



HOKKAIDO UNIVERSITY

Title	Water-column light utilization efficiency of phytoplankton and transparent exopolymer particles in the western subarctic Pacific
Author(s)	野坂, 裕一
Degree Grantor	北海道大学
Degree Name	博士(環境科学)
Dissertation Number	甲第11344号
Issue Date	2014-03-25
DOI	https://doi.org/10.14943/doctoral.k11344
Doc URL	https://hdl.handle.net/2115/55434
Type	doctoral thesis
File Information	Yuichi_Nosaka.pdf



**Water-column light utilization efficiency of phytoplankton
and transparent exopolymer particles
in the western subarctic Pacific**

Yuichi NOSAKA

DOCTORAL DISSERTATION

Graduate School of Environmental Science, Hokkaido University

2014

Table of contents

LIST OF TABLES	V
LIST OF FIGURES	IX
LIST OF PHOTOS	XIV
LIST OF SYMBOLS	XV
LIST OF ABBREVIATIONS	XVI

CHAPTER 1 – GENERAL INTRODUCTION

1.1 OVERVIEWS OF THE OCEAN AND PHOTOSYNTHESIS	1
1.2 PRIMARY PRODUCTION OF PHYTOPLANKTON AND CARBON CYCLE	4
1.3 PRIMARY PRODUCTION AND WATER-COLUMN LIGHT UTILIZATION EFFICIENCY (Ψ) OF PHYTOPLANKTON IN THE WESTERN SUBARCTIC PACIFIC	6
1.4 BIOLOGICAL CARBON PUMP AND TRANSPARENT EXOPOLYMER PARTICLES (TEP) IN THE OYASHIO REGION	7
1.5 PURPOSE OF THIS STUDY	9

CHAPTER 2 – LIGHT UTILIZATION EFFICIENCY OF PHYTOPLANKTON IN THE WESTERN SUBARCTIC GYRE OF THE NORTH PACIFIC DURING SUMMER

2.1 INTRODUCTION	11
2.2 MATERIALS AND METHODS	12
2.2.1 KH08-2 CRUISE	12
2.2.1.1 SEAWATER SAMPLING	12
2.2.1.2 PHYTOPLANKTON PIGMENTS AND CHEMTAX PROCESSING	13
2.2.1.3 FLOW CYTOMETRY	14
2.2.1.4 CELL ABUNDANCE OF PHYTOPLANKTON	15
2.2.1.5 DAILY PRIMARY PRODUCTION	15
2.2.1.6 WATER-COLUMN LIGHT UTILIZATION EFFICIENCY (Ψ) OF PHYTOPLANKTON PHOTOSYNTHESIS	16
2.2.2 SEEDS-I AND SEEDS-II	16

2.3	RESULTS	17
2.3.1	KH08-2 CRUISE	17
2.3.1.1	HYDROGRAPHY	17
2.3.1.2	PIGMENTS AND CHEMTAX OUTPUTS	17
2.3.1.3	ABUNDANCE AND COMMUNITY COMPOSITION OF PHYTOPLANKTON ESTIMATED BY FLOW CYTOMETRY OR SCANNING ELECTRON MICROSCOPY	17
2.3.1.4	PRIMARY PRODUCTION	18
2.3.1.5	LIGHT UTILIZATION EFFICIENCY (Ψ)	19
2.3.2	Ψ IN SEEDS-I AND SEEDS-II	19
2.4	DISCUSSION	20
2.4.1	FACTORS CONTROLLING Ψ VALUES IN THE WSG DURING THE SUMMER	20
2.4.2	RELATIONSHIP BETWEEN Ψ AND DAILY PAR	23

CHAPTER 3 – DYNAMICS OF TRANSPARENT EXOPOLYMER PARTICLES IN THE OYASHIO REGION OF THE WESTERN SUBARCTIC PACIFIC DURING THE SPRING DIATOM BLOOMS **34**

3.1	INTRODUCTION	34
3.2	MATERIALS AND METHODS	36
3.2.1	RESEARCH CRUISES	36
3.2.2	PHYTOPLANKTON PIGMENTS AND CHEMTAX PROCESSING	37
3.2.3	PHYTOPLANKTON SPECIFIC ABSORPTION COEFFICIENT	38
3.2.4	CELL ABUNDANCE OF PHYTOPLANKTON	39
3.2.5	FLOW CYTOMETRY	39
3.2.6	DISSOLVED ORGANIC CARBON (DOC) ANALYSIS	40
3.2.7	PULSE AMPLITUDE MODULATION (PAM) FLUOROMETER MEASUREMENTS	40
3.2.8	PARTICULATE ORGANIC CARBON (POC) PRODUCTION	40
3.2.9	DOC PRODUCTION	41
3.2.10	PHOTOSYNTHESIS-IRRADIANCE ($P-E$) CURVE EXPERIMENTS	42
3.2.11	TEP ANALYSIS	44
3.3	RESULTS	45
3.3.1	HYDROGRAPHY	45
3.3.2	PHYTOPLANKTON PIGMENTS AND COMMUNITY COMPOSITION AS ESTIMATED BY CHEMTAX PROGRAM	46

3.3.3	CELL ABUNDANCES AND COMPOSITIONS OF DIATOMS AND COCCOLITHOPHORES BY ESTIMATED SEM	46
3.3.4	CELL ABUNDANCES OF EUKARYOTIC ULTRAPHYTOPLANKTON AND <i>SYNECHOCOCCUS</i> ESTIMATED BY FLOW CYTEMETERY	47
3.3.5	BACTERIA ABUNDANCE ESTIMATED BY FLOW CYTEMETERY	47
3.3.6	POC CONCENTRATION	48
3.3.7	DOC CONCENTRATION	48
3.3.8	MAXIMUM PHOTOCHEMICAL QUANTUM EFFICIENCY (F_v/F_m) OF PHOTOSYSTEM II FOR PHYTOPLANKTON	48
3.3.9	POC AND DOC PRODUCTION	48
3.3.10	PHYTOPLANKTON SPECIFIC ABSORPTION COEFFICIENT	49
3.3.11	<i>P-E</i> PARAMETERS AND THE MAXIMUM QUANTUM YIELD ($\Phi_{C\text{MAX}}$) OF CARBON FIXATION FOR PHOTOSYNTHESIS	49
3.3.12	TEP LEVELS	50
3.4	DISCUSSION	51
3.4.1	COMPARISONS OF TEP LEVELS BETWEEN THE OYASHIO REGION AND OTHER OCEANS	51
3.4.2	TEP LEVEL IN THE OYASHIO REGION DURING THE SPRING DIATOM BLOOMS	52
3.4.3	CONTRIBUTION OF TEP TO BIOLOGICAL CARBON PUMP IN THE OYASHIO REGION DURING THE SPRING BLOOMS	54
3.4.4	TEP PRODUCTION DURING THE SPRING DIATOM BLOOMS IN THE OYASHIO REGION	54
 CHAPTER 4 – FORMATION OF TRANSPARENT EXOPOLYMER PARTICLES FROM THE DIATOM <i>THALASSIOSIRA NORDENSKIOELDII</i> STRAIN		82
4.1	INTRODUCTION	82
4.2	MATERIALS AND METHODS	84
4.2.1	DESIGN OF LABORATORY CULTURE EXPERIMENT	84
4.2.1.1	ISOLATION, STERILIZATION AND ACCLIMATION OF <i>THALASSIOSIRA NORDENSKIOELDII</i>	84
4.2.1.2	PREPARATION OF THE CULTURE EXPERIMENT	85
4.2.1.3	START OF THE CULTURE EXPERIMENT AND SAMPLING	85
4.2.2	SAMPLES OF EVERY OTHER DAY	86
4.2.2.1	NUTRIENTS	86
4.2.2.2	CELL SIZE AND COUNT	86

4.2.2.3	TRANSPARENT EXOPOLYMER PARTICLE (TEP) LEVELS	88
4.2.2.4	TEP PRODUCTIVITY	89
4.2.3	SAMPLES OF ONCE IN FOUR DAYS	89
4.2.3.1	PARTICULATE ORGANIC CARBON (POC) AND PARTICULATE NITROGEN (PN)	89
4.2.3.2	DISSOLVED ORGANIC CARBON (DOC)	89
4.2.3.3	DOC PRODUCTIVITY ESTIMATED FROM DOC CONCENTRATION	90
4.2.3.4	PIGMENTS	90
4.2.4	PHOTOSYNTHESIS-IRRADIANCE (<i>P-E</i>) CURVE EXPERIMENTS IN THE EXPONENTIAL AND STATIONARY PHASES	90
4.2.4.1	PHYTOPLANKTON SPECIFIC ABSORPTION COEFFICIENTS	90
4.2.4.2	<i>P-E</i> CURVE EXPERIMENT FOR POC AND DOC	91
4.3	RESULTS AND DISCUSSION	93
4.3.1	CELL ABUNDANCE AND CONDITION DURING THE INCUBATION	93
4.3.2	PIGMENTS	95
4.3.3	TEP LEVELS AND TEP PRODUCTIVITIES	97
4.3.4	RELATIONSHIP BETWEEN DOC AND TEP PRODUCTIVITIES	98
4.3.5	POC AND PN CONCENTRATIONS	99
4.3.6	RELATIONSHIP BETWEEN THE LIGHT LEVELS, AND DOC AND POC PRODUCTIVITIES	100
 CHAPTER 5 – GENERAL CONCLUSIONS AND PERSPECTIVES		 122
5.1	GENERAL CONCLUSIONS	122
5.2	PERSPECTIVES	125
 ACKNOWLEDGEMENTS		 126
 REFERENCES		 128

List of tables

Chapter 2		page
Table 2.1	Accessory pigment:chlorophyll <i>a</i> ratio matrices: (A) Initial ratio matrix in the 100–10% light depths; (B) Final ratio matrix obtained by CHEMTAX in the 100–10% light depths; (C) Initial ratio matrix in the 10–1% light depths; (D) Final ratio matrix obtained by CHEMTAX in the 10–1% light depths.	25
Table 2.2	Hydrographic conditions and phytoplankton productivity during Leg 1 of the KH 08-2 cruise. TD: transition domain, WSG: Western Subarctic Gyre, Z_{mix} : surface mixed layer depth, $N_{\text{mix_mean}}$: mean nitrite and nitrate concentrations within the surface mixed layer, Z_{eu} : euphotic layer depth, $N_{\text{eu_mean}}$: mean nitrite and nitrate concentrations within the euphotic layer, $T_{\text{inc_start}}$: start time of incubations, Ψ : water-column light utilization efficiency of phytoplankton photosynthesis.	26
Table 2.3	Size-fractionated chlorophyll <i>a</i> concentrations at 5 m and 5% (or 3%) light depth at each station.	27
Table 2.4	List of the phytoplankton species identified. Genus and species names are arranged alphabetically, not systematically. The asterisk indicates the most dominant species in the diatoms or coccolithophores.	28
Table 2.5	Summary of chlorophyll <i>a</i> concentration, primary production, PAR, Chl <i>a</i> -specific primary production and Ψ during SEEDS-I and SEEDS-II. Fe-in: inside the iron-fertilized patch, Fe-in _{growth} : growth phase based on the F_v/F_m levels in the Fe-in, Fe-out: outside the iron-fertilized patch, PAR: photosynthetic available radiation, Ψ : water-column light utilization efficiency of phytoplankton photosynthesis.	29
Chapter 3		
Table 3.1	Final accessory pigment:chlorophyll <i>a</i> ratio matrices obtained	56

by CHEMTAX: (A) Initial ratio matrix in the 5–20 m depths; (B) Final ratio matrix obtained by CHEMTAX in the 5–20 m depths; (C) Initial ratio matrix in the 30–50 m depths; (D) Final ratio matrix obtained by CHEMTAX in the 30–50 m depths.

