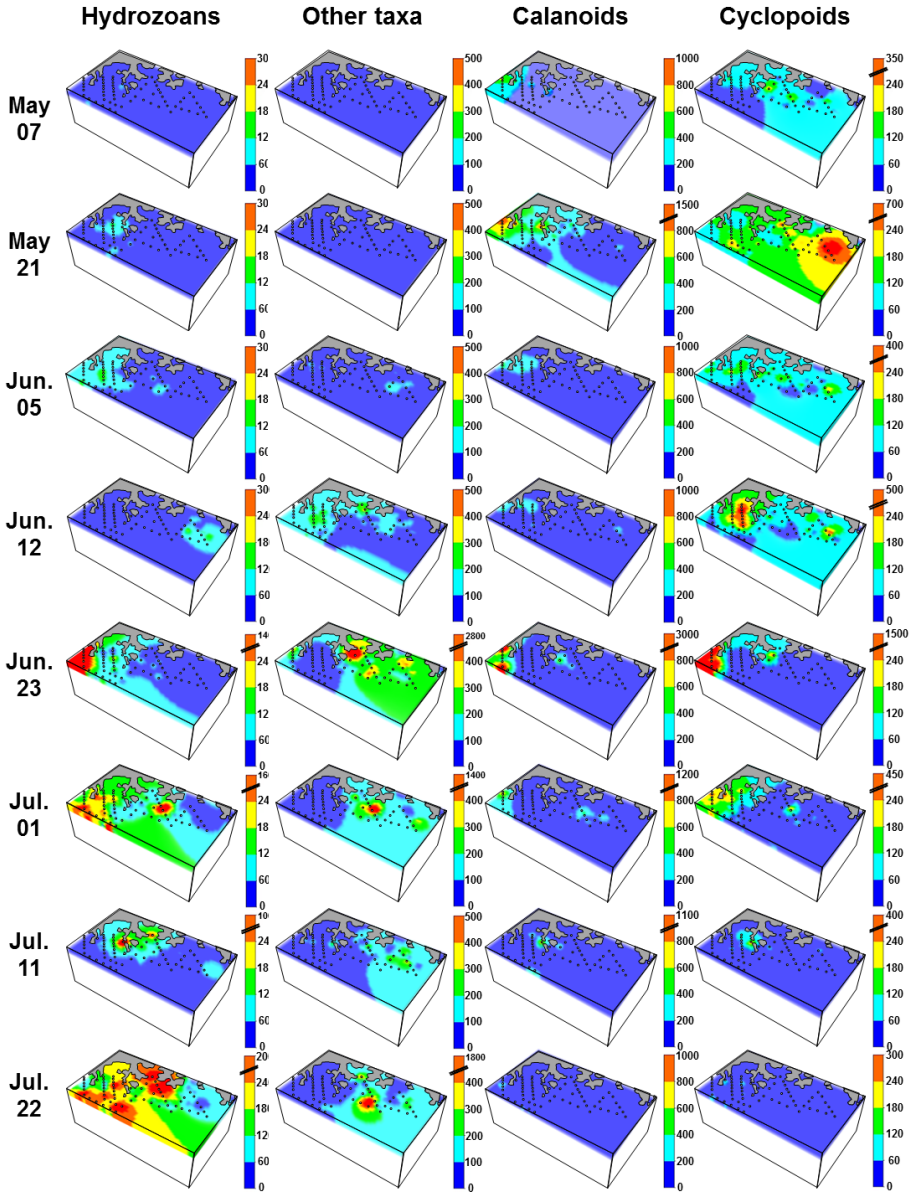
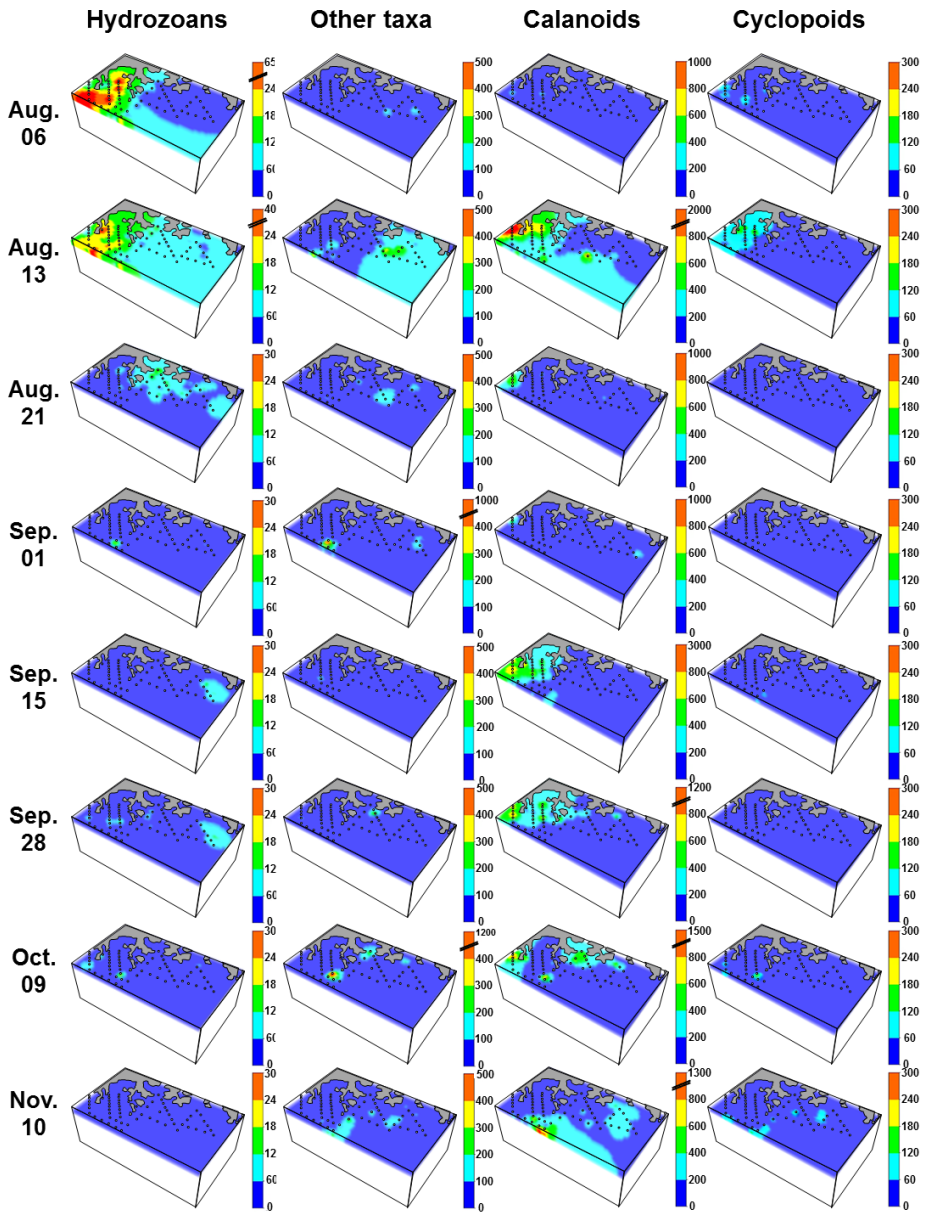



Fig. 4.13 Spatio-temporal distributions of the hydrozoans (A), the other taxa of metazooplankton (B), calanoid copepods (C) and cyclopoid copepods (D) concentrations in the study area

(continued)


Hydrozoans
Other taxa
Calanoids
Cyclopoids
Aug. 06
Aug. 13
Aug. 21
Sep. 01
Sep. 15
Sep. 28
Oct. 09
Nov. 10