Table 3.2	Conductivity and DOC concentrations at before (original) and after (desalted) of the desalination. The parentheses show the percentages between the before and after. The conductivity decreased to ca. 6% of the initial conductivity, whereas the recovery percentages of DOC concentration ranged from 62 to 96%.	57
Table 3.3	Comparisons of the TEP standard curves between this study and previous studies. The slopes (calibration factor) were shown for an inverse number (f^{-1}) of the regressions of Alcian blue absorbance vs. xanthan gum level. The slopes in this study ranged within those of previous studies. It is reported that the slopes vary according to the batch of staining solution (Passow and Alldredge, 1995).	58
Table 3.4	Hydrographic conditions, Chl <i>a</i> , POC, POC/Chl <i>a</i> ratio, and diatom abundances. They were shown in order to the Chl <i>a</i> concentrations, that is alignment sequence of April, 2010, May, 2011 and June, 2010 cruises.	59
Table 3.5	List of the phytoplankton species identified. Genus and species names are arranged alphabetically, not systematically. Dominant species in the April, May and June showed as red, purple and blue colors, respectively.	60
Table 3.6	Maximum photochemical quantum efficiency (F_v/F_m) of photosystem II for phytoplankton, POC production and DOC production. PER was the percentage of DOC production/(DOC plus POC production). They were shown in order to the Chl <i>a</i> concentrations, that is alignment sequence of April, 2010, May, 2011 and June, 2010 cruises.	61

Table 3.7	Summary of phytoplankton specific absorption coefficient ($\bar{a}_{ph}^*$) ($m^2 [mg\ Chl\ a]^{-1}$), the maximum photosynthesis rate of P - E curve (P_{max}^*) ($mg\ C\ [Chl\ a]^{-1}\ h^{-1}$), the initial slope (α^*) ($mg\ C\ [Chl\ a]^{-1}\ h^{-1}\ [\mu mol\ photon\ m^{-2}\ s^{-1}]^{-1}$), the photoinhibition index (β^*) ($mg\ C\ [Chl\ a]^{-1}\ h^{-1}\ [\mu mol\ photon\ m^{-2}\ s^{-1}]^{-1}$), the light saturation index (E_k) ($\mu mol\ photons\ m^{-2}\ s^{-1}$), the coefficient of determination for the P - E fitting curve (r^2) and the maximum quantum yield of carbon fixation ($\Phi_{c,max}$) ($mol\ C\ [mol\ photon]^{-1}$) at 5 m depth. They were shown in order to the Chl a concentrations, that is alignment sequence of April, 2010, May, 2011 and June, 2010 cruises.	62
Table 3.8	The levels of TEP, and the ratios of TEP/Chl a , TEP/POC and TEP-C/POC at 5 m depth, and the integrated levels from 5 to 300 m depths. They were shown in order to the Chl a concentrations, that is alignment sequence of April, 2010, May, 2011 and June, 2010 cruises.	63
Table 3.9	Relationships between TEP and other parameters, and between TEP* and other parameters. A significant relationship showed by boldface.	64
Table 3.10	Summary of the TEP surveys from 1995 to early 2013. This summary was only listed the TEP levels reported for the photometric (i.e., unit: Xanthan equivalent).	66–67
Table 3.11	Summary of TEP-C/POC ratios from 2001 to early 2013.	68
Chapter 4		
Table 4.1	Summary of the results in the exponential and stationary phases. μ : specific growth rate; M : division rate; POC: particulate organic carbon; PN: particulate nitrogen; C: carbon; Chl a : chlorophyll a ; TEP: transparent exopolymer particles; DOC: dissolved organic carbon.	103
Table 4.2	Summary of the results obtained in the Chl a -normalized photosynthetic–irradiance (P – E) curve experiments. $\bar{a}_{ph}^*$: mean chlorophyll (Chl) a -specific absorption coefficient of	104

phytoplankton; $P_{\max}^*$: Chl a -normalized maximum photosynthetic rate; α^* : the initial slope; β^* : the photoinhibition index; E_k : light saturation index; $\Phi_{\text{Chl-a-c-max}}$: the maximum quantum yield of carbon fixation.

Table 4.3	Summary of the results obtained in the cell-normalized photosynthetic–irradiance (P – E) curve experiments. $\bar{a}_{\text{ph}}^{*\text{cell}}$: mean cell-specific absorption coefficient of phytoplankton; $P_{\max}^{\text{cell}}$: cell-normalized maximum photosynthetic rate; α^{cell} : the initial slope; β^{cell} : the photoinhibition index; E_k : light saturation index; $\Phi_{\text{cell-c-max}}$: the maximum quantum yield of carbon fixation.	105
------------------	--	-----

List of figures

Chapter 1	page
Fig. 1.1 Schematic diagram of the light chemical reaction in photosynthesis. (A) Light energy is excited the photosystem (PS) II reaction center, and charge separation occur. (B) The lost electrons in the chlorophyll can acquire by splitting water (H_2O) at PS II oxygen-evolving center (OEC). The electrons flows into the PS I throughout cytochrome b_6f complex. (C) The cytochrome b_6f complex transports the proton (H^+) from stroma to lumen. (D) On the other hand, the entered electrons into the PS I are re-excited by light energy. (E) The PS I synthesize the nicotinamide adenine dinucleotide phosphate (NADPH) from the NADP^+ by using the electrons. The O_2 emitted into the lumen by the H_2O splitting is eventually released to the extracellular (F), whereas the H^+ levels in the lumen increase as the water cleavage occurs (G). The difference of the H^+ levels between the stroma and lumen drive the adenosine triphosphate (ATP) synthase (H), and generate the ATP from both of the adenosine diphosphate (ADP) and the phosphoric acid (Pi) (I). Referred from Taiz and Zeiger (2002).	3
Fig. 1.2 Calvin cycle progress by the three sections. (A) CO_2 and H_2O are fixed into 3-phosphoglycerate (PGA) by the enzyme reaction of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). (B) The generated 3-phosphoglycerate synthesizes the carbohydrates using the reducing power of the ATP and NADPH obtained by the light chemical reaction. (C) Ribulose-1,5-bisphosphate (RuBP) is regenerated by the enzyme reaction of phosphoribulokinase by using the ATP. Redrawn from Taiz and Zeiger (2002).	4
Fig. 1.3 Schematic diagram of the biological carbon pump. (A) Large and small phytoplankton fixes the aqueous CO_2 (aqCO_2) in the seawater. (B) A large fraction of the fixed organic carbon is released again in the form of CO_2 from the surface water to the atmosphere because of respiration in the grazing food chain, and of decomposition and respiration in the microbial loop. (C) On	9

the other hand, a part of the fixed organic carbon is transferred from the surface to deep ocean, and released in the form of CO₂ throughout respiration by the deep consumers and decomposition by bacteria. Redrawn from Chisholm (2000).

Chapter 2

- Fig. 2.1** Sampling stations during the KH08-2 cruise in the western subarctic Pacific. The locations of Stn KNOT, SEEDS-I and SEEDS-II are also indicated. The surface current is drawn with arrows following Yasuda (2003). 30
- Fig. 2.2** Contributions of each phytoplankton group to the chlorophyll *a* biomass within the euphotic zone in the WSG (Stns 5, 6, 8, 9 and 10) and TD (Stns 1, 2, 3 and 11). 31
- Fig. 2.3** Euphotic-depth-integrated cell abundances of eukaryotic ultraphytoplankton and *Synechococcus* in the WSG (Stns 5, 6, 8, 9 and 10) and TD (Stns 1, 2, 3 and 11). 32
- Fig. 2.4** Relationships between Ψ and daily PAR during the KH08-2 cruise (WSG and TD), other studies in the WSG, and the world's oceans. (A) The fitting curves obtained from the Falkowski and Raven (2007), (B) the fitting curve using the WSG data obtained from the KH08-2 cruise and (C) the fitting curve using all WSG data. The fitting curves (A), (B) and (C) correspond to equations (3), (4) and (5), respectively. 33

Chapter 3

- Fig. 3.1** Sampling locations in the TEP survey cruises during the Oyashio spring diatom blooms. The stations in April and June, 2010 were shown with red color (A1 and A2) and white color (J1, J2, J3 and J4), respectively. The stations in May, 2011 were also shown with yellow color (M1, M2 and M3). 69
- Fig. 3.2** Chlorophyll *a* vertical profile in the Oyashio spring phytoplankton blooms. 70
- Fig. 3.3** Average contributions of each phytoplankton group to the Chl *a* 71

biomass within 5–50 m depths. They were shown in order of the Chl *a* concentrations, that is alignment sequence of April, 2010, May, 2011 and June, 2010 cruises.

Fig. 3.4	Vertical distributions of eukaryotes (A, B), <i>Synechococcus</i> (C, D) and bacteria (E, F).	72
Fig. 3.5	Vertical profiles of dissolved organic carbon (DOC) concentrations in April and May cruises (A), and June cruises (B).	73
Fig. 3.6	Vertical profiles of TEP levels in April (A), May (B) and June (C), and of TEP/Chl <i>a</i> ratios in April and May (D) and June (E).	74
Fig. 3.7	TEP levels	76–77
Fig. 3.8	Relationship between TEP and Chl <i>a</i> concentrations within the mixed layer.	78
Fig. 3.9	Relationships between TEP and Chl <i>a</i> concentrations obtained in the various region. This study (A), Hong et al. (1997) (B), Kiørboe (1996) and Passow (2002a) (C), Engel (1998) (D), Average of the diatom strains (Passow, 2002a) (E).	79

Chapter 4

Fig. 4.1	Schematic figure in this experiment. The two 20-L culture vessels were stored in the incubator maintained at 5°C. Six fluorescent lamps were mounted to the upper part in the incubator, and photosynthetic available radiation (PAR) of ca. 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ at the base of the bottle was exposed with light dark-cycle of 12 hours vs. 12 hours.	106
Fig. 4.2	Explanation of the sampling system. The culture experiment was conducted with the 20-L culture vessels (A) with four ports (B). Two ports of the four ports were used for the vent port (B_v) to exchange the air between inside and outside the vessel, and for the sampling port (B_s), respectively. The vent port was mounted the two disposable inline filters (C_v). The sampling port was installed	107

a three-way cock (D) with the inline filter (C_s). When sampling is carried out, the sampling tubing (F) extended from a sampling bottle (G) was connected with the joint (E) extended from the three-way cock (D). Subsequently, the three-way cock was twisted from the atmosphere opening through the inline filter (C_s) to the sampling bottle (G), and an aspirator was connected with the outlet tubing (H) of the sampling bottle (G). The air pressure in the sampling bottle (G) was lowered with the aspirator. Therefore, the water sample was transferred from the 20-L culture vessel to the sampling bottle. After sampling, the three-way cock (D) was re-twisted to the atmosphere opening through the inline filter (C_s), and then the sampling tubing (F) was removed from the joint (E).

Fig. 4.3	Cell abundances in the culture vessels 1 and 2. The error bar shows the standard deviation ($n = 2$).	108
Fig. 4.4	Nitrate (NO_3) plus nitrite (NO_2), and silicate (Si(OH)_4) concentrations in the culture vessels 1 and 2 during this experiment. The error bars show the standard deviation ($n = 2$).	109
Fig. 4.5	Lengths of the averaged cell diameter and pervalvar axis (A), and the averaged area and volume (B). The error bars show the standard deviation ($n = 11$ for days 0–10; $n = 21$ for days 11–40).	110
Fig. 4.6	Relationships between the pigment concentrations and the cell abundances. All pigments were carried out the linear fitting.	111
Fig. 4.7	Figure of the TEP levels. The levels increased with days. Unfortunately, the data of days 38 and 40 in the vessel 1 were lost by a mistake during the sampling process. The error bar shows the standard deviation ($n = 3$).	112
Fig. 4.8	Dissolved organic carbon (DOC) concentrations. Unfortunately, the data of days 40 in the vessel 1 were lost by a mistake during the sampling process. The error bar shows the standard deviation ($n = 5$).	113
Fig. 4.9	Relationship between the cellular TEP and DOC production.	114

Fig. 4.10	Particulate organic carbon (POC) and particulate nitrogen (PN) concentrations. For the PN, the concentrations during days 0–16 could be not detected due to the detection limit.	115
Fig. 4.11	Percentages of the TEP-C/POC concentrations in the vessels 1 and 2.	116
Fig. 4.12	Chl <i>a</i> -normalized particulate organic carbon (POC) productivity (A) and dissolved organic carbon (DOC) productivity (B) in the exponential and stationary phases. The error bars show the standard deviation ($n = 2$).	117
Fig. 4.13	Cell-normalized particulate organic carbon (POC) productivity (A) and dissolved organic carbon (DOC) productivity (B) in the exponential and stationary phases. The error bars show the standard deviation ($n = 2$).	118
Fig. 4.14	Ratios (PER) of the DOC/Total production. The error bar shows the standard deviation ($n = 2$).	119

List of photos

Chapter 3		page
Photo 3.1	Photos of <i>Chaetoceros</i> sp.1 (A) and <i>Chaetoceros</i> sp. 6 (B).	80
Photo 3.2	A Photo of massive TEP (marine “snowflake”) in the Adriatic Sea (Kaiser et al., 2011).	81
Chapter 4		
Photo 4.1	<i>Thalassiosira nordenskioeldii</i> photographed with Scanning electronic microscope (SEM).	120
Photo 4.2	<i>Thalassiosira nordenskioeldii</i> and TEP in this experiment were photographed with a optical microscope. The TEP were attaching to the surface of <i>T. nordenskioeldii</i> . The four cells of the center in the photo were <i>T. nordenskioeldii</i> . The Blue substances were TEP stained by the Alcian blue.	121

List of symbols

Symbol	Definition	Unit
$\bar{a}^*_{ph}$	Mean Chl <i>a</i> -specific Absorption Coefficient of Phytoplankton	m ² (mg Chl <i>a</i>) ⁻¹
$\bar{a}^{cell}_{ph}$	Mean Cell-specific Absorption Coefficient of Phytoplankton	m ² (cell) ⁻¹
E_k	Light Saturation Index	μmol photon m ⁻² s ⁻¹
M	Growth Rate per Day	division d ⁻¹
N_{eu_mean}	Mean Nitrate and Nitrite Concentrations within the Euphotic Zone	μM
N_{mix_mean}	Mean Nitrate and Nitrite Concentrations within the Mixed Layer	μM
P^*_{max}	Chl <i>a</i> -normalized Maximum Photosynthetic Rate of the <i>P-E</i> curve	mg C (Chl <i>a</i>) ⁻¹ h ⁻¹
P^{cell}_{max}	Cell-normalized Maximum Photosynthetic Rate of the <i>P-E</i> curve	mg C (cell) ⁻¹ h ⁻¹
TEP*	Chl <i>a</i> -normalized TEP concentration	μg Xanthan gum (μg Chl <i>a</i>) ⁻¹
T_{inc_start}	Start Time of Incubations	
Z_{eu}	Euphotic Layer Depth	m
Z_{mix}	Surface Mixed Layer Depth	m
α^B or α^*	Chl <i>a</i> -normalized Initial Slope of the <i>P-E</i> curve	mg C (Chl <i>a</i>) ⁻¹ h ⁻¹ (μmol photon m ⁻² s ⁻¹) ⁻¹
α^{cell}	Cell-normalized Initial Slope of the <i>P-E</i> curve	mg C (cell) ⁻¹ h ⁻¹ (μmol photon m ⁻² s ⁻¹) ⁻¹
β^*	Chl <i>a</i> -normalized Photoinhibition Index of the <i>P-E</i> curve	mg C (Chl <i>a</i>) ⁻¹ h ⁻¹ (μmol photon m ⁻² s ⁻¹) ⁻¹
β^{cell}	Cell-normalized Photoinhibition Index of the <i>P-E</i> curve	mg C (cell) ⁻¹ h ⁻¹ (μmol photon m ⁻² s ⁻¹) ⁻¹
μ	Specific Growth Rate	d ⁻¹
ρ	Correlation Coefficient of Spearman's Rank Correlation	
$\Phi_{c\ max}, \Phi_{chl-a-e\ max}$ or $\Phi_{cell-c\ max}$	Maximum Quantum Yield of Carbon Fixation	mol C (mol photon) ⁻¹
Ψ	Water-Column Light Utilization Efficiency	g C (g Chl <i>a</i>) ⁻¹ (mol photon) ⁻¹ m ²

List of abbreviations

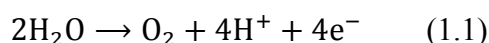
Abbreviation	Definition
^{13}C	Carbon-13
^{14}C	Carbon-14
A1, A2	The Station Names (April) of Chapter 3
ADP	Adenosine Diphosphate
aqCO_2	Aqueous CO_2
ATP	Adenosine Triphosphate
CHEMTAX	Chemical Taxonomy (computer program)
Chl <i>a</i>	Chlorophyll <i>a</i>
Chl <i>b</i>	Chlorophyll <i>b</i>
Chl $c_2 + c_1$	Chlorophyll $c_2 + c_1$
DIC	Dissolved Inorganic Carbon
DMF	<i>N, N</i> -dimethylformamide
DOC	Dissolved Organic Carbon
EPS	Extracellular Polymeric Substances
Fd	Ferredoxin
Fe-in	Inside the Iron-Enriched Patch during the SEEDS-I and SEEDS-II
Fe-in _{growth}	The Growth Phase of phytoplankton assemblages Based on F_v/F_m in Fe-in
Fe-out	Outside the Iron-Enriched Patch during the SEEDS-I and SEEDS-II
FNR	Ferredoxin-NADP Reductase
F_v/F_m	The Photochemical Quantum Efficiency of Algal PS II
HNLC	High-Nitrate, Low-Chlorophyll
HPLC	High-Performance Liquid Chromatography
J1, J2, J3, J4	The Station Names (June) of Chapter 3
KH08-2	The Cruise Name of Ocean Survey by <i>R/V Hakuho-Maru</i> in summer 2008
KNOT	Kyodo North Pacific Ocean Time Series (44°N, 155°E)
M1, M2, M3	The Station Names (May) of Chapter 3
NADP ⁺	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate (the reduced form of NADP ⁺)
NW	North West
OEC	Oxygen-Evolving Center
<i>P-E</i>	Photosynthesis-Irradiance
PAR	Photosynthetically Active Radiation
$p\text{CO}_2$	Partial Pressure of CO_2
PER	Percentage of Extracellular Release (Ratio of the DOC productivity/DOC + POC productivities)
PET	Photosynthetic Electron Transport
Pi	Inorganic Phosphate
PN	Particulate Nitrogen
POC	Particulate Organic Carbon
PS I	Photosystem I
PS II	Photosystem II
<i>R/V</i>	Research Vessel
RMSE	Root Mean Square Error
Rubisco	Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase
RuBP	Ribulose-1,5-Bisphosphate
SAB	Subarctic Boundary
SAF	Subarctic Front
SEEDS-I	Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study I
SEEDS-II	Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study II
SEM	Scanning Electron Microscope
TBAA	Tetrabutyl Ammonium Acetate
TD	Transition Domain
TEP	Transparent Exopolymer Particles
TEP-C	Carbon content in the TEP
TIC	Total Inorganic Carbon
WSG	Western Subarctic Gyre

Chapter 1 – General introduction

1.1 Overviews of the ocean and photosynthesis

The ocean occupies about 71% of the Earth's surface. The mean depth, area and volume is estimated about 3,700 m, $362 \times 10^6 \text{ km}^2$ and $1.33 \times 10^9 \text{ km}^3$, respectively (Charette and Smith, 2010). Many organisms are living in the ocean, and depend upon the primary producers which can convert inorganic materials to organic matters by the photosynthesis. Main primary producers in the ocean is phytoplankton (Raven, 2010), which are planktonic and living throughout the euphotic zones of all water bodies including under ice in polar areas. On the other hand, phytoplankton can be also benthic, living within sediments and on rocks and so on, but their habitat is limited to shallow areas because of the rapid attenuation of the sunlight with depth (Barsanti and Gualtieri, 2006). The euphotic zone is defined as the portion of the water column supporting net primary production. In general the euphotic zone seldom extends down to 150 m depth, and in coastal waters light can penetrate up to ca. 50 m (Kaiser et al., 2011). Hence, photosynthesis rises only in the thin layer of the upper ocean. The spectra between 400 and 700 nm called photosynthetically active radiation (PAR) are used for photosynthesis of phytoplankton (Falkowski and Raven, 2007). To absorb the light energy within the PAR range, algae contain specialized light-sensitive pigments such as chlorophylls and xanthophylls in the thylakoid membrane (Falkowski and Raven, 2007; Kark, 2011).

The light energy captured by the pigments is transferred to the chlorophyll special pair (reaction center) in the photosystem II (PS II), and the chlorophyll is excited by the accumulated light energy (Fig. 1.1A). Subsequently, the energy of the excited chlorophyll is converted to the electron energy by pass to the near electron acceptor. This is referred as charge separation, and it is a reductive reaction of the electron acceptor by the excited chlorophyll molecule (Pearlstein, 1996; Blankenship, 2002). The lost electrons in the chlorophyll can be acquired by splitting water (H_2O) at PS II oxygen-evolving center (OEC) that consist of a tetranuclear manganese cluster (Umena et al., 2011; Fig. 1.1B). The water cleavage is a chemical reaction as the flowing equation (1.1) (Hoganson and Babcock, 1997).



Then, molecular oxygen (O_2) and protons ($4H^+$) are evolved as by-product. The details of the charge separation and oxygen evolution are described in Barber (2008).

The electrons obtained by the charge separation are transferred to the downstream of the photosynthetic electron transport (PET) chain. On the route of PET, the electrons enter in the cytochrome b_6f complex, and subsequently are transferred to the photosystem I (PS I). The cytochrome b_6f complex transports the H^+ from outside (stroma) to inside (lumen) of the thylakoid membrane (Fig. 1.1C) (Cramer et al., 1996). On the other hand, the entered electrons into the PS I are re-excited by light energy, and the charge separation occur as described above, again (Fig. 1.1D). Redox potential of those electrons in the PS I is higher than that of PS II (Blankenship and Prince, 1985; Prince, 1985), and the electrons reduce the ferredoxin (Fd) in the stroma. Finally, the reduced Fd and the ferredoxin-NADP reductase (FNR) is generated the nicotinamide adenine dinucleotide phosphate (NADPH) by reducing the $NADP^+$ (Fig. 1.1E) (Shin and Arnon, 1965; Arakaki et al., 1997).

On the other hand, O_2 and $4H^+$ in the equation 1.1 are emitted into the lumen (Falkowski and Raven, 2007). The O_2 is eventually released to the extracellular (Fig. 1.1F), whereas the inside pH of the thylakoid membrane decreases as the water cleavage occurs, since the emitted $4H^+$ are remained in the lumen (Fig. 1.1G). Moreover, because the H^+ transportation from the stroma to lumen also occurs in the cytochrome b_6f complex, the electrochemical potential is higher in the lumen than stroma. The electrochemical potential slope drives the adenosine triphosphate (ATP) synthase by the H^+ transportation from the lumen to stroma (Fig. 1.1H) (Junge, 1999), and ATP is synthesized from both of the adenosine diphosphate (ADP) and the inorganic phosphate (Pi) (Fig. 1.1I) (Junge et al., 1997; Junge, 2004).

The ATP and NADPH synthesized by a series of the light chemical reaction are used for the reaction energy of carbon fixation. The carbon fixation system is called the Calvin cycle (e.g., Calvin, 1989). The Calvin cycle initiates by reacting carbon dioxide (CO_2) with ribulose-1,5-bisphosphate (RuBP) and yield 3-phosphoglycerate (PGA) catalyzed by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Fig. 1.2A). The generated 3-phosphoglycerate synthesizes the carbohydrates using the reducing power of the ATP and NADPH obtained by the light chemical reaction (Fig. 1.2B). The used ATP is decomposed to the ADP and Pi by hydrolysis, and the NADPH is oxidized to the $NADP^+$. The ADP, Pi and $NADP^+$ may be recycled for the light

chemical reaction as described above. The Calvin cycle concludes with the RuBP reproduction by the enzyme reaction of phosphoribulokinase using ATP (Fig. 1.2C).

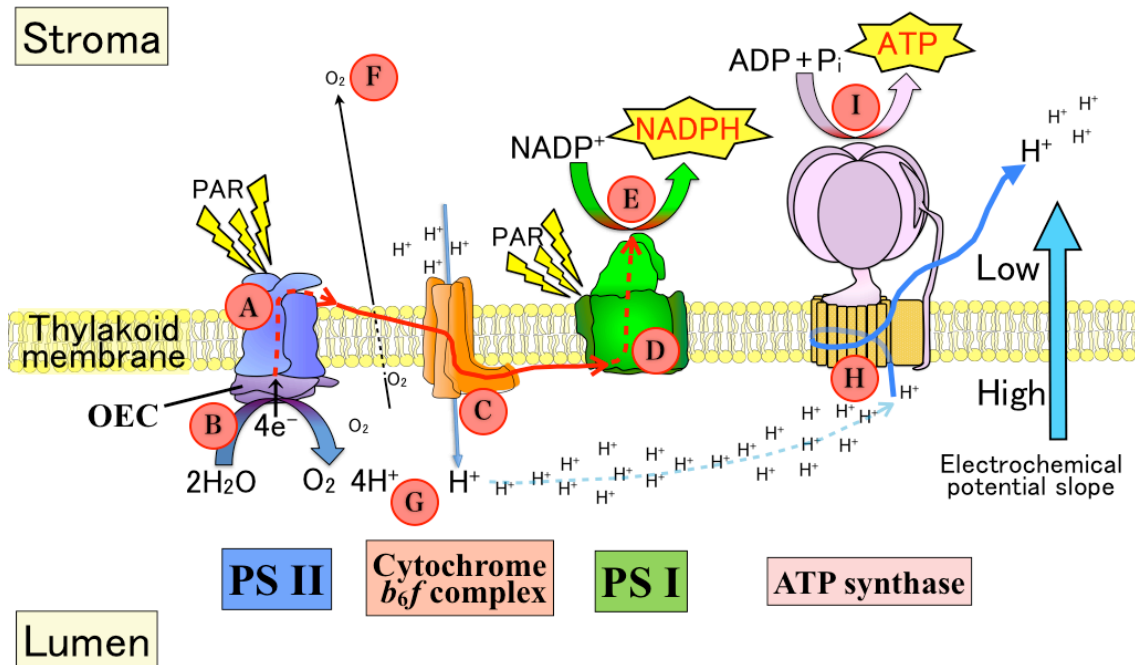


Fig. 1.1 Schematic diagram of the light chemical reaction in photosynthesis. (A) Light energy is excited the photosystem (PS) II reaction center, and charge separation occur. (B) The lost electrons in the chlorophyll can acquire by splitting water (H_2O) at PS II oxygen-evolving center (OEC). The electrons flows into the PS I throughout cytochrome b_6f complex. (C) The cytochrome b_6f complex transports the proton (H^+) from stroma to lumen. (D) On the other hand, the entered electrons into the PS I are re-excited by light energy. (E) The PS I synthesize the nicotinamide adenine dinucleotide phosphate (NADPH) from the NADP^+ by using the electrons. The O_2 emitted into the lumen by the H_2O splitting is eventually released to the extracellular (F), whereas the H^+ levels in the lumen increase as the water cleavage occurs (G). The difference of the H^+ levels between the stroma and lumen drive the adenosine triphosphate (ATP) synthase (H), and generate the ATP from both of the adenosine diphosphate (ADP) and the phosphoric acid (P_i) (I). Referred from Taiz and Zeiger (2002).