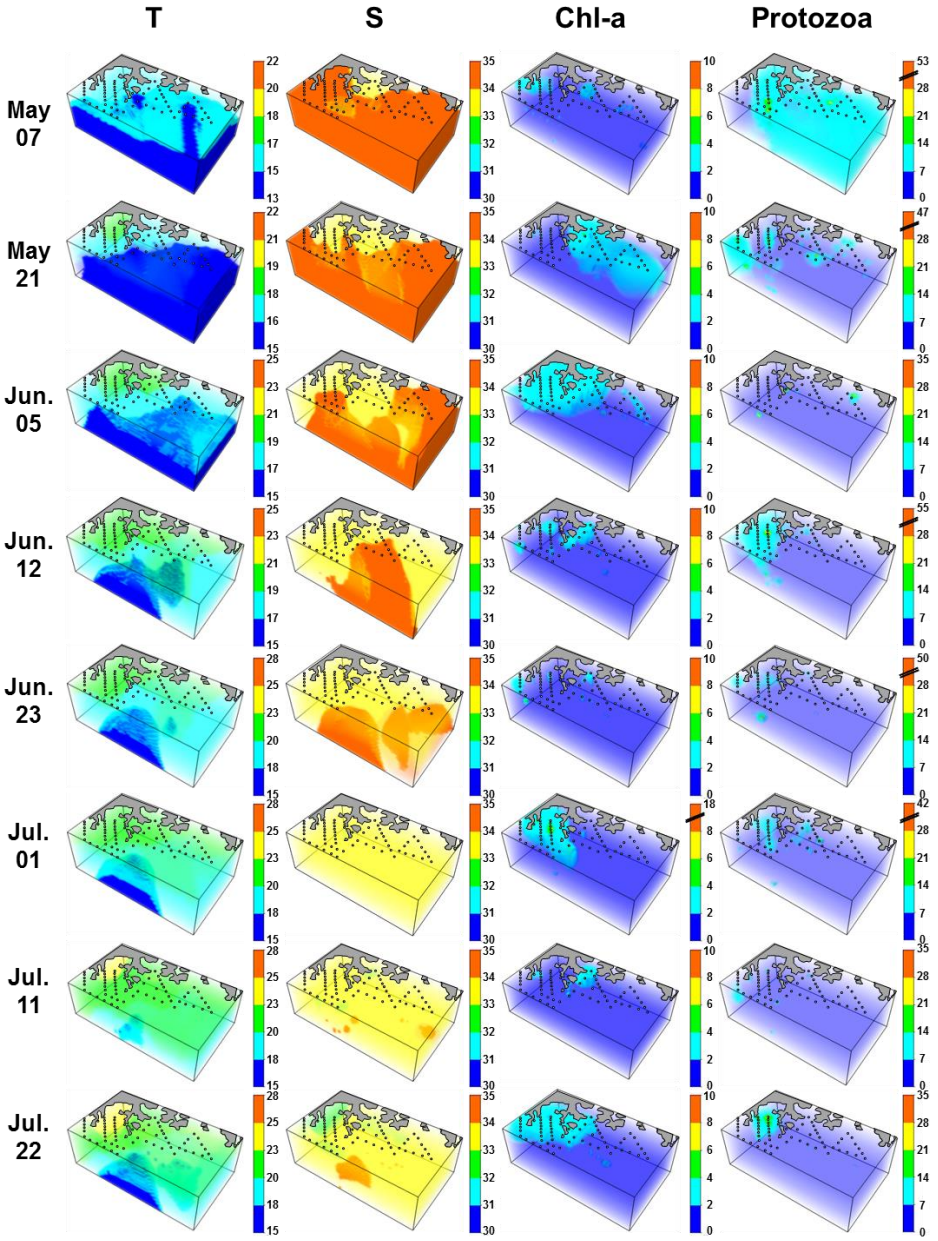
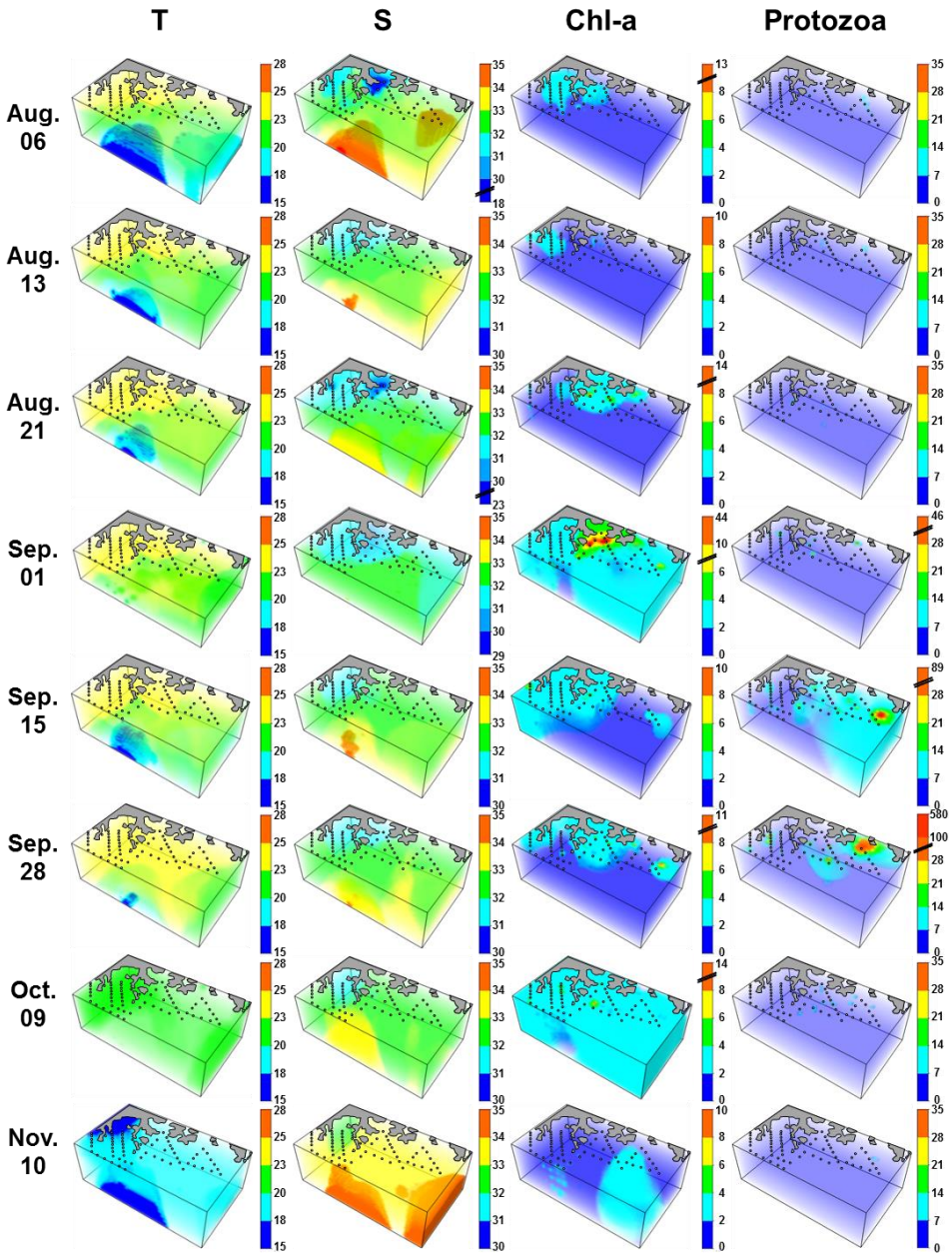



Fig. 4.14 Spatio-temporal distributions of the water temperature (A), salinity (B), chlorophyll-a (C) and total protozoa(D) concentrations in the study area

(continued)


T
S
Chl-a
Protozoa
Aug. 06
Aug. 13
Aug. 21
Sep. 01
Sep. 15
Sep. 28
Oct. 09
Nov. 10

Increasing of $CO_2$ concentration in atmosphere lead the more reaction that bicarbonate ions dissociate to carbonate [$CO_3^{-2}$] and [$H^+$] in the seawater (Niehoff et al., 2013). Thus, the increasing of $CO_2$ concentration brings decreasing of pH in the ocean. The pH variations in the seawater directly affect to especially in calcifying organisms or indirectly affect to the metazooplankton community by change the prey quality and concentration (Tortell et al., 2002, Nielsen et al., 2010). Recently, Lischka et al., (2015) reported that they did not detect significant effect of $CO_2$ for total abundance and diversity of metazooplankton but some of study documented the negative effects of acidification on *Acartia* sp. (Vehmaa, 2015). They found the incubated female *Acartia bifilosa* were smaller in some of high $CO_2$ experimental condition while the acidification condition negatively effects on egg hatching and development of the copepod. In contrast, in the present study, the abundance of total metazooplankton, copepods, larvae of invertebrates, chaetognaths and hydrozoans are significantly negatively correlated with pH (Table 4.4). The different result with earlier studies about the $CO_2$ concentration and pH effect on the metazooplankton community that may be caused by the variation ranges in the experimental condition or study area. In case of the present study, the variability of the pH was relatively low (< 0.2) during the study. Thus, the variations of pH might only directly influence on the amount of primary production of algal prey species which were the potential preys of the metazooplankton. As a result, the pH indirectly affected to the populations and distributions of metazooplankton. (e.g. If pH increase, the primary production decrease, consequentially abundance of metazooplankton predators finally decrease.)

Therefore, among the variety physicochemical factors which are able to potentially directly or indirectly affect to the populations and composition of metazooplankton taxa, even though only water temperature and pH might considerably influenced to the metazooplankton community, nutrients

concentrations and dissolved oxygen might not considerably affect to the metazooplankton community during the study.

### 4.4.3. The effects of the biological factors on the metazooplankton community and the multidirectional grazing impacts of metazooplankton on red tide dinoflgealltes

Among the biological factors such as the chlorophyll-a and concentrations of each plankton taxa used for correlation analysis with the abundance of metazooplankton taxa in the study area (Table 4.4). The abundance of total dinoflagellate were the only factor which is significantly positively correlated with the abundance of the total metazooplankton (Table 4.4). At the species level, predominant dinoflagellate *Prorocentrum donghaiense* that formed a red tides from June to July is significantly positively correlated with the abundances of 4 copepod species (e.g., *Corycaeus affinis, Labidocera euchaeta, Labidocera rotund* and *Paracalanus parvus*) and 7 metazooplankton taxa (e.g., *Penilia avirostris*, barnacle nauplius, decapod zoea, fish larvae, chaetognatha, hydromedusa, siphonophora) (Table 4.5). On the other hand, one of the dominant calanoid copepod *P. parvus* and *Calanus sinicus* were negatively correlated with some species of predominant diatom genera *Chaetoceros* including *Chaetoceros affinis, Chaetoceros compressus* and *Chaetoceros curvisetus.* Generally, metazooplankton actively select or avoid the prey items depend on the size, concentration, nutritional quality, swimming behavior and toxin (Chen, 2012). Furthermore, some of the chain-forming diatom species are too large to be grazed by metazooplankton (Dam et al., 1995). Thus, although the abundance of predominant dinoflagellate *Prorocentrum donhaiense* (ESD 13.3 μm) significantly positively correlated with the abundances of numerous metazooplankton taxa, the abundance of predominant diatom species of the *Chaetoceros* spp. which are generally chain-formed (length > 100 μm ), were negatively correlated with *C. sinicus* and *P. parvus*, in the South Sea of Korea.

Cladocerans including the genus *Evaden, Peniila and Podon* are the predominant cladoceran species during the present study. They are known to be able to feed on various species of small diatoms (<10um) such as *Skeletonema costatum*. Furthermore, they prefer to feed on large dinoflagellate (> 200μm) (Bainbridge, 1958, Kim et al., 1989). As earlier studies, *Evadne terestina* and *Penilia avirostris* were significantly positively correlated with the some species of large dinoflagellate such as *Ceratium* spp., *Protoperidinium* spp. and tintinnid ciliates in the present study (Table 4.5).

The temporal variations of cladocerans were widely spread and observed in high concentration (from June 23 to July 22) which were overlapped with the extinction phase of occurred *Prorocentrum* red tides (from June 12 to July 1) and the beginning of *Ceratium* red tides occurred on July 11th (Jeong et al. 2016, Fig. 4.8), in sequence. In addition, the hydrozoans including hydromedusa and siphonophora observed in high concentrations and widely exist from June 23 to August 13 (Fig. 4.13). However, the concentrations of cladocerans gradually decreased and almost disappear on August 6 when the red tides of *Ceratium* spp. was peak (Fig. 4.3, 4.12). Nevertheless, the hydrozoans including carnivorous predator hydromedusa and siphonophora were broadly spread at the same time.

Cladocerans are known to be able to feed on diverse algal species in wide range of the size including *Prorocentrum* spp. and *Ceratium* spp. (from smaller than 10μm to 200μm) (Bainbridge, 1958, Katechakis and Stibor, 2004). Thus, initially they might utilize the *Prorocentrum donhaiense* as food and partly controlled the extinction phase of *P. donhaiense* red tides in June by direct feeding. Moreover, the extinction of the *P. donhaiense* red tides might lead to occur the next red tides event by *Ceratim* spp. which species is the 2nd advantageous competitor to occur algal bloom in the South sea of Korea during the study (Jeong et al., 2016). Although, *Ceratium* spp. are generally known to too big to grazed by most copepods such as *Acartia*, *Paracalanus* and *Oithina* and they are also believed to constitute poor prey

items for copepods (Hargrave and Green, 1970), the genus of predominant cladocerans such as *Evadne*, *Penilia* and *Podon* are known to prefer to feed on large dinoflagellate including *Ceratium furca* which species was the mainly predominant during the *Ceratium* red tides in the study area (Bainbridge, 1958, Katechakis and Stibor, 2004). Thus, *Ceratium* spp. population might be grazed and partly negatively influenced by cladoceran predators during the developing stage of *Ceratium* spp. bloom formation in July. However, the hydrozoans which are carnivorous metazooplankton predators (Regula et al., 2009) might actively feed on the populations of cladocerans in July. Consequentially, decrease of the abundance and distribution of cladocerans by the predation led the cladocerans could not negatively much influence on the red tides of *Ceratium* spp. until to before they were faced with the next red tides competitor species such as *Alexandrium* spp. and *Cochlodinium polykrikoides* (Jeong et al., 2016). In addition, the caldocerans were regionally temporarily appeared in high concentration once again on September 1 (Fig. 4.12). The temporal reappearance of cladocerans occurred with not only the peak of the red tides event of *Cochlodinium polykrikoides* in the same region, but also the decline of hydrozoans concentration (Fig. 4.12, 4.13). Thus, in common with the previous red tides event of *Prorocentrum* spp. and *Ceratium* spp. during the study, the predation by cladocerans might negatively effect on the *C. polykrikoides* red tides population. Moreover, the little negative effect of hydrozoan predators on cladocerans by predation might lead to the cladocerans negatively more influenced on *C. polykrikoides* red tides by direct predation.