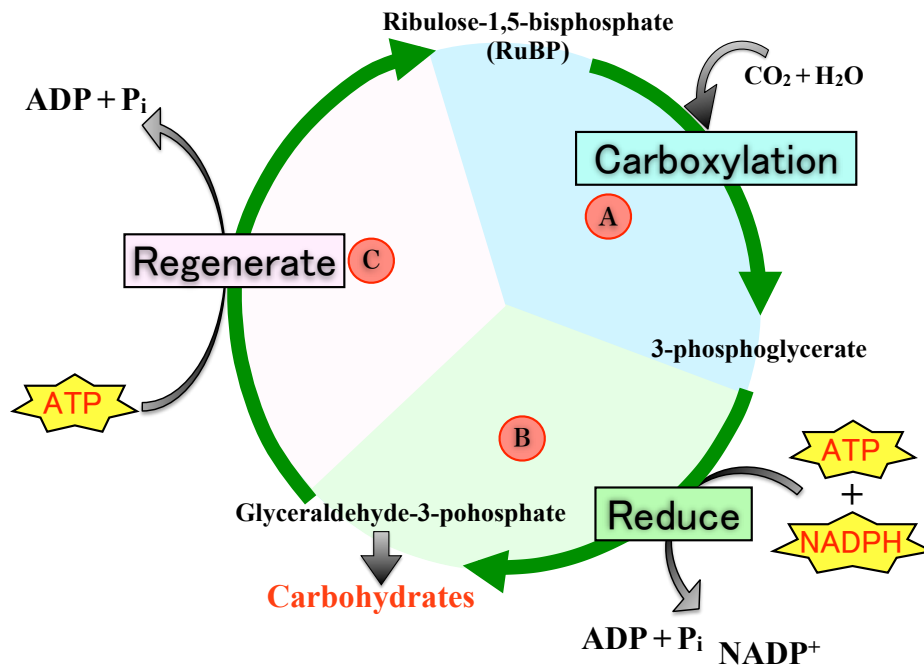
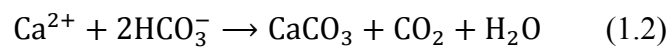


Fig. 1.2 Calvin cycle progress by the three sections. (A) CO₂ and H₂O are fixed into 3-phosphoglycerate (PGA) by the enzyme reaction of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). (B) The generated 3-phosphoglycerate synthesizes the carbohydrates using the reducing power of the ATP and NADPH obtained by the light chemical reaction. (C) Ribulose-1,5-bisphosphate (RuBP) is regenerated by the enzyme reaction of phosphoribulokinase by using the ATP. Redrawn from Taiz and Zeiger (2002).

1.2 Primary production of phytoplankton and carbon cycle

Phytoplankton is autotrophs, and varies considerably in size ranging from about 0.7 µm to over 200 µm in diameter (Chisholm et al., 1988; Kaiser et al., 2011). Although the carbon biomass of the phytoplankton in the ocean account for less than 1% of the 600 billion tons of the all photosynthetic carbon biomass on the Earth, it is reported that amount of carbon fixed by phytoplankton is nearly equal to that of terrestrial plants (Field et al., 1998; Falkowski, 2002). Diatoms contribute predominantly to the global carbon fixation (Roberts et al., 2007). They have the tests of silica, and two phylogenetic groups: the centric and pennate diatoms, which can be identified by the symmetry of their frustules. The centric and pennate diatoms are characterized by radial

and bilaterally symmetrical shape, respectively. The amount of the carbon fixation in phytoplankton reaches to ca. 50 Pg C per year (1 Pg = 10¹⁵ g) (Field et al., 1998; Granum et al. 2005; Roberts et al., 2007), and diatoms can correspond to 40% of the total primary production in the ocean (Nelson et al., 1995; Tréguer and Pondaven, 2000; Sarthou et al., 2005). On the other hand, coccolithophores are dressed in tests of calcium carbonate (CaCO₃). When calcification of CaCO₃ occurs in the aquatic environment, CO₂ is released into the surrounding water as following equation (1.2) (Frankignoulle et al., 1994, 1995).



Therefore, it ultimately increases the atmospheric CO₂ level, and gives the positive feedback to global warming. Moreover, this leads to decrease in the oceanic pH (ocean acidification), and may change the abundance and community composition of phytoplankton (e.g., Endo et al., 2013).

Atmospheric CO₂ has increased from the industrial revolution. This originates in the burning of fossil fuels. The level rose 140% from 280 ppm at pre-industrial revolution (1750) to 390 ppm at present (2011) (WMO, 2012). Meanwhile, the ocean is a major reservoir of CO₂. In spite of the fact that it already contains 50 times the mass of CO₂ in the atmosphere, the ocean has more capacity for string it (Siedler et al., 2001). The ocean surface exchanges CO₂ with the atmosphere for the difference of the partial pressure of CO₂ (*p*CO₂) between the seawater and atmosphere (called solubility carbon pump). The CO₂ dissolved in the ocean surface can use for photosynthesis by phytoplankton. A part of the CO₂ fixed into organic carbon by the phytoplankton may be transported from the surface to deeper layer (called the biological carbon pump), and the organic carbon is decomposed by the respiration of heterotrophic organisms such as bacteria in the deep ocean. Then, the dissolved inorganic carbon (DIC) levels are higher in the deeper layer than in the surface layer (e.g., Key et al., 2004; Emerson and Hedges, 2008). In the deep layer, it is considered that the seawater is transported by deep circulation (i.e., the great ocean conveyor belt) (Broecker, 1991). Seawater in the vicinity of the Iceland is cooled by contact with the cold winter air masses that sweep from the Canadian Arctic. The cooling increases the density of the surface water in the area. The high-density water sink to the abyss, and the water flows southward. Finally, the water rises from the deep layer to the surface in the north regions of the Indian and

Pacific oceans, called upwelling. In the past, the term between the sink and upwelling has estimated up to ca. 2,000 years by ^{14}C analysis of a radioactive isotope of dissolved inorganic carbon (Key et al., 2004; Emerson and Hedges, 2008). However, recently, the term is reported to be ca. 1,000 years (Matsumoto, 2007). Since the upwelling current transports the various substances such as nutrients from the deep layer to the surface, the regions are the higher primary production than other oceans (Lalli and Parsons, 1997).

1.3 Primary production and water-column light utilization efficiency (Ψ) of phytoplankton in the western subarctic Pacific

The Western subarctic Pacific is located in the upwelling region. Massive phytoplankton blooms in the spring occurs by both of the stratification within the water column and the relief of light limitation (Kasai et al., 1997). The depth-integrated primary production during the spring phytoplankton blooms are reported to reach $3200 \text{ mg C m}^{-2} \text{ d}^{-1}$ in the Oyashio region of the near costal region of Hokkaido, Japan (Isada et al., 2010), $1700 \text{ mg C m}^{-2} \text{ d}^{-1}$ in the off shore of the costal region (Shiomoto, 2000), and $1600 \text{ mg C m}^{-2} \text{ d}^{-1}$ in the western subarctic gyre (WSG) (Imai et al., 2002). During summer in the WSG, the levels of macronutrients (nitrate, silicate and phosphate) in the surface are relatively high (nitrate: $9.9 \mu\text{M}$, silicate: $17.3 \mu\text{M}$ and phosphate: $1.02 \mu\text{M}$) (Whitney, 2011), whereas the levels of Chl *a* maintain relatively low value ($0.5\text{--}0.7 \text{ mg m}^{-3}$) (Imai et al., 2002). This has been recognized as high-nitrate, low-chlorophyll (HNLC) waters, and the HNLC phenomenon in the WSG mainly attributable to low iron availability of phytoplankton and high zooplankton grazing (Tsuda et al., 2003; 2007). The assessments of the iron availability in the WSG were carried out by two *in situ* iron fertilization experiments: the Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study I (SEEDS-I) and II (SEEDS-II). Those experiments certainly showed that the iron for phytoplankton in the HNLC water was insufficient (e.g., Tsuda et al., 2003; Kudo et al., 2005, 2009). The occurrence of the algal blooms in the edge of the WSG can be ascribed to higher iron supply into the edge of the WSG. The plausible iron sources are a proximity to Asian atmospheric dust sources (Mahowald et al., 2005), iron-rich continental Kuril/Kamchatka margin (Lam and Bishop, 2008) and Okhotsk Sea Intermediate Water (Nishioka et al., 2007, 2011).

The western subarctic Pacific including the WSG showed the highest seasonal

biological drawdown of $p\text{CO}_2$ in the waters among the world's oceans (Takahashi et al., 2002), and that was probably attributable to the phytoplankton bloom (Midorikawa et al., 2003; Ayers and Lozier, 2012). These indicate that the phytoplankton assemblages play a key role in the carbon cycle. Similarly, Shiimoto (2000) also showed that the water-column light utilization efficiency (Ψ) of phytoplankton photosynthesis (Falkowski, 1981) in the western subarctic Pacific during the spring blooms was the highest among the world's oceans. On the other hand, Imai et al. (2002) was reported that the Ψ values at the Station KNOT (44°N, 155°E) in the edge of the WSG were constantly low throughout the year. It is represented that the Ψ values could be influenced by the PAR (Falkowski and Raven, 2007) and phytoplankton community composition (Hashimoto and Shiimoto, 2002; Isada et al., 2009). In addition, the low iron availability in the HNLC water can increase carbon:Chl *a* ratio of the phytoplankton (Sunda and Huntsman, 1997), and this possibly affect the Ψ value. The Ψ value also may contribute to the high biological carbon pump efficiency in this region, because the phytoplankton plays a key role in the biological carbon pump.

1.4 Biological carbon pump and transparent exopolymer particles (TEP) in the Oyashio region

The biological carbon pump efficiency in the western subarctic Pacific was higher than in other areas of the world's oceans (Kawakami et al., 2004). The biological carbon pump can define as transportation of the organic carbon from surface to deeper layer throughout biological activities in the ocean. The biological carbon pump commences by conversion from the aqueous CO_2 (aqCO_2) in the seawater to the organic carbon by phytoplankton (Fig. 1.3A). A large fraction of the fixed organic carbon is released again in the form of CO_2 from the surface water throughout respiration in the grazing food chain (Lalli and Parsons, 1997), and also throughout decomposition and respiration in the microbial loop (Azam et al., 1983) (Fig. 1.3B). On the other hand, a part of the fixed organic carbon is transferred to the deep ocean, and released in the form of CO_2 throughout respiration by the deep consumers and decomposition by bacteria (Fig. 1.3C). Since the deep circulation is transporting the seawater in the deep ocean, the CO_2 released in the deep ocean can preserve in the deep ocean during up to 2200 years as described in the section 1.2. Therefore, the biological carbon pump is very important for the carbon cycle in this planet.

One of the substances to closely relate with the biological carbon pump efficiency is the transparent exopolymer particles (TEP). TEP are defined as $>0.4\ \mu\text{m}$ transparent particles that consist of acid polysaccharides, and are stainable with Alcian blue dye (Alldredge et al., 1993). TEP are very sticky particles that exhibit the characteristics of gel (Passow, 2002). Hence, TEP can act as “bonding agent” and condense various particles existing in the ocean. Moreover, the aggregated particles may increase the sinking velocity in the water column. Therefore, it is thought that TEP can increase biological carbon pump efficiency. In the marine systems, TEP be formed from dissolved organic precursors, which are mainly released by phytoplankton (Passow, 2002; Engel et al., 2004; Oosstende et al., 2013).

Again, the high biological carbon pump efficiency is reported in the western subarctic Pacific including the Oyashio region (Kawakami et al., 2004). In addition, the massive diatom bloom in the Oyashio region occurs every spring with the high primary production (Kasai et al., 1997). However, the investigation of TEP in the Oyashio region has never been carried out to date. It is known that diatoms can produce TEP precursors during the growth phase of the bloom (Alldredge et al., 1993), and then, TEP levels in the water may rise (e.g., Passow and Alldredge, 1994). In contrast, diatoms can produce such TEP precursors in the post-boom phase (Staats et al., 2000), and thereby, TEP levels in the water may increase (e.g., Passow and Alldredge, 1995). Therefore, little is known about TEP production mechanisms during the bloom.

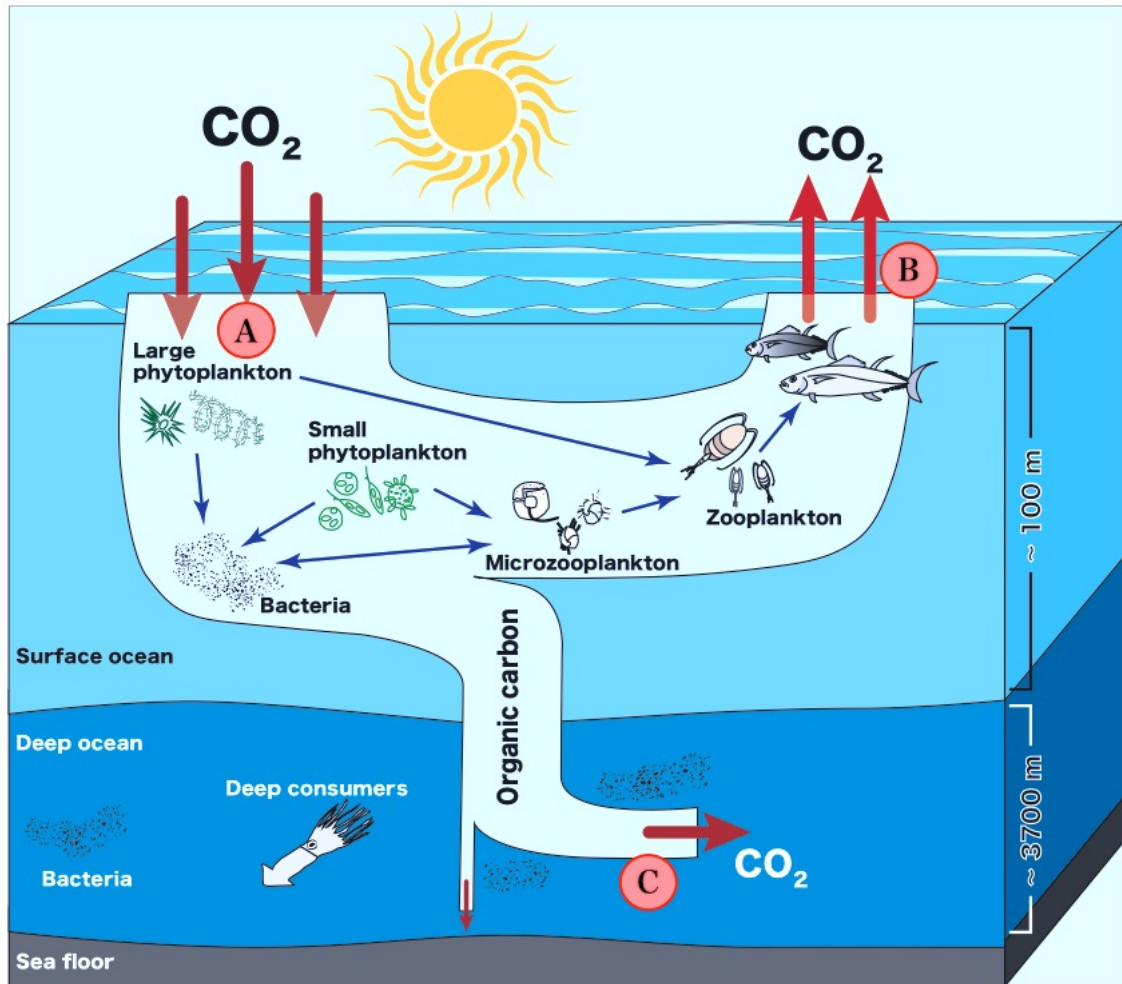


Fig. 1.3 Schematic diagram of the biological carbon pump. (A) Large and small phytoplankton fixes the aqueous CO₂ ($a_q\text{CO}_2$) in the seawater. (B) A large fraction of the fixed organic carbon is released again in the form of CO₂ from the surface water to the atmosphere because of respiration in the grazing food chain, and of decomposition and respiration in the microbial loop. (C) On the other hand, a part of the fixed organic carbon is transferred from the surface to deep ocean, and released in the form of CO₂ throughout respiration by the deep consumers and decomposition by bacteria. Redrawn from Chisholm (2000).

1.5 Purpose of this study

Firstly, this dissertation aims to characterize the water-column light utilization efficiency (Ψ) in the western subarctic gyre (WSG) of the western subarctic Pacific during summer. The second is to assess the levels of transparent exopolymer particles

(TEP) in the Oyashio region in the western subarctic Pacific during the spring diatom blooms.

In the Chapter 2, I reported the Ψ values in the WSG during 2008 summer, and discussed the relationships between the Ψ values and the PAR levels and between the Ψ values and the phytoplankton community composition. Moreover, in order to assess the importance of iron availability for the Ψ values in the WSG during summer, the Ψ values in the SEEDS-I and SEEDS-II were estimated. I made a comparison between the inside and outside of the iron patch. Also I summarized the Ψ data in the WSG during summer to date and discussed its variations.

In the Chapter 3, I reported the TEP levels in the Oyashio region from the spring diatom blooms to post-bloom in the 2010 and 2011, and discussed the relationships between the TEP levels in the seawater and the phytoplankton biomass and between the TEP levels and the DOC production rates by phytoplankton.

Subsequently in the Chapter 4, in order to assess the relationship between TEP production and DOC production by the Oyashio diatoms more in depth, a laboratory experiment was carried out by using the diatom strain *Thalassiosira nordenskioeldii* isolated from the diatom bloom in the 2011 Oyashio expedition.

In the Chapter 5, I concluded the results from Chapter 2 to 4, and pointed out the remarkable points in my studies.

Chapter 2 – Light utilization efficiency of phytoplankton in the Western Subarctic Gyre of the North Pacific during summer

2.1 Introduction

The Western Subarctic Gyre (WSG) in the western North Pacific is surrounded by the current systems composed of the Alaska Current, the East Kamchatka Current, the Oyashio Current and the Subarctic Current (Favorite *et al.*, 1976; Yasuda, 2003). The unique hydrographic conditions significantly affect biogeochemical processes in the WSG. For example, during the spring and summer, chlorophyll (Chl) *a* concentrations in the surface waters of the central WSG are generally $<1 \text{ mg m}^{-3}$, whereas occasional phytoplankton blooms with $>2 \text{ mg m}^{-3}$ Chl *a* occur at the southwest edge of the WSG (e.g., Imai *et al.*, 2002; Goes *et al.*, 2004). Because the subarctic waters generally contain high levels of macronutrients, including nitrate and silicic acid, in the mixed layer (Whitney, 2011), the central WSG has been recognized as a high-nitrate, low-chlorophyll (HNLC) body of water. The HNLC phenomenon in the WSG is mainly attributable to low iron availability and high zooplankton grazing (Tsuda *et al.*, 2003; 2007). In contrast, the occurrence of algal blooms at the edge of the WSG can be ascribed to a higher iron supply there. The plausible iron sources are Asian atmospheric dust sources (Mahowald *et al.*, 2005), the iron-rich continental Kuril/Kamchatka margin (Lam and Bishop, 2008) and the Okhotsk Sea Intermediate Water (Nishioka *et al.*, 2007, 2011).

The western subarctic Pacific, including the edge of the WSG, shows the highest seasonal biological drawdown of partial pressure of CO_2 ($p\text{CO}_2$) in surface waters among the world's oceans (Takahashi *et al.*, 2002). The drawdown of surface $p\text{CO}_2$ is probably attributable to the phytoplankton bloom (Midorikawa *et al.*, 2003; Ayers and Lozier, 2012). These results indicate that the phytoplankton assemblages play a key role in the carbon cycle in this area. Similarly, Shiimoto (2000) also showed that the water-column light utilization efficiency (Ψ) of phytoplankton photosynthesis (Falkowski, 1981) in the western subarctic Pacific during the spring blooms was the highest among the world's oceans. Hashimoto and Shiimoto (2002) subsequently showed that large diatoms ($>10 \text{ }\mu\text{m}$ in size) contributed to these high Ψ values in the western subarctic Pacific during spring. It was previously believed that Ψ values were

relatively constant, averaging approximately $0.4 \text{ g C (g Chl } a)^{-1} (\text{mol photon})^{-1} \text{ m}^2$ (Platt, 1986; Platt *et al.*, 1988). However, closer inspection and analyses using various data from the world's oceans revealed that Ψ is not constant, but it increases with decreasing average daily irradiance (Falkowski and Raven, 2007). However, Imai *et al.* (2002) reported that Ψ values were consistently low ($0.3 \pm 0.1 \text{ g C [g Chl } a]^{-1} [\text{mol photon}]^{-1} \text{ m}^2$) throughout the year at the KNOT Station (44°N, 155°E) in the WSG, where Chl *a* concentrations in the euphotic zone kept relatively constant. These lower Ψ values might be due to low iron availability in the WSG, but that has never been confirmed. At present, little is known about the spatiotemporal variation of Ψ in the WSG.

In this study, we examined Chl *a* concentrations, primary production, and photosynthetic available radiation (PAR) to obtain Ψ values in the WSG and in a transition domain (TD) between the Subarctic Front (SAF) and the Subarctic Boundary (SAB) in the summer of 2008. The data from the TD was used for comparisons with those of WSG. Phytoplankton community composition was also estimated because Ψ can be influenced by this parameter (Hashimoto and Shiimoto, 2002; Isada *et al.*, 2009). To assess the importance of iron availability for Ψ values in the WSG, data from two *in situ* iron enrichment experiments called the Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study I (SEEDS-I) and II (SEEDS-II) in the summers of 2001 and 2004, respectively (Kudo *et al.*, 2005, 2009), were used in this study. In SEEDS-I, a large diatom bloom occurred after iron enrichment (Tsuda *et al.*, 2003). In contrast, during SEEDS-II, iron fertilization yielded a bloom consisting of autotrophic nanoflagellates such as cryptophytes and prasinophytes (Suzuki *et al.*, 2009). The different phytoplankton composition in SEEDS-I and SEEDS-II were correlated with Chl *a* levels and primary production (Kudo *et al.*, 2005, 2009). Therefore, these two experiments were excellent opportunities to examine the effects of both iron availability and phytoplankton community structure on Ψ values in the WSG during the summer.

2.2 Materials and methods

2.2.1 KH08-2 cruise

2.2.1.1 Seawater sampling

Samples were collected from nine stations in the western North Pacific (Fig. 1) from July 29 to August 20, 2008 on board the R/V *Hakuho-Maru* (KH08-2 Leg. 1) of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC). Prior to

sampling, vertical profiles of photosynthetic available radiation (PAR; 400–700 nm) and spectra of downward PAR were obtained with a HyperPro profiling reflectance radiometer (Satlantic Inc.). Incident PAR above the sea surface was continuously measured on deck with a PAR sensor (ML-020P, EKO Instruments Co., Ltd.) every 10 min on average, and the values were recorded with a data logger. Seawater sampling at all stations was accomplished using a CTD-CMS attached with acid-cleaned Niskin bottles. Discrete water samples were collected at five light depths corresponding to 100% (5 m in practice), 30%, 10%, 5% (or 3% at stations (Stns) 5 and 11) and 1% of the surface irradiance with reference to the PAR profile. In this study, euphotic zone depth was defined as the depth at which the PAR value was 1% of the surface value. Nutrients (nitrite plus nitrate—hereafter denoted as nitrate, phosphate and silicate) were determined with a BRAN + LUEBBE auto-analyzer following the manufacturer's protocol. In this study, the Subarctic Front (SAF), located south of the WSG, was defined as the 4 °C isotherm at 100 m, and the Subarctic Boundary (SAB), located south of the SAF, was defined as a near-surface salinity front of 34 (Favorite *et al.*, 1976; Yasuda, 2003). The area between the SAF and SAB was defined as a Transition Domain (TD) (Favorite *et al.*, 1976; Yasuda, 2003).

2.2.1.2 Phytoplankton pigments and CHEMTAX processing

Concentrations of Chl *a* were determined using a Turner Design Model 10-AU fluorometer, according to the Welschmeyer method (1994). Water samples (110 mL) were taken from 12 depth layers between 0 and 200 m and filtered onto Whatman GF/F filters (25 mm in diameter). Water samples (500 mL) collected from the 100% and 5% (or 3%) light depths were size-fractionated using 47 mm Whatman GF/F filters and 47 mm Nuclepore filters (10 µm pore-size), to estimate the contributions of the <10 and >10 µm fractions. Algal pigments were immediately extracted by soaking each filter in *N,N*-dimethylformamide (DMF) at –20 °C for 24 h and were analyzed on board.

For the analysis of phytoplankton pigments using high-performance liquid chromatography (HPLC), water samples (500 mL) collected from five light depths (100%, 30%, 10%, 5% (or 3%) and 1%) were filtered onto 25 mm Whatman GF/F filters under gentle vacuum (<100 mm Hg). The filter samples were folded, blotted with filter paper and stored in a deep-freezer (–80°C). Phytoplankton pigments were extracted with sonication in DMF according to the protocol of Suzuki *et al.* (2005).

HPLC pigment analysis was performed according to the method of Van Heukelem and Thomas (2001), except the flow rate was 1.2 mL min⁻¹. Before injection, 250 µL of algal extracts was mixed with 250 µL ion-pair solution (28 mM tetrabutyl ammonium acetate (TBAA), pH 6.5), and equilibrated for 5 min at 5°C. Two hundred fifty µL of the mixture was injected into a Shimadzu HPLC (CLASS-VP) system incorporating an Agilent Eclipse XDB-C8 column (3.5 µm particle size, 4.6 × 150 mm). The HPLC solvent system was as follows: solvent A was 70% methanol and 30% 2.8 mM TBAA (pH 6.5) aqueous solution, and solvent B was methanol. A linear gradient with an isocratic eluent method was used: 0 min 95%A/5%B, 22 min 5%A/95%B, 22–30 min 5%A/95%B.

To estimate the contributions of each algal group in the two layers (100–10% and 10–1% light depths) to the total Chl *a* biomass, all data were interpreted by factorization using the CHEMTAX program (Mackey *et al.*, 1996). For the value at 10% light depth, we averaged the values obtained from the 100–10% and 10–1% layers. Initial and final pigment ratios were calculated following the method of Latasa (2007) (Table 1), and the initial ratios were based on Suzuki *et al.* (2002, 2005, 2009) who estimated the community composition of phytoplankton in the NW subarctic Pacific during the summer. Prymnesiophytes, pelagophytes and prasinophytes in our CHEMTAX analysis are synonymous with type 3 haptophytes, type 2 chrysophytes and type 3 prasinophytes of Mackey *et al.* (1996), respectively.

2.2.1.3 Flow cytometry

Duplicate water samples (each 2 mL) from the 100% to 1% light depths (i.e., within the euphotic zone) were preserved with paraformaldehyde (0.2% final concentration) and stored in a deep-freezer at –80 °C until analysis on land. An EPICS flow cytometer (XL ADC system, Beckman Coulter) equipped with a 15 mW air-cooled argon laser exciting at 488 nm and a standard filter setup was used to enumerate eukaryotic ultraphytoplankton (<5 µm in size). Prior to analysis, samples were thawed and drawn through a 35 µm nylon-mesh-capped Falcon cell strainer (Becton-Dickinson) to remove larger cells. For the enumeration of eukaryotic ultraphytoplankton, a certain volume of Flow-Count fluorospheres (Beckman Coulter) and 2.0 µm Fluoresbrite YG beads (Poly Sciences) were added to each sample. The details of flow cytometric analysis are described in Suzuki *et al.* (2005).