Some of earlier studies suggested that the grazing of cladocerans is not the significant effect on phytoplankton bloom dynamics (Graneli et al., 1989, Turner and Graneli, 1992). However, cladocerans were frequently observed with bloom of dinoflagellate that is similar to the present study, but even it was not clear whether due to the grazing interactions between algal preys and carnivorous predators or not (Morey-Gaines, 1979, White, 1980). On the other hand, although herbivorous filter

feeder cladocerans are considered to play less important roles in marine food web, they frequently consume much portions of primary production in marine environment (Bosch and Taylor, 1973, Raymont, 1983, Brendelberger et al., 1986, Egloff et al., 1997). Likewise, during the present study in the South sea of Korea, although the spatio-temporal distributions of cladocerans were not regionally perfectly coincide with the red tides organisms and predators, their variations of abundance and co-occurring patterns make us to predict that they play diverse and very important roles such as not only control the populations of co-occurring red tides organisms including *Prorocentrum* spp., *Ceratium* spp. and *C. polykrikoides* by direct feeding, but also the trophic link between primary productions of algal species and upper levels carnivorous predators in trophic pathway of the marine ecosystem.

Calanoid and cyclopoid copepods including genera *Acartia*, *Paracalanus*, *Oithona* and *Corycaeus* are the most abundant metazooplankton in worldwide, and their upper trophic level consumers such as carnivorous zooplankton and fish larvae prefer to primarily utilize calanoids and cyclopoids as a prey. Therefore, among the metazooplankton taxa, copepods are generally the most important links from the marine primary production to higher trophic levels in the marine ecosystem (Conway et al., 1998, Sundby and Fossum, 1990, Ringuette et al., 2002, Turner, 2004). However, there is no doubt about that the grazing impact of microzooplankton on red tides organisms is much bigger than the negative effect of mesoooplankton by feeding on red tides organisms (Calbet, 2001, Strom et al., 2001, Calbet and Landry, 2003). Thus, in order to investigate whether the calanoid copepods directly negatively influenced on the algal bloom dynamics by grazing on red tides organisms, or they indirectly positively affected on the red tides population by feeding on predominant microzooplankton species which are significant potential predators of red tides organisms, we calculated and compared the maximum grazing coefficients attributable to calanoid copepods on co-occurring

red tides organisms such as *Prorocentrum, Alexandrium* and *Cochlodinium* and the predominant potential grazers of red tides organisms including *Gyrodinium, Polykrikos* and total ciliates, respectively.

The heterotrophic dinoflagellate *Gyrodinium* spp. are ubiquitous and abundance many coastal waters (Haigh and Taylor, 1991, Yoo et al., 2013). They are often observed during the red tides of *Prorocentrum* spp. (Sournia et al., 1991, Borkman et al., 1993, Fiala et al., 1998, Johnson et al., 2003). Furthermore, *Gyrodinium* spp. are known to feed well on *Prorocentrum* spp. and sometimes have a considerable grazing impact on population of *Prorocentrum* spp. in the marine environment (Kim and Jeong, 2004, Yoo et al., 2013). Likewise, *Gyrodinium* spp. observed in relatively high concentration and widely distributed near the coastal waters of Goheung-Yoesu region while the *Prorocentrum* spp. formed a red tides in the same region from June 12 to July 1 (Fig. 4.14, included in microzooplankton). In addition, at the same time, calanoid copepods abundant near the coastal water of Goheung region (Fig. 4.13). The maximum grazing coefficients attributable to calanoid copepods on *Gyrodinium* spp. and *Prorocentrum* spp. were calculated at the same time on June 23 (0.047 $d^{-1}$ and 0.029 $d^{-1}$, respectively) (Fig. 4.10, 4.11). When the June 23 was the middle stage of the *Prorocentrum* spp. red tides event. Although, the calculated each grazing coefficients of calanoid copepods on *Gyrodinium* spp. and *Prorocentrum* spp. might not enough to considerably control and remove the red tides of *Prorocentrum* spp. and the distribution of *Gyrodiniuum* spp. (i.e. only up to 2.9 % and 4.6 % of *Prorocentrum* and *Gyrodinium* populations were able to be removed in 1d, respectively), the grazing coefficients attributable to calanoid copepods on *Gyrodinium* spp. was more than 50% higher than the grazing coefficients attributable to calanoid copepods on *Prorocentrum* spp. during the middle stage of *Prorocentrum* spp. red tides. Previously, many studies suggested calanoid copepods prefer to feed on protists, rather than feeding on phytoplankton, and they also eventually do not restrict phytoplankton blooms (Stoecker and

Campuzzo, 1990, Kleppel, 1993, Paffenhofer, 1998). Likewise, our study show that the feeding activity of calanoid copepods during the *Prorocentrum* spp. red tides may positively indirectly influenced on *Prorocentrum* spp. red tides population by grazing on the *Gyrodinium* spp. that were the potential predators of *Prorocentrum* spp. during the red tides.

The ichthyotoxic *Cochlodinium polykrikoides* red tidess have caused great economic losses in the aquaculture industry in the world wide including Korea (Whyte et al., 2001, Fukuyo et al., 2002, Yan et al., 2002, Gárate-Lizárraga et al., 2004, Vargas-Montero et al., 2006, Anton et al., 2008, Azanza et al., 2008, Hamzehei et al., 2013, Park et al., 2013, Jeong et al., 2016). *Polykrikos* spp. has become sometimes abundant at the declining stage of *C. polykrikoides* red tidess in the South Sea of Korea without its abundance (NFRDI, 2014, Lee et al., 2015) while they may have a considerable grazing impact on populations of co-occurring *C. polykrikoides* (Lee et al., 2015). During the present study, *C. polykrikoides* red tides occurred from August 21 to October 9 (Jeong et al. 2016) (Fig. 4.8). Furthermore, *Polykrikos* spp. were the predominant microzooplankton and regionally abundant at the declining stage of *C. polykrikoides* in the coastal waters of Yoesu-Tongyoun region on Septembetr 15 (Fig. 4.8, 4.14). At the same time, by contrast, calanoid copepods were only abundant in the coastal waters of Goheung (Fig. 4.13). Both of the two regional districts where the *Polykrikos* spp. and calanoids abundant, occurred *C. polykrikoides* red tides (Fig. 4.8, 4.13). The maximum grazing coefficients attributable to calanoid copepods on *C. polykrikoides* and *Polykrikos* spp. were calculated on August 13 and September 15, respectively (0.018 $d^{-1}$ and 0.008 $d^{-1}$, respectively). The maximum grazing coefficients attributable to calanoid copepods on *C. polykrikoides* was calculated at the early stage of *C. polykrikoides* red tides (i.e. up to 1.8% of *C. polykrikoides* populations were removed in 1 d) but the maximum grazing coefficients attributable to calanoid copepods on *Polykrikos* spp. was contrastively calculated at

the declining stage of *C. polykrikoides* red tides (i.e. up to 0.8% of *Polykrikos* spp. populations were removed in 1 d) (Fig. 4.9, 4.10). Although the calculated maximum grazing impact of calanoids on *C. polykrikoides* was morderate on August 13 (0.018 $d^{-1}$), it was much higher than the maximum grazing coefficients attributable to calanoid copepods on *Polykrikos* spp. (< 0.002 g $d^{-1}$) at the same period. Whereas, the calculated maximum grazing impact of calanoids on *Polykrikos* spp. was comparatively low on September 15 (0.008 $d^{-1}$), but it was similar to the maximum grazing coefficients attributable to calanoid copepods on *C. polykrikoides* at the same time (0.009 $d^{-1}$) (Fig. 4.9, 4.10). Therefore, even though the grazing coefficients attributable to calanoid copepods on *C. polykrikoides* at the developing stage of *C. polykrikoides* red tides was not much big and it might not enough to prevent the expansion of *C. polykrikoides* red tides, calanoid copepods lightly negatively influenced on the developing of *C. polykrikoides* red tides by direct feeding. However, both of the multidirectional negative or positive grazing impacts of calanoids on *C. polykrikoide* red tides by the direct feeding and indirect removal the potential predators of *C. polykrikoides* may little effect on the *C. polykrikoides* red tides population at the declining stage of the red tides. In addition, the grazing impact of calanoid copepods on diatoms *Skeletonema costatum* and *Chaetoceros* spp., which are known to be able to delay or prevent the outbreak of *C. polykrikoides* red tides by inhibit the growth rate and swimming speed of *C. polykrikoides* (Lim et al., 2014) were much greater (up to 0.05 $d^{-1}$ and 0.032$d^{-1}$, respectively) than their predation impacts on *C. polykrikoides* at the very early stage of *C. polykrikoides* red tides on August 13 (Fig. 4.8, 4.9). Therefore, the calanoid copepods may help the red tide dinoflagellates by suppressing the abundances of the heterotrophic dinoflagellate predator and the diatom inhibitors.

In the present study, we investigated which kind of environmental factors including physicochemical and biological properties are able to potentially influence on the metazooplankton community and, by extension, multidirectional

ecological impact of metazooplankton on the red-tides organisms. Although the amount of multidirectional ecological impacts of metazooplankton on red tides are not enough to perfectly control or terminate the red tides event, we observed the variations of metazooplankton community including herbivorous and carnivorous feeders definitely involve between the most changing and shift of the causative organisms of sequentially occurred red tides through the trophic pathway.