2.2.1.4 Cell abundance of phytoplankton

Water samples (500 mL) were taken from the 100% and 5% (or 3%) light depths for counting diatoms and coccolithophores and were fixed with buffered formalin (pH 7.8, 1% final concentration). An aliquot (14–45 mL) of the sample was filtered onto a Nuclepore membrane (0.8 μm pore size) filter set in a glass funnel (8 mm in diameter at the base) under vacuum (100–200 mm Hg). The filter membrane was rinsed with distilled water to remove salts and immediately dried for three hours in an oven at 60°C. To count and identify the algal cells, the whole area of membrane ($>50\text{ mm}^2$) was examined with a scanning electron microscope (SEM, JMS-840A, JEOL Ltd.) at a magnification of approximately 2,000 $\times$ (Hattori *et al.*, 2004). Species of phytoplankton were identified according to Fukuyo (1990), Tomas (1997), Young *et al.* (2003) and Scott and Marchant (2005).

2.2.1.5 Daily primary production

Daily primary production ($\text{mg C m}^{-2} \text{ d}^{-1}$) was estimated using *in situ* (Stns 5 and 11) or simulated *in situ* (Stns 1, 2, 3, 6, 8, 9 and 10) incubation techniques. For the simulated *in situ* method, irradiance in the incubators was adjusted with blue film screens, where blue-green light (370–575 nm, center at 460 nm) can be transmitted in water. Temperatures in the incubators for the 100%, 30% and 10% light levels were maintained by flowing the surface seawater, and temperatures in the 5% and 1% light level incubators were controlled with a cooling system to simulate the 1% light depth temperature. The samples were dispensed into three 275-mL acid-cleaned polycarbonate bottles (two light and one dark) and were inoculated with a solution of $\text{NaH}^{13}\text{CO}_3$ (99 atom% ^{13}C), which was equivalent to ca. 10% of the total inorganic carbon (TIC) in the seawater. The concentrations of TIC were measured by coulometry (CM5012, UIC). All bottles were incubated for ca. 24 hours. The start time of the incubations varied according to the sampling time (Table 2). The concentrations of particulate organic carbon and ^{13}C abundance were determined using a mass spectrometer (DELTA V, Thermo Fisher Scientific Inc.) with an on-line elemental analyzer (FlashEA1112, Thermo Fisher Scientific Inc.). Daily primary production was calculated according to Hama *et al.* (1983).

2.2.1.6 Water-column light utilization efficiency (Ψ) of phytoplankton photosynthesis

To investigate the relationship between daily primary production and PAR in the water-column, the water-column light utilization efficiency (Ψ ; g C [g Chl *a*]⁻¹ [mol photon]⁻¹ m², Falkowski, 1981) of phytoplankton photosynthesis was determined using the following equation (2.1):

$$\Psi = \frac{\int_{t_0}^{t_d} \int_{z_{0m}}^{z_{1\%}} P(t, z) dz dt}{\int_{z_{0m}}^{z_{1\%}} B(z) dz \cdot \int_{t_0}^{t_d} I_0(t) dt} \quad (2.1)$$

where $\int_{t_0}^{t_d} \int_{z_{0m}}^{z_{1\%}} P(t, z) dz dt$ (mg C m⁻² d⁻¹) is primary production integrated from the surface (0 m) to the 1% light (i.e., euphotic zone) depth over day, $\int_{z_{0m}}^{z_{1\%}} B(z) dz$ (mg Chl *a* m⁻²) is Chl *a* concentration integrated within the euphotic zone. For the calculations of primary production and Chl *a* concentration, the values at 100% light depth (i.e., 5 m depth in this study) were extrapolated to the surface. $\int_{t_0}^{t_d} I_0(t) dt$ (mol photons m⁻² d⁻¹) is the PAR incident on the sea surface integrated over the day.

2.2.2 SEEDS-I and SEEDS-II

The SEEDS-I and SEEDS-II cruises were conducted from June 28 to August 6 in 2001 aboard the FR/V *Kaiyo-Maru* (Fisheries Agency of Japan) and from July 20 to August 22 in 2004 aboard the R/V *Hakuho-Maru* (JAMSTEC, Japan) and R/V *Kilo Moana* (University of Hawaii, USA). The iron fertilizations in the WSG were conducted at 48.5°N, 165°E in SEEDS-I and at 48°N, 166°E in SEEDS-II. Overviews of the two experiments were described in Tsuda *et al.* (2003, 2007). During the experiments, Chl *a* concentrations were determined using Turner Design 10-AU fluorometers following the method of Welschmeyer (1994) after pigment extraction in DMF. PAR data were continuously measured with quantum light sensors set on the upper deck of the vessels. Primary production was estimated using a ¹³C tracer method (Hama *et al.*, 1983), and the procedures were described in Kudo *et al.* (2005, 2009).

2.3 Results

2.3.1 KH08-2 cruise

2.3.1.1 Hydrography

Hydrographic conditions are summarized in Table 2. According to the temperature and the salinity data (Favorite *et al.*, 1976; Yasuda, 2003), Stns 5, 6, 8, 9 and 10 were classified as being in the WSG. The other stations (Stns 1, 2, 3 and 11) were located in the TD between the WSG and the SAB. Euphotic layer depth (average $\pm$ standard deviation) was 46 ± 7 m in the WSG and 41 ± 7 m in the TD. The mean nitrate concentrations (hereafter N_{eu_mean}) within the euphotic zone ranged from 11.0 to 17.6 μM at the WSG stations, and from 4.7 to 7.8 μM at the TD stations. The lowest and highest daily PAR levels were observed at Stn 8 ($13.3 \text{ mol photons m}^{-2} \text{ d}^{-1}$) and at Stn 11 ($36.8 \text{ mol photons m}^{-2} \text{ d}^{-1}$), respectively.

2.3.1.2 Pigments and CHEMTAX outputs

Concentrations of Chl *a* within the euphotic zone were 22.6–32.6 (27.0 average) mg m^{-2} in the WSG and 24.6–35.2 (31.6) mg m^{-2} in the TD (Table 2). The percentages of the Chl *a* in the $<10 \mu\text{m}$ fraction ranged from 72 to 84% ($78 \pm 5\%$) at 5 m and from 60 to 90% ($81 \pm 12\%$) at the 5% (or 3%) light depth in the WSG (Table 3). In the TD, the relatively low percentages of the Chl *a* in the $<10 \mu\text{m}$ were found between 39 and 62% ($50 \pm 9\%$) at 5 m and between 45 and 92% ($70 \pm 21\%$) at the 5% (or 3%) light depth.

The final ratio matrix in the 100 (5 m) – 10% light depths, calculated by the CHEMTAX program (Table 1B), was within the range of Mackey *et al.* (1996). Although the final ratio matrix in the 10–1% light depths (Table 1D) was almost within the range of the values shown by Mackey *et al.* (1996), the Chl *b*/Chl *a* ratio for chlorophytes (0.61) was slightly higher than the maximum value (0.57) reported by Mackey *et al.* (1996). In the WSG, the major phytoplankton groups in terms of Chl *a* biomass, as estimated by CHEMTAX calculations, were prymnesiophytes ($28 \pm 11\%$), pelagophytes ($22 \pm 9\%$) and diatoms ($20 \pm 8\%$) (Fig. 2). In the TD, relatively high contributions of prymnesiophytes ($32 \pm 17\%$) and chlorophytes ($26 \pm 12\%$) to Chl *a* biomass were found. The contributions of diatoms in the TD ranged from 0–61%.

2.3.1.3 Abundance and community composition of phytoplankton estimated by flow

cytometry or scanning electron microscopy

The results of flow cytometry showed that Stn 1 had the maximum abundance (9.3×10^{12} cells m^{-2}) of eukaryotic ultraphytoplankton within the euphotic zone (Fig. 3). In contrast, the abundance at Stn 5 was the lowest (3.7×10^{12} cells m^{-2}). Average cell abundances of eukaryotic ultraphytoplankton within the euphotic zone in the WSG and TD were $4.3 \pm 0.6 \times 10^{12}$ cells m^{-2} and $8.6 \pm 0.7 \times 10^{12}$ cells m^{-2} , respectively. The cell abundance of eukaryotic ultraphytoplankton was significantly higher in the TD than in the WSG (Wilcoxon rank sum test, $p < 0.05$, $n = 9$). The cell abundance of *Synechococcus* within the euphotic zone ranged from 0.9×10^{12} cells m^{-2} at Stn 5 to 8.8×10^{12} cells m^{-2} at Stn 6 (Fig. 3). Mean cell abundance of *Synechococcus* within the euphotic zone was $4.7 \pm 3.1 \times 10^{12}$ cells m^{-2} in the WSG and $4.8 \pm 1.6 \times 10^{12}$ cells m^{-2} in the TD, and no significant difference between the WSG and the TD was found (Wilcoxon rank sum test, $p = 0.90$, $n = 9$).

Scanning electron microscopy (SEM) identified 39 centric diatom species, 20 pennate diatom species and 13 coccolithophore species (Table 4). The highest cell abundances of diatoms at the 100% and 5% (or 3%) light depths were found at Stn 2 in the TD (151×10^3 cells L^{-1} and 99×10^3 cells L^{-1} , respectively) and were dominated by the centric diatom *Corethron criophilum* (91% and 98%, respectively). Coccolithophores in 100% and 5% (or 3%) light depths showed maxima at Stn 10 (29×10^3 cells L^{-1}) and at Stn 5 in the WSG (36×10^3 cells L^{-1}), respectively. Coccolithophores were dominated by *Emiliania huxleyi* type C (98% and 83%, respectively). The diatom or coccolithophore cell abundances between the WSG and the TD were not significantly different at the 100% light depth (Wilcoxon rank sum test, diatoms: $p = 0.41$; coccolithophores: $p = 0.11$, $n = 9$) or the 5% (or 3%) light depth (Wilcoxon rank sum test, diatoms: $p = 0.06$; coccolithophores: $p = 0.56$, $n = 9$).

2.3.1.4 Primary production

Primary production within the euphotic zone ranged from 566 to 1,535 mg C $m^{-2} d^{-1}$. The average values in the WSG and the TD were 683 ± 128 mg C $m^{-2} d^{-1}$ and $1,107 \pm 405$ mg C $m^{-2} d^{-1}$ (Table 2), respectively. Primary production was not significantly different between the WSG and the TD (Wilcoxon rank sum test, $p = 0.19$, $n = 9$). Primary production at each station changed concomitantly with the Chl *a* level (Spearman's rank test, $\rho = 0.88$, $p < 0.005$, $n = 9$). The values of Chl *a*-specific primary

production the assimilation number) changed little (23–27 mg C [mg Chl *a*]⁻¹ d⁻¹) in the WSG, whereas the values ranged widely (24–44 mg C [mg Chl *a*]⁻¹ d⁻¹) in the TD (Table 2). No significant difference in Chl *a*-specific primary production was found between the WSG and the TD (Wilcoxon rank sum test, $p = 0.19$, $n = 9$).

2.3.1.5 Light utilization efficiency (Ψ)

The Ψ values obtained in the WSG and TD were between 0.64 and 1.86 g C (g Chl *a*)⁻¹ (mol photon)⁻¹ m² (Table 2). The average values were 1.31 ± 0.38 g C (g Chl *a*)⁻¹ (mol photon)⁻¹ m² in the WSG and 1.17 ± 0.40 g C (g Chl *a*)⁻¹ (mol photon)⁻¹ m² in the TD. No significant difference in the Ψ values was found between the WSG and the TD (Wilcoxon rank sum test, $p = 0.73$, $n = 9$). The Ψ values in the WSG in this study significantly increased with a decrease in the daily PAR (Spearman's rank test, $\rho = -1.0$, $p < 0.05$, $n = 5$) (Fig. 4). However, no such relationship was found in the TD (Spearman's rank test, $\rho = -0.80$, $p = 0.33$, $n = 4$) and the total area (WSG plus TD) (Spearman's rank test, $\rho = -0.68$, $p = 0.05$, $n = 9$).

2.3.2 Ψ in SEEDS-I and SEEDS-II

The data obtained from SEEDS-I and SEEDS-II are summarized in Table 5. In SEEDS-I and SEEDS-II, the growth phase of phytoplankton assemblages inside the iron-enriched patch (hereafter Fe-in_{growth}) was defined in terms of the increase in F_v/F_m levels (Tsuda *et al.* 2003; Suzuki *et al.* 2009), which occurred on days 2–9 during SEEDS-I and days 2–11 during SEEDS-II. Therefore, to examine the effects of iron enrichment on Ψ values, we compared not only Ψ data between inside the iron-patch (hereafter Fe-in) and outside the iron-patch (hereafter Fe-out) in the two experiments but also Ψ data between the Fe-in_{growth} and Fe-out, assuming that the Fe-in_{growth} was not limited by availability during the growth phase.