# Chapter 5: Development of effective mass cultivation system of microalgae for the prey of dinoflagellate predators.

## 5.1. Introduction

The biotechnological development of microalgae biomass production has been growing rapidly in recent years (Chisti 2007, Hu et al. 2008, Lam and Lee 2012, Wijffels et al. 2013). Diverse microalgae are known to be able potentially to synthesize 30-fold more oil per hectare than terrestrial plants (US Depart. of Energy 2009). They are also currently widely used to synthesize valuable pigment, materials and bioresources (Fuentes-Grünewald et al. 2009). In comparison with the other materials of usable bioresources, marine microalgae have several more advantages such as high growth rate, tolerance to high CO2 concentrations and high efficiency CO2 mitigation (Chang and Yang 2003, Hsueh et al. 2007). Among the various valuable microalgae, dinoflagellates have comparatively much greater biovolume up to 88,000 $\mu m^3$, which are several orders magnitude higher than green algae (Tang 1995, Smayda 1997, Stolte and Graces 2006, Olenina et al. 2006). Furthermore, heterotrophic dinoflagellate are known to have unsaturated fatty acids such as EPA [eicosapentaenoic acid] and DHA [docosahexaenoic acid] which are not present in the prey species (Klein Breteler et al. 1999, Broglio et al. 2003, Veloza et al. 2006). Moreover, humans even cannot synthesize DHA ourselves. In addition, DHA is considered to be helpful for development of brain and eye in infants, accordingly, the market of DHA globally well formed (Kroes et al. 2003, Ward and Singh 2005).

The production of biomass and microalgal lipid is influenced by various cultivation conditions and environmental factors such as temperature, salinity and

CO2 concentration (Sosik and Mitchell 1994, Fu et al. 2007). In order to cultivate microalgae in extremely high concentrations and to bulk the total capacity of the target culture enough to commercially use, the effective mass cultivation method must be developed. Furthermore, mass cultivation of heterotrophic dinoflagellates in high concentrations to utilize valuable bioresources such as DHA and EPA from them, preferentially the other algal prey species should be prepared to supply in much more high concentrations than the target heterotrophic species.

Thus, I newly designed the effective photo bioreactor (PBR) to mass cultivate the phototrophic microalgal prey to grow heterptrophic dinoflagellates in high concentrations such as *Oxyrrhis marina* and *Gyrodinium dominans*. They are known to be able to synthesize valuable materials when it feed on *Dunaliella tertiolecta* (Chu et al. 2008a, 2008b). Furthermore, in this study, the overall structure of newly desined PBRs and the cultivation systems, cultivation conditions and maximum grown concentrations were investigated and compared with the prospective studies. In addition, I investigated the different responses of intracellular pigment and extracellular glycerol production according to the supplied CO2 concentrations of *D. tertiolecta* when the species were cultivated in the newly developed PBR in extremely high concentration.

## 5.2. Materials and methods

### 5.2.1. Newly designed photo bioreactors (PBRs)

The newly designed PBR is indoor type of airlift bubble column made from polycarbonate (PC) material. The PBR is consisting 5 pieces that can be assembly together (Fig. 5.1). Moreover, by assembly the additional pieces of the PBR, which can be increased the length and total cultivation volume (Fig. 5.1). 5cm in diameter PC column was made to minimaize the self-light-shading in extreamly high

concentrations by their own pigment and 60cm long that can be cultivated and contained maximum one liter of microalgae with a column assembly. Furthermore, all the disassembled pieces of PBR can be sterilized using autoclave (121°C) and permanently reusable without contamination by the inhibitors such as the other non-target phototrophic microalgae and ciliates. Additional materials such as nutrient or food can be supplied through the holes on the cap (Fig. 5.1). CO2 gas or air bubbles supply under the conical bottom via the stainless link, and the grown and concentrated algae will be harvested at very bottom (Fig 5.1, 5.2)

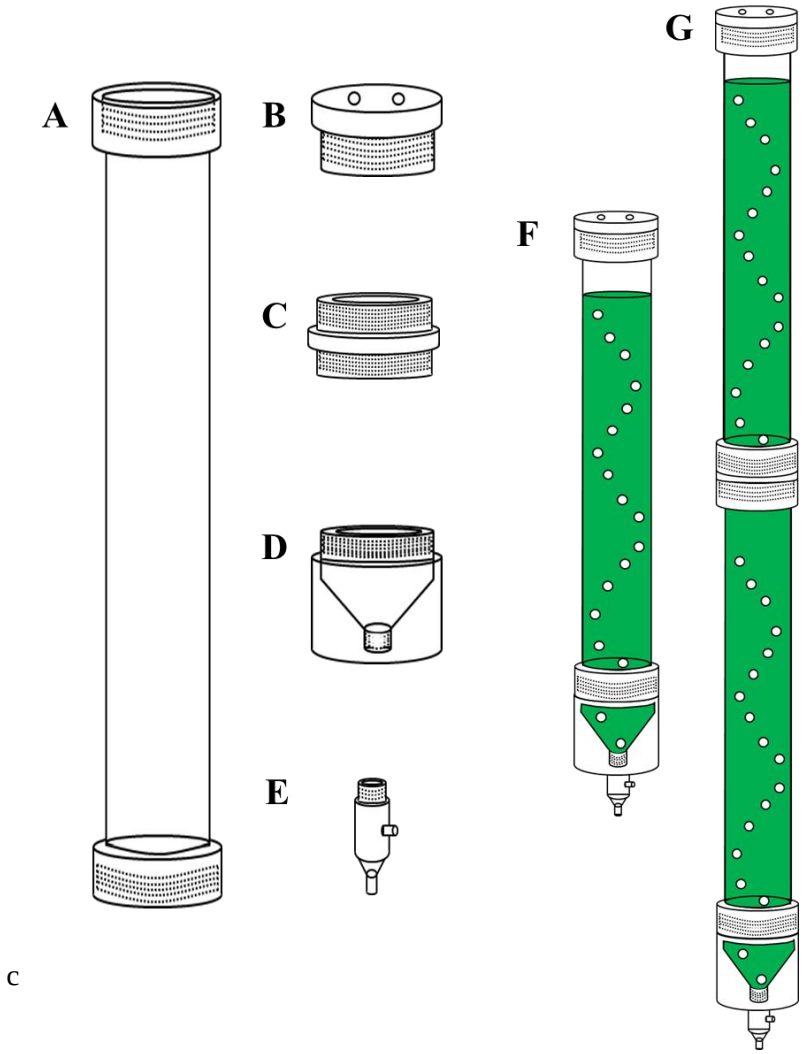



Fig. 5.1 Exploded diagrams and assembly view of the newly designed PBRs. main column (A), cap (B), column connection (C), conical bottom (D), stainless link (E), complete assemblies (F,G)

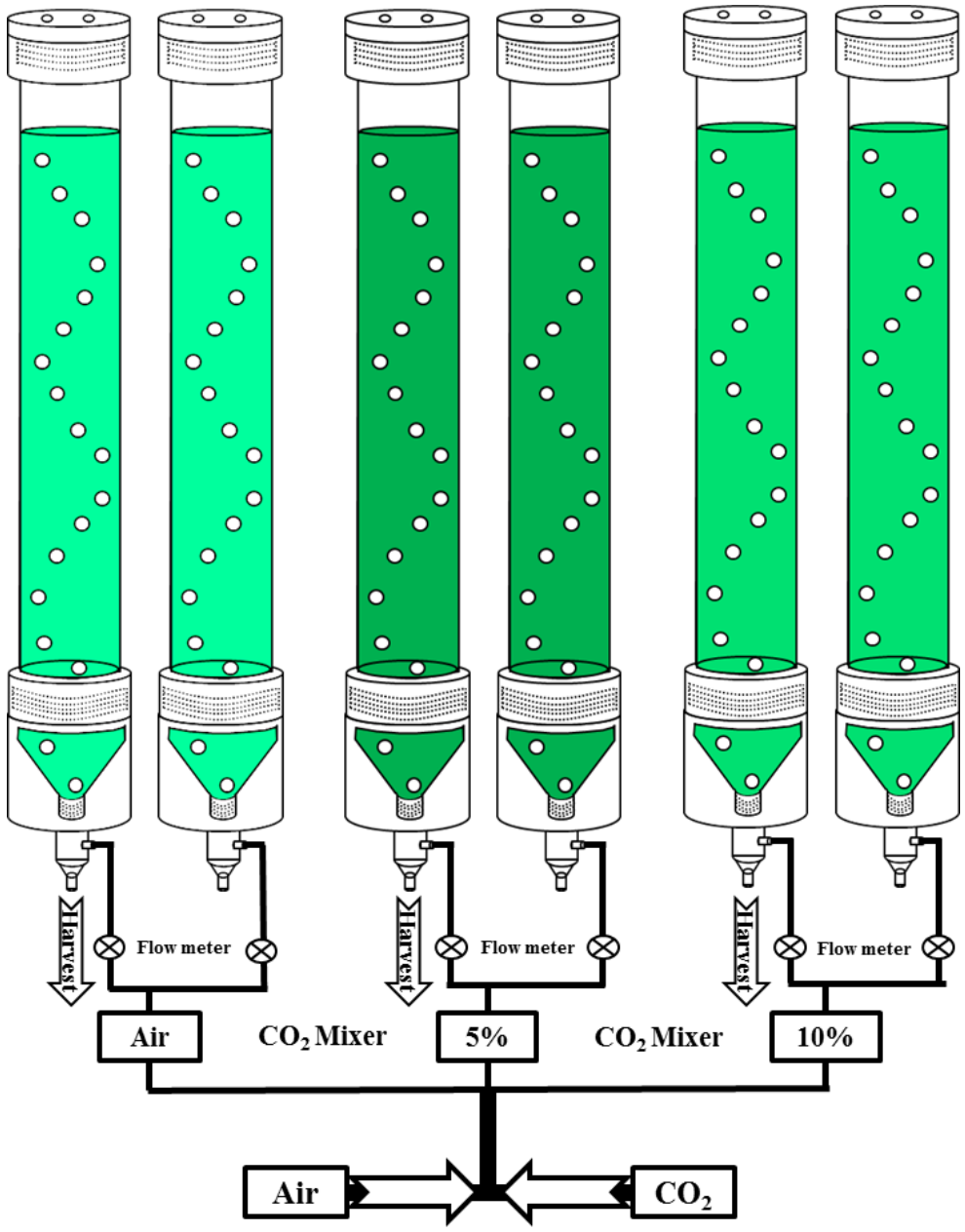



Fig. 5.2 Cultivation and experimental setup for *Dunaliella tertiolecta* with different CO2 concentrations

### 5.2.2. Culture conditions (species, strain, nutrients, light intensity, $CO_2$)

The strain of investigated *Dunaliella tertiolecta* was obtained from the Culture Collection of Algae at the University of Texa (UTEX), strain number LB-999. Before the experiment the *D. tertiolecta* was cultured in culture flask with F/2 seawater medium. The mass cultivation and experiment started with inoculating the 100 ml of the seed culture into the columns that were contained culture media, which *D. tertiolecta* concentrations were around 3 X $10^5$ cells $ml^{-1}$ and the samples took every in 15 days. The nutrient concentrations of culture media used in the experiment were maintained between 100 mg $l^{-1}$ to 500 mg $l^{-1}$ based on $NO_3$ concentration. The $NO_3$ concentrations were tested in every experiment everyday after sampling using $NO_3$ test strips (Easy test, JBL). Air and mixed $CO_2$ such as air only, 5% and 10% were separately injected in the PBRs at 50cc $min^{-1}$, and the light(LED) intensity was 200μE $m^{-2}s^{-1}$ (Light : Dark = 24 : 0) at 20°C.