In SEEDS-I, the depth-integrated Chl *a* concentration and primary production within the euphotic zone were 37.6–232.2 mg m⁻² and 394–2,350 mg C m⁻² d⁻¹ in the Fe-in, 37.6–232.2 mg m⁻² and 394–2,033 mg C m⁻² d⁻¹ in the Fe-in_{growth}, and 31.5–40.1 mg m⁻² and 409–515 mg C m⁻² d⁻¹ in the Fe-out. The primary production values did not change concomitantly with the Chl *a* level in the Fe-in, Fe-in_{growth} and Fe-out (Spearman's rank test, Fe-in: $\rho = 0.83$, $p = 0.06$, $n = 6$; Fe-in_{growth}: $\rho = 0.80$, $p = 0.33$, $n = 4$; Fe-out: $\rho = 0.20$, $p = 0.92$, $n = 4$). The 24-hour-integrated PAR on the sea surface

in the Fe-in, Fe-in_{growth} and Fe-out was 17.5–38.8, 17.5–38.8 and 17.6–38.4 mol photons m⁻² d⁻¹, respectively. As a result, Ψ ranged between 0.25–0.60 g C [Chl *a*]⁻¹ mol photon⁻¹ m² in the Fe-in, 0.25–0.59 g C [Chl *a*]⁻¹ mol photon⁻¹ m² in the Fe-in_{growth} and 0.3–0.6 g C [Chl *a*]⁻¹ mol photon⁻¹ m² in the Fe-out. No significant difference in the Ψ values was found between the Fe-in and the Fe-out (Wilcoxon rank sum test, $p = 0.76$, $n = 10$) or between the Fe-in_{growth} and the Fe-out (Wilcoxon single-rank sum test, $p = 0.89$, $n = 4$).

Similarly, in SEEDS-II, the depth-integrated Chl *a* concentration and primary production within the euphotic zone were 20.2–69.5 mg m⁻² and 557–1,446 mg C m⁻² d⁻¹ in the Fe-in, 31.8–69.5 mg m⁻² and 887–1,446 mg C m⁻² d⁻¹ in the Fe-in_{growth}, and 16.7–42.6 mg m⁻² and 324–909 mg C m⁻² d⁻¹ in the Fe-out. The primary production also did not change concomitantly with the Chl *a* level in the Fe-in, Fe-in_{growth} and Fe-out (Spearman's rank test, Fe-in: $\rho = 0.71$, $p = 0.09$, $n = 7$; Fe-in_{growth}: $\rho = 0.80$, $p = 0.33$, $n = 4$; Fe-out: $\rho = 0.54$, $p = 0.30$, $n = 6$). The 24 hour-integrated PAR values on the sea surface in the Fe-in, Fe-in_{growth} and Fe-out were 12.1–41.5, 12.1–34.3 and 20.2–35.2 mol photons m⁻² d⁻¹, respectively. The values of Ψ were 0.34–1.65 g C [Chl *a*]⁻¹ mol photon⁻¹ m² in the Fe-in, 0.58–1.33 g C [Chl *a*]⁻¹ mol photon⁻¹ m² in the Fe-in_{growth} and 0.61–1.24 g C [Chl *a*]⁻¹ mol photon⁻¹ m² in the Fe-out. Again, no significant difference in the Ψ values was found between the Fe-in and the Fe-out or between the Fe-in_{growth} and the Fe-out (Wilcoxon rank sum test, Fe-in vs. Fe-out: $p = 1.0$, $n = 13$; Fe-in_{growth} vs. Fe-out: $p = 0.91$, $n = 10$).

The Chl *a* levels, primary production and daily PAR were not significantly different between SEEDS-I and SEEDS-II (Wilcoxon rank sum test, primary production: $p = 0.98$, Chl *a*: 0.10, $n = 23$, daily PAR: $p = 0.92$, $n = 23$). However, the Chl *a*-specific primary production and Ψ in SEEDS-I were significantly lower than those in SEEDS-II (Table 5) (Wilcoxon rank sum test, Chl *a*-specific primary production: $p < 0.001$, Ψ : $p < 0.001$, $n = 23$).

2.4 Discussion

2.4.1 Factors controlling Ψ values in the WSG during the summer

As described above, it has been reported that Ψ is not constant, but it increases with a decrease in daily PAR (Falkowski and Raven, 2007). Similar results were also found in previous reports from the WSG during the summer (Shiomoto, 2000;

Honda *et al.*, 2009) and during the KH08-2 cruise in this study. In contrast, relatively constant Ψ values, irrespective of daily PAR, were reported in the WSG over a year (Imai *et al.*, 2002). For constant Ψ values, Imai *et al.* (2002) noted that Chl *a*-specific primary production was proportional to the daily PAR. However, such a relationship was not found in our KH08-2 cruise (Spearman's rank test, $\rho = -0.10$, $p = 0.95$, $n = 9$). In addition, the Ψ values ($0.2\text{--}0.4 \text{ g C [g Chl } a]^{-1} \text{ mol photon}^{-1} \text{ m}^2$) of Imai *et al.* (2002) were significantly lower than those found during the KH08-2 cruise (Table 2) (Wilcoxon rank sum test, $p < 0.05$, $n = 9$). Therefore, the differences in the Ψ -related parameters between Imai *et al.* (2002) and our KH08-2 cruise were examined. The euphotic layer depth changed little between Imai *et al.* (2002) ($53 \pm 6 \text{ m}$) and the KH08-2 cruise ($46 \pm 7 \text{ m}$) (Wilcoxon rank sum test, $p = 0.14$, $n = 9$). Primary production ($194\text{--}304 \text{ mg C m}^{-2} \text{ d}^{-1}$) measured by Imai *et al.* (2002) was lower, compared to the results ($683 \pm 128 \text{ mg C m}^{-2} \text{ d}^{-1}$) of our KH08-2 cruise (Wilcoxon rank sum test, $p < 0.05$, $n = 9$). However, the Chl *a* concentrations and the daily PAR were not significantly different between the two studies (Wilcoxon rank sum test, Chl *a*: $p = 0.41$, $n = 9$; daily PAR: $p = 0.11$, $n = 9$). As a result, the Chl *a*-specific primary production in Imai *et al.* (2002) showed relatively low values ($6\text{--}12 \text{ mg C [mg Chl } a]^{-1} \text{ d}^{-1}$) compared to our KH08-2 cruise (Table 2; Wilcoxon rank sum test, $p < 0.05$, $n = 9$). The low primary production of Imai *et al.* (2002) may be due to the lower (ca. 1%) inoculation percentage of a solution of $\text{NaH}^{13}\text{CO}_3$ to the total inorganic carbon in the seawater compared to our inoculation percentage (ca. 10%). However, the higher inoculation of $\text{NaH}^{13}\text{CO}_3$ increases seawater pH, which can decrease primary production (Raven, 2006; Raven *et al.*, 2011) because an increase in seawater pH reduces aqueous CO_2 available for phytoplankton.

In general, high primary production was associated with diatoms in the subarctic Pacific (Harrison *et al.*, 1999). In the KH08-2 cruise, the diatoms were one of the major phytoplankton groups in the WSG (Fig. 2). At Stn KNOT, Mochizuki *et al.* (2002), using light microscopy, reported that large diatoms ($>10 \text{ }\mu\text{m}$) such as *Chaetoceros concavicornis* and *Corethron criophilum* contributed to the increase in primary production. However, Komuro *et al.* (2005) showed that small phytoplankton ($2\text{--}10 \text{ }\mu\text{m}$; small diatoms, coccolithophores and parmales) were also important primary producers at Stn KNOT as revealed by SEM. The cell abundance of coccolithophores in this study was highest at 5 m at Stn 5 and at the deeper layer of Stn 10 in the WSG, and

the prymnesiophytes including coccolithophores, and pelagophytes, were also major phytoplankton groups in the WSG (Fig. 2). Therefore, the relatively high primary production compared to Imai *et al.* (2002) could be derived from these phytoplankton groups.

According to Hashimoto and Shiomoto (2002), Ψ value of algal cells with 2–10 μm was higher than that of $<2 \mu\text{m}$ or $>10 \mu\text{m}$ in the WSG during summer. Although the size-fractionated Chl *a* data in the KH08-2 cruise were only taken from the layers at 5 m and 5% (or 3%) light depth, the Chl *a* fractions of $<10 \mu\text{m}$ were $78 \pm 5\%$ at 5 m and $81 \pm 12\%$ at 5% (or 3%) light depth in the WSG (Table 3). Those results suggested that the high Ψ values in the WSG during summer were mainly attributable to the small phytoplankton. The phytoplankton size may be related with ambient nutrient levels (Thingstad, 1998; Krap-Boss *et al.*, 1996). Although relatively high macronutrient levels were observed in the WSG during the summer (Whitney, 2011), the growth of large-sized phytoplankton is often limited by iron availability (Tsuda *et al.* 2003). Unfortunately, iron data were seldom available from the KH08-2 cruise.

Therefore, we examined Ψ values in Fe-in and Fe-out areas during SEEDS-I and SEEDS-II. We found that iron availability did not affect Ψ values in the WSG in these studies. Suzuki *et al.* (2009) reported that the initial slope (α^B) of the photosynthesis–irradiance ($P-E$) curve little changed between Fe-in and Fe-out during SEEDS-II. Because the α^B has the same dimension as Ψ , this can support our results that iron availability did not affect Ψ values.

As discussed above, the Ψ values in the WSG were influenced by the magnitude of primary production. In SEEDS-I and SEEDS-II, however, the Ψ values did not increase with primary production (Table 5). Compared with the Ψ values of Falkowski and Raven (2007), Shiomoto (2000) found that high Ψ values were caused by high values of Chl *a*-specific primary production. In SEEDS-I and SEEDS-II, the increases in Chl *a*-specific primary production increased the Ψ values and these results were similar to previous studies (Shiomoto, 2000; Honda *et al.*, 2009; Isada *et al.*, 2009).

Interestingly, the Ψ values during SEEDS-II were higher than those during SEEDS-I (Table 5). Autotrophic flagellates predominated in the phytoplankton assemblages in Fe-in during SEEDS-II (Suzuki *et al.*, 2009), whereas the chain-forming diatom *Chaetoceros debilis* ($>10 \mu\text{m}$ in size) bloomed during SEEDS-I (Tsuda *et al.*, 2003). In the WSG during the KH08-2 cruise, autotrophic flagellates were also dominant (Fig. 2).

Previous studies (e.g., Harrison and Platt, 1980; Furnas, 1982) showed that higher Chl *a*-specific primary production was observed when nano-sized phytoplankton (2–20 μm), which include most autotrophic flagellates observed in the WSG (Suzuki *et al.*, 2002), rather than micro-sized phytoplankton (20–200 μm) dominated. As mentioned above, Ψ values become lower with an increase in PAR. In this study, we found higher Ψ values in the WSG during summer when moderate PAR levels (ca. 10–30 $\text{mol photons m}^{-2} \text{ d}^{-1}$) existed and autotrophic flagellates predominated in the phytoplankton assemblages.

In the KH08-2 cruise, the data of the TD were used as reference to the WSG. Unexpectedly, we found that the primary production, Chl *a*-specific primary production and Ψ were not significantly different between the TD and the WSG, representing that the light utilization efficiency of phytoplankton photosynthesis was also high in the TD during summer. Although Ψ values in the TD have been reported previously by Shiimoto (2000) and Isada *et al.* (2009), the data from TD are clearly fewer than those from the WSG. Comprehensive understanding of the variability of Ψ values and its controlling mechanisms in the TD are also required as future work.

2.4.2 Relationship between Ψ and daily PAR

Recently, Honda *et al.* (2009) calculated Ψ values on the basis of primary production, Chl *a* concentration and PAR values in the northern North Pacific, including stations in the Bering Sea and in the eastern North Pacific. The Ψ values ranged from 0.2 to 1.5 $\text{g C (g Chl } a)^{-1} (\text{mol photon})^{-1} \text{ m}^2$. Furthermore, the Ψ values significantly correlated with surface PAR ($R^2 = 0.86$, $p < 0.00001$) and tended to increase with a decrease in surface PAR. The relationship was shown using the following power function (Eq. 2.2).

$$\Psi = 3.41 (\text{surface daily PAR})^{-0.68} \quad (2.2)$$

We calculated the power function of Ψ values in the world's oceans (Falkowski and Raven, 2007) where the data obtained from the subarctic North Pacific were not included. As a result, Eq. 2.3 was obtained ($R^2 = 0.35$, $p < 0.001$ for slope, root mean square error (RMSE) = 0.17, $n = 211$).

$$\Psi = 1.85 (\text{surface daily PAR})^{-0.56} \quad (2.3)$$

Similarly, Eq. 2.4 was also derived from the WSG in the KH08-2 cruise ($R^2 = 0.96$, $p <$

0.001, for slope, RMSE = 0.08, $n = 5$).

$$\Psi = 28.71 (\text{surface daily PAR})^{-1.04} \quad (2.4)$$

The slope (-1.04) and intercept (28.71) of Eq. 2.4 are lower and higher than those (slope: -0.56 , intercept: 1.85) of Eq. 2.3 in Falkowski and Raven (2007).

In addition, we estimated the relationship between Ψ and daily PAR in the WSG during the summers from 1993 to 2008 using Shiimoto (2000), Hashimoto and Shiimoto, (2002), Imai *et al.* (2002) and the KH08-2 cruise. The Ψ values varied largely year by year; however, the values showed a significant correlation with the daily PAR (Eq. 2.5, $R^2 = 0.31$, $p < 0.05$ for slope, RMSE = 0.39, $n = 23$).

$$\Psi = 7.44 (\text{surface daily PAR})^{-0.73} \quad (2.5)$$

Eq. 2.5 is useful for estimating Ψ values from the daily PAR in the WSG during summer (June–August). All Ψ data in the WSG and Falkowski and Raven (2007) are plotted in Fig. 4, and the fitting curves of Eq. 2.3, 2.4 and 2.5 are also drawn. Shiimoto (2000) reported that the Ψ values in the western subarctic Pacific during the spring blooms were the highest among the world's oceans as compared them under the same daily PAR level. Recently, Isada *et al.* (2009) showed that relatively high Ψ values in the Oyashio region in both May and September as compared with those of Falkowski and Raven (2007). In this study, the Ψ values (curve B in Fig. 4) in the WSG during the summer of 2008 were clearly higher than the previous data. Moreover, the Ψ values in the WSG during the summers between 1993–2008 (curve C in the Fig. 4) were also generally higher than those of the world's oceans derived from Falkowski and Raven (2007). Therefore, these results indicate that Ψ in the WSG during the summer is the highest among the world's oceans. The high values of the water-column light utilization efficiency (Ψ) of phytoplankton photosynthesis can contribute to the high efficiency of biological carbon pumping observed in the WSG (Honda, 2003).