### 5.2.3. Operational efficiency analyses

For the experimental analyses, 40-ml aliquots were removed from the each column every 24 hours. The columns were filled with 40 ml of filtered seawater to the final volume of 1L after every sampling and added F/2 medium stock if it needed. Among the aliquot 5ml of them fixed with 5% Lugol's solution, and the cells in 1-ml Sedgwick-Rafter chambers (SRCs) were enumerated by light microscopy to measure cell concentrations. The other 20-ml, 10-ml and 5-ml of the remained aliquots were used to measure the variations of pH, extracellular glycerol production with biomass and intracellular pigment, respectively.

pH was measured using a YSI Professional Plus instrument (YSI Inc., Yellow Springs, OH, USA). Collected cells to measure the extracellular glycerol and biomass (measured during only 8 days) were centrifusged at 13,000 rpm for 10min, and the supernatants were retained to assay for extracellular glycerol and remained

pellets were used to mesure the biomass. The Glycerol content was measured using the Free Glycerol Determination kit (FG0100, sigma-Aldrich, St Louis, MO, US), and the biomass was determimed from dry weigh (24 hours dry in 15-ml conical tubes at 60°C). Intracellular pigment variations were measured as in APHA (1995), the method of Fluorometric Determination of Chlorophyll-a was used (excitation wavelength : 430nm, emission wasvelength : 663nm).

## 5.3. Results

### 5.3.1. Growth and biomass yields of *Dunaliella tertiolecta* in the improved PBRs

Cell concentrations of *Dunaliella tertiolecta* cultivated in the PBRs rapidly increased at the beginning in all of the experimental conditions such as only air, 5% and 10% $CO_2$ supplied (Fig. 5.3. The cell concentration cultivated in the PBRs with only air supplied was increased up to 22 x $10^5$ cells $ml^{-1}$ only 2days after the experiment. Furthermore, the *D. tertiolecta* concentration in the PBRs with only air supplied was almost saturated 4days after the experiment, but 10 days after the cultivation the almost saturated cell concentration increased once again at the cell concentration more than 3.0 x $10^6$ (Fig. 5.3. The *D. tertiolecta* cell concentration in the PBRs with 5% $CO_2$ supplied grew extreamly faster than the any other experimental conditions. The PBRs with 5% $CO_2$ supplied exponentially increased during 10 days after the cultivation, the cell concentration was up to 72 x $10^6$. In addition, the *D. tertiolecta* cell concentration in the PBRs with 10% $CO_2$ supplied exponentially consistently increased during 10 days after began. However, the cell concentration of *D. tertiolecta* in the PBRs with 10% $CO_2$ supplied were saturated at more than 52 x $10^6$ cells $ml^{-1}$ (Fig. 5.3.

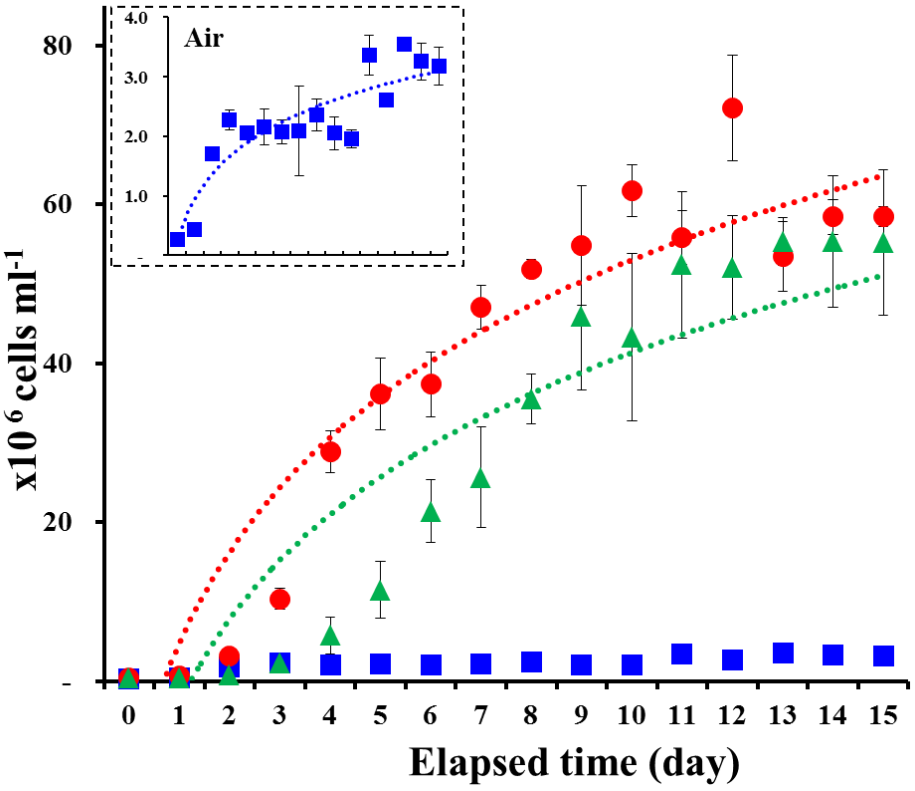



Fig. 5.3 Variations of the *Dunaliella tertiolecta* cell concentrations in the PBRs. Symbols represent treatment means $\pm$ SD (Blue : Air only, Red : 5% $CO_2$ , Green: 10% $CO_2$)

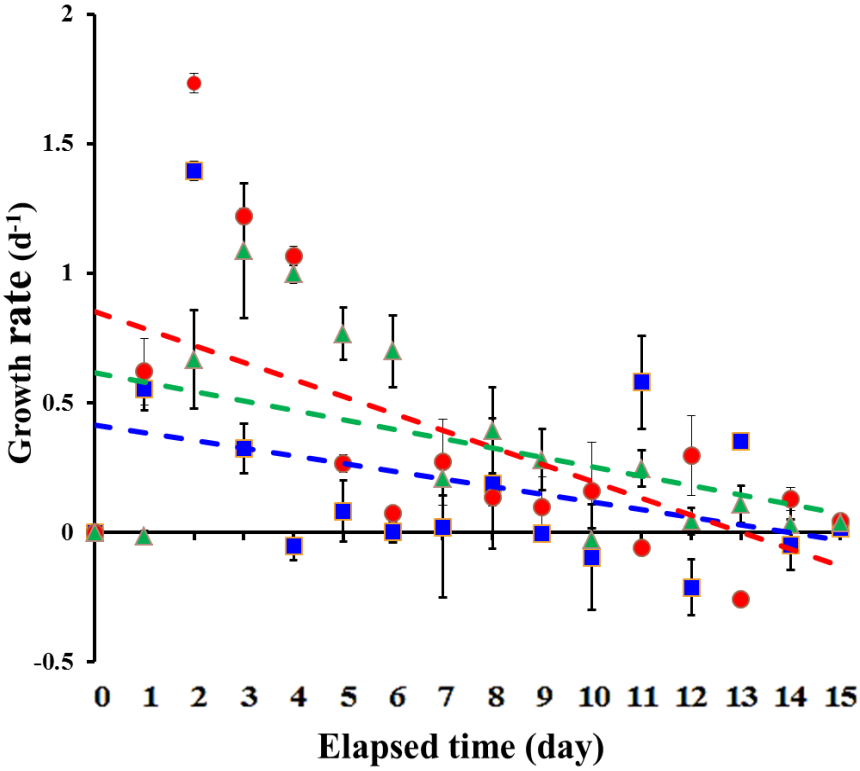



Fig. 5.4 Specific growth rate ($d^{-1}$) of *Dunaliella tertiolecta* in the PBRs. Symbols represent treatment means + SD (Blue : Air only, Red : 5% $CO_2$ , Green: 10% $CO_2$)

The specific growth rate of *Dunaliella tertiolecta* cultivated in the PBRs rapidly increased at the beginning in all of the experimental conditions as same as the cell concentraion (Fig. 5.4. The growth rate of *D. tertiolecta* cultivated in the PBRs with only air supplied that peaked 2days after the cultivation (up to 1.4 $d^{-1}$). After the maximum peak their growth rate was consistently very low except between from 11d to 13d. The growth rates of *D. tertiolecta* in the PBRs with 5% $CO_2$ supplied were extreamly high (up to 1.7 $d^{-1}$) and the growth rate consistently decreased. Moreover, the growth rate of the *D. tertiolecta* cultivated in the PBRs with 10% $CO_2$ supplied was up to 1.1 $d^{-1}$ 4days after cultivation (Fig. 5.2).

### 5.3.2. Variations in the intracellular pigment and extracellular glycerol composition of *Dunaliella tertiolecta*

Determined total fluorescence intensities increased with the growth of the *Dunaliella tertiolecta*. The detected fluorescence intensity sustainedly increased from the beginning of the cultivation in the PBRs with $CO_2$ supplied 5% and 10% (Fig. 5.3A). In the PBRs air only supplied, the fluorescence intensities increased during the first 3days and decreased on the experiment day of 4th but the intensity continuously re-increased again 5 days after the experiment (Fig. 5.5). Furthemore, the fluorescence intensities per a *D. tertiolecta* cell were relatively high (maximum 0.48 ng $L^{-1}cell^{-1}$) at the start of cultivation in the every condition of PBRs (Fig. 5.5). However, the fluorescence intensities per a *D. tertiolecta* cell rapidly declined 2 days after the experiment in the every PBRs. After the rapid decline of the fluorescence value, the fluorescence intensities per a *D. tertiolecta* cell that cultivated in the PBRs with air and 5% $CO_2$ supplied were determiened between 0.11 ng $L^{-1}cell^{-1}$ and 0.28 ng $L^{-1}cell^{-1}$, which maintained without much variations until the end of the experiment (Fig. 5.5). However, in the PBRs with 10% $CO_2$ supplied, the fluorescence intensities per a *D. tertiolecta* cell gradually increased again from experiment day of five to the end of cultivation (up to 0.34 ng $L^{-1}cell^{-1}$) (Fig. 5.5).

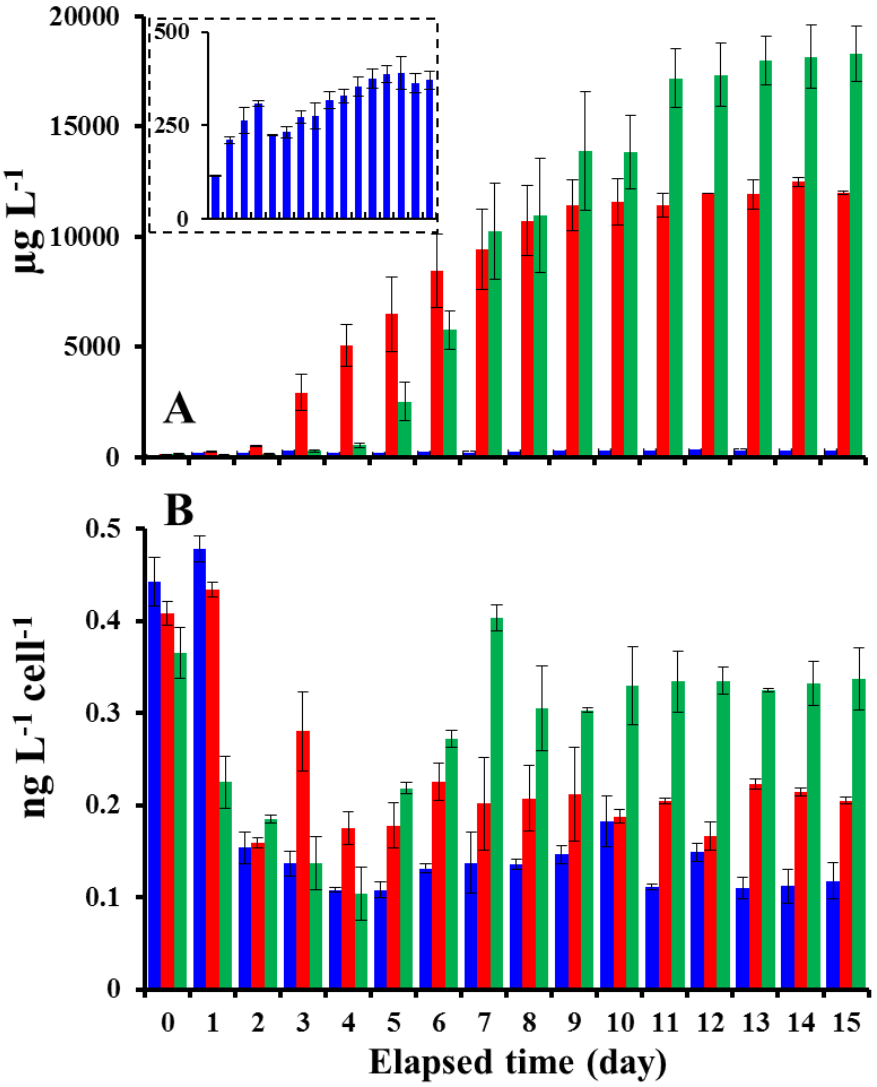



Fig. 5.5 Variations of fluorescence intensities (μg $L^{-1}$, ng $L^{-1}$ $cell^{-1}$) of *Dunaliella tertiolecta* in the PBRs. Error bars indicate SD. Blue : Air only, Red : 5% $CO_2$ , Green: 10% $CO_2$

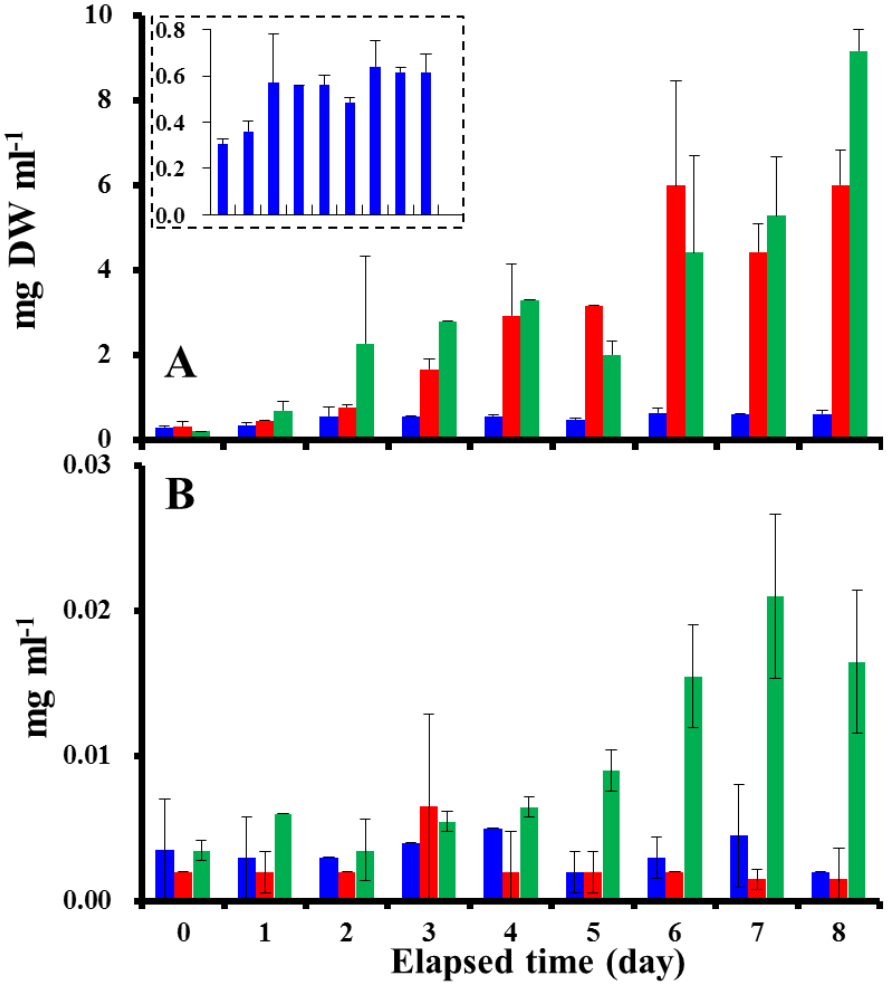



Fig. 5.6 Dry weight (mg DW 10ml-1) of *Dunaliella tertiolecta* and extracellular glycerol (mg ml-1) produced by *D. tertiolecta* in the PBRs. Error bars indicate SD. Blue : Air only, Red : 5% $CO_2$ , Green: 10% $CO_2$

Among the 15 days of entire experiment, the variations of total biomass (mg Dry Weight $ml^{-1}$) and amount of extracellular glycerol production (mg $ml^{-1}$) were determined during only 8 days after cultivation started. Total biomass of *Dunaliella tertiolecta* cultivated in the PBRs gradually increased at the beginning in all of the experimental conditions such as only air, 5% and 10% $CO_2$ supplied (Fig. 5.4A). The total biomass cultivated in the PBRs with only air supplied increased up to 0.64 mg DW $ml^{-1}$. Total biomass of the *D. tertiolecta* in the PBRs with 5% $CO_2$ supplied increased up to 6.0 mg DW $ml^{-1}$ (Fig. 5.6). In addition, the maximum total biomass of *D. tertiolecta* in the PBRs with 10% $CO_2$ supplied was 9.2 mg DW $ml^{-1}$. Moreover, the extracellular glycerol productions were not much varied during the entire experiment in the PBRs with air and 5% $CO_2$ supplied. However, in the experiment of PBRs with 10% $CO_2$ supplied, the extracellular glycerol production was much greater than the other PBRs from 5 days incubation to the end of the cultivation (Fig. 5.6).

## 5.4. Discussion

Microalgae such as dinoflagellates and greenalgae are the prospective and valuable raw materials for biofuel feedstocks (Mohimani 2013). Priviously, many microalgal species were tried to mass cultivate such as *Dunaliella* and *Spirulina* (Belay 1997, Borowitzka et al. 1984). Their potential oil productivity is known to at least four times higher than the other sources (Duarte et al. 2010). Furthermore, heterotrophic dinoflagellates usually syntheses much amount of unsaturated fatty acids themselves and save in the cells (Klein Breteler et al. 1999, Broglio et al. 2003, Veloza et al. 2006). The unsaturated fatty acids such as EPA [eicosapentaenoic acid] and DHA [docosahexaenoic acid], which are not present in the prey species and even in the humans body. Thus, the developing an effective method to obtain much amount of valuable materials from heterotrophic

dinoflagellate developing the effective system that can grow the prey species in high concentration to fed on heterotrophic dinoflagellate should be investigated first. The sensitivity of the algal species were well studied that their growth and productivity are very much influenced and depend on the cultivate conditions such as temperature, salinity and light (Borowitzka 1998). Thus, by combining the marine ecological studies and biotechnological studies, optimal micro algal cultivation conditions shold be found and new incubation systems should be developed.

The types of algal cultivation systems were divided into two types, open pond and photo bioreactors (Moheimani 2013). Recently, open pond type of mass cultivations are broadly the most recommended cultivation system that is lower cost to build and much easier to scales and capacity up. However, in case of Korea has clear four seasons so the variations of temperature is too big, and the place to build the huge open pond system outside is limited. Thus, the mass cultivation using PBRs is more recommended in Korea. The method using PBRs is considered to have more advantages to prevent contamination by the other algal species or potential predators than open pond systems (Dodd 1986). Therefore, development and investigation for effective indoor photo bioreactor is adequate for domestic circumstances in Korea.

*Dunaliella tertiolecta* grew very well in the newly developed PBRs in this study with $CO_2$ supply. The cell concentration of *D. tertiolecta* cultivated in the PBRs with air only supplied was the lowest during the experiment, but the cell concentration in the PBRs with air only supplied consistently increased from the beginning to the end of the experiment (maximum cell concentration was $3.3 \times 10^6$). During the study, the maximum cell concentration was observed in the PBRs with 5% $CO_2$ supplied ($7.2 \times 10^7$ cells $ml^{-1}$). The maximum cell concentration observed in the PBRs with 5% $CO_2$ supplied was 20% greater than the maximum cell concentration determined in the PBRs with 10% $CO_2$ supplied. Furthermore, the

maximum cell concentrations observed in the PBRs with 5% $CO_2$ supplied was almost 20 times much higher than the observed maximum concentration in the PBRs with air only supplied. This different maximum cell concentrations and growth in the same PBRs may have been due to the supplied $CO_2$ concentrations and pH (Fig. 5.7. The results of this study is similar to the previous studies, they reported *D. tertiolecta* showed the highest growth rate in the range of 2-6% $CO_2$ supplied (Suzuki et al. 1995, Tang et al. 2011). Moreover, Kim et al (2012) reported that the pH value lower or higher than that of the optimal culture condition inhibits their growth. The maximum cell concentration observed in the newly designed PBRs with 5% $CO_2$ supplied that was greater than the any other reported maximum cell concentration of *D. tertiolecta* cultivated indoor (Table 5.1). Thus, by combining the data determined in this study and previously reported, the optimal concentration of supplied $CO_2$ is 5% and optimal pH range may be between 7 to 7.5 (Fig. 5.7) o grow *D. tertiolecta* in extrealy high concentration. In addition, newly designed and developed PBRs used in the present study and the cultivation method with $CO_2$ supply may have considerable competitiveness to grow micro algal prey species in extreamly high concentrations for heterotrophic dinoflagellates predators.

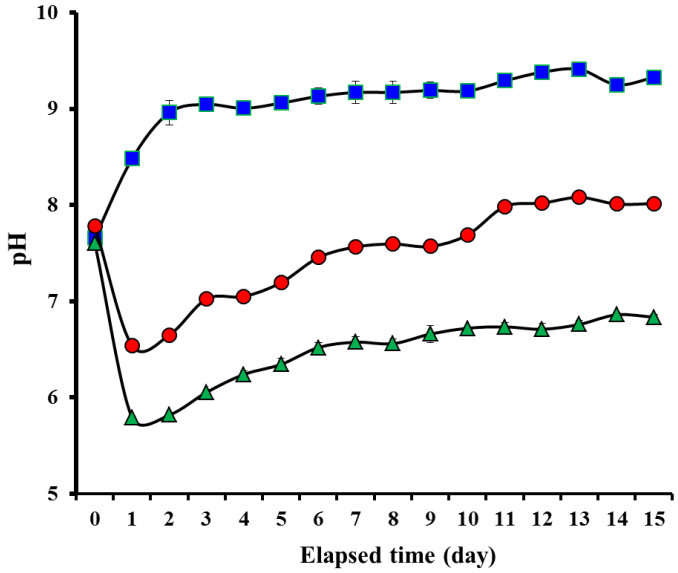



Fig. 5.7 pH variations according to the supplied $CO_2$ concentrations in the PBRs. Symbols represent treatment means ± SD. Blue: Air only, Red: 5% $CO_2$ , Green: 10% $CO_2$

Table 5.1 Comparison of the maximum concentrations of *Dunaliella tertiolecta* cultivated indoor scale

| Reference | Maximum concentration | Incubator | Light intensity | Light : Dark cycle | Temperature | PSU or NaCl (M) | Culture medium | CO2 |
|---|---|---|---|---|---|---|---|---|
| This study | $7.2 \times 10^7$ | PBR | 200 | 12:0 | 20 | 30 | F/2 x (2 to 5) | 5% |
| Jeon et al. 2013 | $2.5 \times 10^7$ | PBR | 120 | 12:12 | 23 | 1.0M | D medium | 5% |
| Chow et al. 2013 | $7.5 \times 10^6$ | Flask | 70 | 17:7 | 25 | 2M | Erlenmyer medium | - |
| Chow et al. 2015 | $3.6 \times 10^7$ | Flask | 50 | 17:7 | 25 | 2M | Erlenmyer medium | - |
| Tang et al. 2011 | $1.2 \times 10^7$ | Flask | 350 | 15:9 | 23 | 30 | Erdschreiber's medium | 4% |
| Fazeli et al. 2005 | 5.6 x 106 | Flask | 150 | 14:10 | 25 | 1.5M | Seawater medium | - |

*Dunaliella tertiolecta* is known to have five kinds of pigments in the cell such as Chlorophyll-a, Chlorophyll-b, β-carotene, Lutein and Neoxanthin (Moon 2016). Among the pigments, natural made β-carotene is preferred bioresources by humans (Choi et al. 2012). *Dunaliella* spp. is known to overproduce carotenoids under stress conditions of high light or high NaCl concentrations (Ben-Amotz et al. 1988, Park et al. 2013). I additionally measured the fluorescence of cultivated *D. tertiolecta* in the PBRs every day. Through the observed fluorescence intensity, I determined the relative value of chlorophyll-a in vivo (APHA, 1995). The total chlorophyll-a concentrations in the every PBRs sustainedly increased with the growth of the cell concentrations during 15 days (Fig. 5.3 5.5) However, the variations of chlorophyll-a concentration per a *D. tertiolecta* cell was different with the cell concentrations. Although the cell concentration in the PBRs with 5% $CO_2$ supplied was always higher than the concentration of 10% $CO_2$ supplied except the experiment day of 13th (Fig. 5.3), the total chlorophyll-a concentrations and chlorophyll-a concentration per a *D. tertiolecta* cell of the PBRs with 10% $CO_2$ supplied was greater than that of 5% $CO_2$ supplied from 7 and 6 days after the cultivation began, respectively (Fig 5.5). Moreover, the measured extracellular glycerol production of 10% $CO_2$ supplied PBRs that evidently increased alone 5 days after the cultivation. Fazeli et al. (2005) and Moon (2016) reported the salinity and high concentrations of $CO_2$ supply, which make the *D. tertiolecta* to change their own pigment composition such as chlorophyll-a and β-carotene. Furthermore, *D. tertiolecta* produce intracellular and extraceullular glycerol to prevent the damages from osmotic pressure (Chow et al. 2013, 2015). Both of the changes of *D. tertiolecta* such as pigment composition and glycerol production were occured under some of stress conditions. Therefore, the reason why the observed fluorescence intensity and extracellular glycerol production were relatively higher in the PBRs with 10% $CO_2$ supplied, may be caused by the pH.

## 5.5. Conclusion

I developed newly designed photo bioreactor for mass cultivation of microalgae. The developed PBRs are adequate for domestic circumstances in Korea. The PBR is consisting 5 pieces that can be assembly together and it can be increased the length and total cultivation volume. As a result, *Dunaliella tertiolecta* grew very well in the newly developed PBRs. Furthermore, supply of $CO_2$ much stimulates the growth of *D. tertiolecta*. In addition, *D. tertiolecta* may change their pigment composition and amount of glycerol production under pH stress condition. Thus, using the newly designed photo bioreactor and cultivation method with $CO_2$ supply, (1) we can cultivate microalgae in extreamly high concentration, (2) the high concentrated microalgae can feed to the heterotrophic dinoflagellates, (3) we may grow the heterotrophic dinoflagellates in higher concentration (4) finally we may obtain more amounts of valuable bioresoreces from the grown heterotrophic dinoflagellates.

# Chapter 6: Overall conclusion

In the present study, I focused attetion on the diverse roles of dinoflagellates in the Korean coastal ecosystems and the potential relationships between the dinoflagellates and humans. The diverse roles of dinoflagellates around us such as symbionts of corals, mixotrophic predators of red tides species, important tropic links in marine food web and raw materials for the usable bioresources, which are necessarily directly or indirectly closely related to the humans.

The symbiotic dinoflagellates *Symbiodinium* spp. that occur at low concentration in reef-building corals are cryptic spceices. Evidence acquired from extensive sampling, long-term monitoring, and experimental manipulation can allow us to deduce the ecology and functional significance of these populations and whether they might contribute to the response of coral-dinoflagellate mutualisms to climate change. Consistent with broader geographic sampling, *Alveopora japonica* is only one species comprised 99.9 %, or greater, the population of symbionts in every sample. Temporal variations of the other tree more *Symbiodinium* spp. occurred in the *A. japonica* in prevalence and abundances of these background *Symbiodinium* could not be definitively related to any particular environmental factor including seasonality and water chemistry. Many transient *Symbiodinium* spp., which occur only at trace abundances in the coral's microbiome, belong to different functional guilds and likely have little, if any, importance to a coral's physiology. The successful integration between host and symbiont into a stable functional unit should therefore be considered when defining host-symbiont specificity.

Mixotrophic dinoflagellate *Polykrikos hartmannii* have chloroplasts and forms a two-zooid pseudo-colony. The ingestion rate of *P. hartmannii* on red tides specise *Cochlodinium polykrikoide*s increased, but reached saturation at a prey concentration of 945 ng C $ml^{-1}$ (1350 cells $ml^{-1}$). The maximum ingestion rate of *P. hartmannii* on *C. polykrikoides* was 1.9 ng C $predator^{-1}$ $d^{-1}$ (2.7 cells $predator^{-1}$ $d^{-1}$).

However, the maximum mixotrophic growth rate of *P. hartmannii* with added *C. polykrikoides* cells (0.030 $d^{-1}$) was slightly greater than of those without added prey (0.023 $d^{-1}$). The calculated grazing coefficients for *P. hartmannii* on co-occurring *C. polykrikoides* were up to 0.324 $d^{-1}$ (28% of the population of C. *polykrikoides* was removed by *P. hartmannii* populations in 1 d). Thus, *P. hartmannii* can have a considerable grazing impact on the red tide populations of *C. polykrikoides*.

In order to investigate whether the standing stocks, distribution and grazing of the metazooplankton negatively directly effect on the population of red tides species, or positively indirectly influence on the dinoflagellate community by feeding on potential microzooplankton predators of red tides species, water samples were collected from 60 stations in the South Sea of Korea, from May to November 2014. Furthermore, the three-dimensional (3-D) distributions of physicochemical and biological parameters. Sometimes the grazing of metazooplankton on red tides dinofalgellatess is bigger than the grazing impact of metazooplankton on microzooplankton that are the potential key predators of red tide dinoflagellates, which negatively influence on the red tides event, but some times the metazooplankton reversly positively effect on the red tides populations. Although the amount of multidirectional ecological impacts of metazooplankton on red tides are not enough to perfectly control or terminate the red tides event, I observed the variations of metazooplankton community including herbivorous and carnivorous feeders definitely involve between the most changing and shift of the causative organisms of sequentially occurred red tides through the trophic pathway.

Mass cultivation of heterotrophic dinoflagellates in high concentrations to utilize valuable bioresources such as DHA and EPA from them, preferentially the other algal prey species should be prepared to supply in much more high concentrations than the target heterotrophic species. To commercially utilize heterotrophic dinoflagellates to obtain any valuable bioresources, development and choose the type of cultivation system is very important. Thus, I developed newly

designed photo bioreactor for mass cultivation of microalgae prey for heterotrophic dinoflagellates. *Dunaliella tertiolecta* grew very well in the newly developed PBRs. Furthermore, supply of $CO_2$ much stimulates the growth of *D. tertiolecta*. In addition, *D. tertiolecta* may change their pigment composition and amount of glycerol production under pH stress condition. Thus, using the newly designed photo bioreactor and cultivation method with $CO_2$ supply, we can cultivate microalgae in extreamly high concentration, the high concentrated microalgae may feed to the heterotrophic dinoflagellates to mass cultivate the heterotrophic dinoflagellates in higher concentration.

Among the investigated diverse roles of dinoflagellates in this study in Korean coastal ecosystems, the symbiont dinoflagellates live in the tissue of host coral species and support more than 90% of energy that the host coral needed. Coral reefs create various direct economical values derive from humans coastal activities including fishing, tourism and coastal protection. Thus, consequentially, the symbiotic dinoflagellates always positively influence on human by maintain the nature coral reefs healthy in coastal waters of Korea. Furthermore, the feeding activities of mixotrophic dinoflagellate predators on red tides species can reduce the economic losses of humans. However, sometimes they concentrated and bloom themselves it negatively influenced on human. Moreover, the trophic cascade between dinoflagellates-zooplankton may the principal trophic pathway in the transfer biomass to higher levels of marine organisms such as fish, which may be the key determinant of the total economical profit from the sea. In addition, dinoflagellates have much potentials to be directly commercialized that provide various usable bioresources to human such as biodiesel, DHA and valuable pigments.

Thus, I extensively and multidirectionally approached and investigated the diverse ecological roles of dinoflagellates, which was much more helpful to specifically understand the diverse ecological roles of dinoflagellates in the marine

ecosystems. Because, all of their various roles in around us such as primary producers, prey for zooplankton, mixotrophic predators, symbiont of corals and raw materials for bioresources may be consequentially closely related each another. Among the multiple effects of dinoflagellates on humans, some of impacts may difficult to directly feel ourselves because it is indirectly and weakly influence on humans. However, some of negative and positive influences of dinoflagellates on humans such as fish kill by large-scale red tides and development of usable bioresources closely happen around us now. Thus, any roles of the dinoflagellate in the marine ecosystems, marine environment and humans industry may be significantly closely related with humans life. Therefore, the extensive and multidirectional investigations about the diverse roles of dinoflagellates should be progressively continued.

I hope this study stimulate to observe the way to maximize the positive effects of the dinoflagellates and minimize the negative influences of the dinoflagellate on humans activities.

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# 국문초록

해양 와편모조류는 세계적으로 널리 발견되는 종이며, 대형해조류 및 해양 생물 내부 등을 포함하는 다양한 서식처에서 생활하 rh, 생태학적으로는 해양 플랑크톤 군집의 주요 구성 요소이다. 한국 연안생태계에서 발생하는 와편모조류 적조는 매년 큰 경제적 손실을 끼치고 있으나, 이러한 와편모조류의 생태학적 역할에 대한 다방면에 걸친 연구는 거의 없었다. 와편모조류의 다양한 생태학적 역할과 그들이 자연환경 및 인류에게 초래할 수 있는 잠재적 영향력을 이해하기 위해서는 다방면적인 광범위한 연구가 필수적이다. 그래서 본 연구에서는 한국 연안생태계에서 산호 공생생물로서, 적조생물의 혼합 영양 포식자로서, 동물 플랑크톤의 먹이로서 와편모조류의 역할에 대하여 연구하였다. 또한, 종속영양성 와편모조류의 고농도 배양을 위한 먹이생물인 미세조류의 대량배양을 위한 새로운 광생물반응장치를 새롭게 개발하고 그 성능을 실험 하였다.

산호에 공생하는 와편모조류들 중 그 서식밀도가 아주 낮은 *Symbiodinium* 종들의 생태학적 중요성에 대한 연구를 진행하였다. 장기간 연속 채집과 모니터링을 통하여 산호 안에서 낮은 농도로 존재하는 공생성 와편모조류가 숙주산호와의 어떠한 상관관계를 갖는지 예측 하였다. *Symbiodinium* 의 농도 변화와 공존하는 종간의 상대적인 비율 변화를 분석하기 위하여 Q-PCR 을 사용하였다. *Alveopora japonica* 에는 한 종의 *Symbiodinium* 이 99% 이상을 차지하고 있었으며, 다른 세 종의 *Symbiodinium* 또한 함께 발견 되었다. 연구결과, 극히 낮은 농도로 산호안에 존재하는 *Symbiodinium* 의 농도 변화는 주변 환경의 변화와는 무관한 것으로 나타났다. 따라서 산호와

공생관계를 유지하는 여러 공생미세조류 종들 중, 가장 높은 농도로 공생하는 *Symbiodinium* 의 극도 우점 종만이 산호-공생 미세조류 간의 관계에서 중요한 역할을 한다고 할 수 있다.

본 연구에서는 자가영양성 와편모 조류인 *Polykrikos hartmannii* 의 먹이생물 섭식 여부, 섭식 정도, 먹이생물 종 및 섭식 방법 등을 연구하였다. 그리고 *P. hartmannii* 의 적조생물 와편모류 *Cochlodinium polykrikoides* 에 대한섭식률과 성장률을 산출하였는데, *C. polykrikoides* 는 한국연안에서 적조를 일으켜 많은 피해를 야기시키는 대표 와편모조류 이다. 또한 본 연구를 통해 *P. hartmannii* 가 혼합영양성 와편모조류라는것을 처음으로 보고하였다. *P. hartmannii* 는 먹이로 제공된 다양한 잠재적 먹이 종들 중 오직 체인형태의 군체를 만들고 독성을 지닌것으로 알려진 와편모조류 *C. polykrikoides* 와 *Gymnodinium catenatum* 만을 섭식하였다. *P. hartmannii* 의 먹이 섭식 방법은 자신에 몸에 지닌 독침을 먹이생물에 발사하여 기절시킨 후 통째로 삼키는 것이다. *P. hartmannii* 의 *C. polykrikoides* 섭식률은 먹이생물의 농도가 높아짐에 따라 높아지다가 먹이생물 농도가 945 ng C $ml^{-1}$ (1350 cells $ml^{-1}$) 이상 될 경우에는 포화되어 더 이상 증가하지 않았다. *P. hartmannii* 의 *C. polykrikoides* 최대 섭식률은 1.9 ng C $predator^{-1}$ $d^{-1}$ (2.7 cells $predator^{-1}$ $d^{-1}$). 이였다. *P. hartmannii* 의 먹이생물로써 *C. polykrikoides* 에게 미치는 포식압은 0.324 $d^{-1}$ 이였는데 이는 자연상태의 *C. polykrikoides* 군집을 최대 28% 까지 하루에 제거 할 수 있는 수치이다. 결과적으로 *P. hartmannii* 는 적조 원인 생물인 *C. polykrikoides* 군집을 섭식을 통하여 상당 수준 제어 할 수 있는 것으로 나타났다.

해양생태계의 대표적인 일차생산자의 한 종류인 와편모조류가 섭식자인 동물 플랑크톤들의 먹이생물로서 해양생태계 먹이망에서 어떠한 중요한 역할을 하는지 규명하기 위해 2014 년 5 월부터 11 월까지 남해 연안역에서 16 회의 선박채집을 통하여 광범위하고 다각적인 연구를 진행하였다. 현장 해역의 수온, 염분, chl-a 및 식물 플랑크톤 – 원생 동물 – 후생 동물을 포함한 플랑크톤 군집 등의 3 차원 (3-D) 분포를 분석하야 그림으로 시각화하였다. 후생동물플랑크톤 중 가장 우점하는 Calanoid 요각류들의 적조현상 주요 적조 원인 종인 *Prorocentrum donghaiense* 와 *Cochlodinium polykrikoides* 에 대한 포식영향정도 (Grazing impact)를 분석한 결과, Calanoid 요각류들이 직접적인 포식을 통하여 적조현상을 소멸시키기지 못할 것으로 추산되었다 (각 최대 0.029 $d^{-1}$, 0.018$d^{-1}$). Calanoid 요각류들은 적조원인생물인 *Prorocentrum donghaiense* 의 주요 포식자인 종속영양와편모조류 *Gyrodinium* spp. 을 포식함으로써 개체수 감소에 더 많은 영향을 미친다. (0.047 $d^{-1}$). 또한 *Cochlodinium poikrikoides* 의 적조현장 발달 초기 단계에서 *Cochlodinium poikrikoides* 의 성장과 유영을 방해하는 주요 규조류 종들인 *Skeletonema costatum* 과 *Chaetoceros* spp. 등을 포식함으로써 오히려 적조형성에 유리한 영향을 준다 (각 0.05 $d^{-1}$, 0.032 $d^{-1}$). 이로써 Calanoid 요각류들은 적조 원인생물들의 작재적 포식자인 종속영양 와편모조류와 성장방해종들인 규조류들을 포식을함으로써 간접적으로 적조생물의 번식에 긍정적인 영향을 미친다.

와편모조류 및 녹조류와 같은 미세 조류는 바이오 연료의 소재생물로서 유망하고 가치있는 원생생물 종류들이다. 이들의 잠재적인 바이오 오일 생산성은 기타 다른 원천재료들에 비해 보다 적어도 4 배

이상 높다. 또한, 종속영양와편모조류는 많은 양의 불포화 지방산을 합성하는것으로 알려져 있다. 본 연구에서는 한국의 기후 조건에 적합한 종속영양과 와편모조류를 고농도로 배양하기 위한 먹이 미세조류 종을 고농도로 배양할 수 있는 효과적인 광생물반응기를 개발했다. 새롭게 디자인하여 개발한 광생물반응기는 폴리카보네이트 재질로 제작된 관형태의 모델이다. 이는 총 5 개의 부분으로 나뉘어지며, 부품의 추가적인 조립을 통하여 배양액의 총 부피와 배양관의 길이를 조절 할 수 있으며, 모든 부속품들은 고온멸균이 가능하여 대량배양 중의 오염을 예방 할 수 있다. 본 연구에서 *Dunaliella tertiolecta* 가 새로 개발 된 광생물반응기에서 이산화탄소 공급을 통하여 에서 매우 잘 자란다는 것을 확인 하였다. 연구 기간 동안, 5 % CO2 를 공급해 해준 광생물반응관 에서 최대 농도 7.2 x $10^7$ cells $ml^{-1}$ 로 성장하였다. 동일한 광생물반응관에서 세포의 성장을 좌우하는 주된 요인은 공급된 CO2 농도와 pH 등이다. 새로 개발 된 광생물반응관에서 기록한 *Dunaliella tertiolecta* 의 최대 농도는 기존에 보고된 최대배양 농도들보다 높았다. 따라서 5 % CO2 를 공급 하여 pH 범위는 7 ~ 7.5 범위를 유지해주는 것이 고농도로 *D. tertiolecta* 를 배양 할 수 있는 최적 조건으로 사료된다. 결론적으로 본 연구에서 사용 된 새로 설계된 광생물반응관과 CO2 를 공급하는 배양법은 다른 연구들과 비교해 보았을때 경쟁력이 있는 것으로 보여지며, 아주 고농도로 미세조류를 배양 할 수 있고, 아주 높은 농도로 배양된 미세조류들은 먹이생물로써 종속영양성 와편모조류를 고농도로 배양하는데 사용 될 수 있다.

본 연구에서는 해양 생태계에서 와편모조류들의 다양한 역할들에 대하여 입체적 관점에서 이해하기 위하여 다방향적으로 접근을 시도하여 보았다. 그 이유는, 와편모조류의 생태학적 다양한 역할들이 (일차

생산자로, 동물플랑크톤의 먹이생물, 혼합영양성 포식자, 산호의, 생물자원원료) 결과적으로 모두가 직간접적 관련이 있기 때문이다. 인간에게 미칠 수 있는 와편모도류들의 여려 영향들 중에서 일부는 인간에 간접적으로 적은 영향을 주기 때문에 직접적으로 체감되지 않을 수 있다. 하지만 대규모 적조로 인한 어류의 폐사가 야기시키는 부정적 피해나 그와 반대로 산업적인 바이오물질의 개발과 같은 긍정적인 효과들은 인간의 생활과 아주 밀접하게 발생되고 있다. 따라서 이 연구의 결과는 산호의 공생 생물, 혼합 영양 포식자, 해양 생태계에서 후생동물 플랑크톤의 먹이생물 및 먹이망 내의상호 작용자 등으로서의 와편모조류의 생태학적 역할을 규명하는데 기여할 것으로 판단된다.

**Keywords:** 해양생태, 공생생물, 와편모조류, 후생동물플랑크톤, 적조, 혼합영양성

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