Table 2.1 Accessory pigment:chlorophyll *a* ratio matrices: (A) Initial ratio matrix in the 100–10% light depths; (B) Final ratio matrix obtained by CHEMTAX in the 100–10% light depths; (C) Initial ratio matrix in the 10–1% light depths; (D) Final ratio matrix obtained by CHEMTAX in the 10–1% light depths.

	Fucox	19'-But	19'-Hex	Peri	Diadinox	Allox	Violax	Prasinox	Chl <i>b</i>	Zeax	Chl <i>a</i>
(A)											
Diatoms	0.75	0	0	0	0.16	0	0	0	0	0	1
Prymne	0	0	0.58	0	0.10	0	0	0	0	0	1
Pelago	0.49	0.64	0	0	0.17	0	0	0	0	0	1
Chloro	0	0	0	0	0	0	0.049	0	0.29	0.044	1
Prasino	0	0	0	0	0	0	0.13	0.35	0.72	0	1
Crypto	0	0	0	0	0	0.17	0	0	0	0	1
Dino	0	0	0	0.59	0	0	0	0	0	0	1
Cyano	0	0	0	0	0	0	0	0	0	0.37	1
(B)											
Diatoms	0.75	0	0	0	0.16	0	0	0	0	0	1
Prymne	0	0	0.60	0	0.10	0	0	0	0	0	1
Pelago	0.49	0.64	0	0	0.15	0	0	0	0	0	1
Chloro	0	0	0	0	0	0	0.084	0	0.51	0.056	1
Prasino	0	0	0	0	0	0	0.13	0.35	0.72	0	1
Crypto	0	0	0	0	0	0.17	0	0	0	0	1
Dino	0	0	0	0.59	0	0	0	0	0	0	1
Cyano	0	0	0	0	0	0	0	0	0	0.37	1
(C)											
Diatoms	0.44	0	0	0	0.06	0	0	0	0	0	1
Prymne	0	0	1.08	0	0.16	0	0	0	0	0	1
Pelago	0.38	0.40	0	0	0.05	0	0	0	0	0	1
Chloro	0	0	0	0	0	0	0.056	0	0.48	0.066	1
Prasino	0	0	0	0	0	0	0.07	0.36	0.70	0	1
Crypto	0	0	0	0	0	0.11	0	0	0	0	1
Dino	0	0	0	0.59	0	0	0	0	0	0	1
Cyano	0	0	0	0	0	0	0	0	0	0.35	1
(D)											
Diatoms	0.44	0	0	0	0.06	0	0	0	0	0	1
Prymne	0	0	1.08	0	0.16	0	0	0	0	0	1
Pelago	0.38	0.40	0	0	0.03	0	0	0	0	0	1
Chloro	0	0	0	0	0	0	0.069	0	0.61	0.081	1
Prasino	0	0	0	0	0	0	0.07	0.36	0.70	0	1
Crypto	0	0	0	0	0	0.10	0	0	0	0	1
Dino	0	0	0	0.59	0	0	0	0	0	0	1
Cyano	0	0	0	0	0	0	0	0	0	0.35	1

Abbreviations: Prymne, prymnesiophytes; Pelago, pelagophytes; Chloro, chlorophytes; Prasino, prasinophytes; Crypto, cryptophytes; Dino, dinoflagellates; Cyano, cyanobacteria; Fucox, fucoxanthin; 19'-But, 19'-butanoyloxyfucoxanthin; 19'-Hex, 19'-hexanoyloxyfucoxanthin; Peri, peridinin; Diadinox, diadinoxanthin; Allox, alloxanthin; Violax, violaxanthin; Prasinox, prasinoxanthin; Chl *b*, chlorophyll *b*; Zeax, zeaxanthin; Chl *a*, chlorophyll *a*.

Table 2.2 Hydrographic conditions and phytoplankton productivity during Leg 1 of the KH 08-2 cruise. TD: transition domain, WSG: Western Subarctic Gyre, Z_{mix} : surface mixed layer depth, $N_{\text{mix_mean}}$: mean nitrite and nitrate concentrations within the surface mixed layer, Z_{eu} : euphotic layer depth, $N_{\text{eu_mean}}$: mean nitrite and nitrate concentrations within the euphotic layer, $T_{\text{inc_start}}$: start time of incubations, Ψ : water-column light utilization efficiency of phytoplankton photosynthesis.

Station	Temperature (°C)			Salinity			Water mass	Z_{mix} (m)	$N_{\text{mix_mean}}$ (μM)	Z_{eu} (m)	$N_{\text{eu_mean}}$ (μM)	$T_{\text{inc_start}}$	PAR (mol photons $\text{m}^{-2} \text{d}^{-1}$)	Chlorophyll <i>a</i> concentration (mg m^{-2})	Primary production ($\text{mg C m}^{-2} \text{d}^{-1}$)	Chl <i>a</i> -specific primary production ($\text{mg C [mg Chl a]}^{-1} \text{d}^{-1}$)	Ψ ($\text{g C [g Chl a]}^{-1} [\text{mol photon}]^{-1} \text{m}^2$)
	5 m	100 m	5 m	5 m	100 m	100 m											
1	20.2	7.9	33.6	33.9	33.7	TD	TD	10	0.1	45	4.7	14:20	25.4	32.1	1280	40	1.57
2	16.9	6.1	33.2	33.7	33.7	TD	TD	9	2.7	34	6.1	10:45	27.6	34.5	1026	30	1.08
3	16.1	4.2	33.6	33.5	33.5	TD	TD	22	3.3	37	4.9	10:30	31.5	35.2	1535	44	1.39
5	13.0	1.4	32.7	33.2	33.2	WSG	WSG	11	7.2	37	11.0	14:00	26.2	24.9	566	23	0.87
6	11.9	3.2	32.7	33.3	33.3	WSG	WSG	18	15.6	44	17.6	15:15	17.3	22.6	583	26	1.49
8	12.3	3.2	32.6	33.2	33.2	WSG	WSG	15	10.8	50	14.3	19:10	13.3	29.3	726	25	1.86
9	14.3	2.2	33.0	33.0	33.0	WSG	WSG	14	11.5	54	14.5	7:20	21.7	25.7	659	26	1.18
10	12.4	2.0	32.6	33.0	33.0	WSG	WSG	17	13.9	43	15.1	15:45	23.2	32.6	882	27	1.17
11	18.8	6.1	33.1	33.7	33.7	TD	TD	10	2.0	48	7.8	17:10	36.8	24.6	583	24	0.64

Table 2.3 Size-fractionated chlorophyll *a* concentrations at 5 m and 5% (or 3%) light depth at each station.

Station	Water mass	Chlorophyll <i>a</i> (%)			
		5 m		5 % (or 3%) light depth	
		< 10 μm	> 10 μm	< 10 μm	> 10 μm
1	TD	48	52	82	18
2	TD	39	61	45	55
3	TD	49	51	62	38
5	WSG	75	25	90	10
6	WSG	72	28	86	14
8	WSG	84	16	87	13
9	WSG	79	21	80	20
10	WSG	78	22	60	40
11	TD	62	38	92	8

Table 2.4 List of the phytoplankton species identified. Genus and species names are arranged alphabetically, not systematically. The asterisk indicates the most dominant species in the diatoms or coccolithophores.

Centric diatoms	Pennate diatoms	Coccolithophores
<i>Actinocyclus</i> sp. 1	<i>Diploneis</i> sp. 1	<i>Alisphaera</i> sp. 1
<i>Asteromphalus hookeri</i>	<i>Fragilariopsis atlantica</i>	<i>Calcidiscus leptoprus</i> ssp. Leptoporus
<i>Azpeitia</i> sp. 1	<i>F. curta</i>	<i>Coccolithus pelagicus</i> ssp. Braarudii
<i>Bacteriastrum elongatum</i>	<i>F. cylindriiformis</i>	<i>C. pelagicus</i> ssp. Pelagicus
<i>B. hyalinum</i>	<i>F. cylindrus</i>	<i>Coronosphaera mediterranea</i>
<i>Bacteriastrum</i> sp. 1	<i>F. oceanica</i>	<i>Cyrtosphaera lecaliae</i>
<i>Chaetoceros affinis</i>	<i>F. pseudonana</i>	<i>Emiliana huxleyi</i> type A
<i>Ch. concavicornis</i>	<i>Fragilariopsis</i> sp. 1	<i>E. huxleyi</i> type B
<i>Ch. debilis</i>	<i>Lioloma delicatulum</i>	<i>E. huxleyi</i> type C*
<i>Ch. messanensis</i>	<i>Navicula</i> sp. 1	<i>Syracosphaera corolla</i>
<i>Chaetoceros</i> sp. 1	<i>Neodenticula seminae</i>	<i>S. molischii</i> type 1
<i>Chaetoceros</i> sp. 2	<i>Nitzschia bicaipitata</i>	<i>S. nana</i>
<i>Corethron criophilum</i> *	<i>Ni. braarudii</i>	<i>S. pulchra</i>
<i>Co. inerme</i>	<i>Nitzschia</i> sp. 1	
<i>Coscinodiscus</i> sp. 1	<i>Pseudonitzschia pseudodelicatissima</i>	
<i>Eucampia zodiacus</i>	<i>P. subfraudulenta</i>	
<i>Hemiaulus hauckii</i>	<i>Pseudonitzschia</i> sp. 1	
<i>Odontella longicuris</i>	<i>Pseudonitzschia</i> sp. 2	
<i>Rhizosolenia</i> sp. 1	<i>Pseudonitzschia</i> sp. 3	
<i>Rhizosolenia</i> sp. 2	<i>Thalassionema nitzschioides</i>	
<i>Skeletonema costatum</i>		
<i>Thalassiosira allenii</i>		
<i>Th. angulata</i>		
<i>Th. anguste-lineata</i>		
<i>Th. curviseriata</i>		
<i>Th. eccentrica</i>		
<i>Th. gracilis</i>		
<i>Th. lineata</i>		
<i>Th. mala</i>		
<i>Th. nordenskiöldii</i>		
<i>Th. oestrupii</i>		
<i>Th. pacifica</i>		
<i>Th. proschkinae</i>		
<i>Th. trifulta</i>		
<i>Thalassiosira</i> sp. 1		
<i>Thalassiosira</i> sp. 2		
<i>Thalassiosira</i> sp. 3		
<i>Thalassiosira</i> sp. 4		
<i>Thalassiosira</i> sp. 5		

Table 2.5 Summary of chlorophyll *a* concentration, primary production, PAR, Chl *a*-specific primary production and Ψ during SEEDS-I and SEEDS-II. Fe-in: inside the iron-fertilized patch, Fe-in_{growth}: growth phase based on the F_v/F_m levels in the Fe-in, Fe-out: outside the iron-fertilized patch, PAR: photosynthetic available radiation, Ψ : water-column light utilization efficiency of phytoplankton photosynthesis.

Iron-fertilized patch	Day	Chlorophyll <i>a</i> concentration (mg m ⁻²)	Primary production (mg C m ⁻² d ⁻¹)	PAR (mol photons m ⁻² d ⁻¹)	Chl <i>a</i> -specific primary production (mg C [mg Chl <i>a</i>] ⁻¹ d ⁻¹)	Ψ (g C [g Chl <i>a</i>] ⁻¹ mol photon ⁻¹ m ²)
SEEDS-I						
Initial	0	38.8	441	30.2	11	0.38
Fe-in _{growth}	2	37.6	520	31.7	14	0.44
Fe-in _{growth}	4	41.0	394	38.8	10	0.25
Fe-in _{growth}	7	115.0	1851	27.5	16	0.59
Fe-in _{growth}	9	232.2	2033	17.5	9	0.50
Fe-in	11	221.0	2350	35.2	11	0.30
Fe-in	13	131.7	1690	21.4	13	0.60
Fe-out	4	38.7	515	38.4	13	0.35
Fe-out	9	40.1	424	17.6	11	0.60
Fe-out	13	31.5	409	23.4	13	0.55
SEEDS-II						
Initial	0	33.9	527	20.2	16	0.77
Fe-in _{growth}	2	31.8	887	34.3	28	0.81
Fe-in _{growth}	5	62.8	1161	31.8	19	0.58
Fe-in _{growth}	8	65.5	1052	12.1	16	1.33
Fe-in _{growth}	11	69.5	1446	24.1	21	0.86
Fe-in	17	45.3	557	36.3	12	0.34
Fe-in	23	20.2	633	41.5	31	0.75
Fe-in	25	24.8	629	15.4	25	1.65
Fe-out	11	42.6	909	23.9	21	0.89
Fe-out	15	42.5	723	26.0	17	0.65
Fe-out	24	18.2	794	35.2	44	1.24
Fe-out	31	16.7	378	26.8	23	0.84
Fe-out	32	20.5	324	25.8	16	0.61

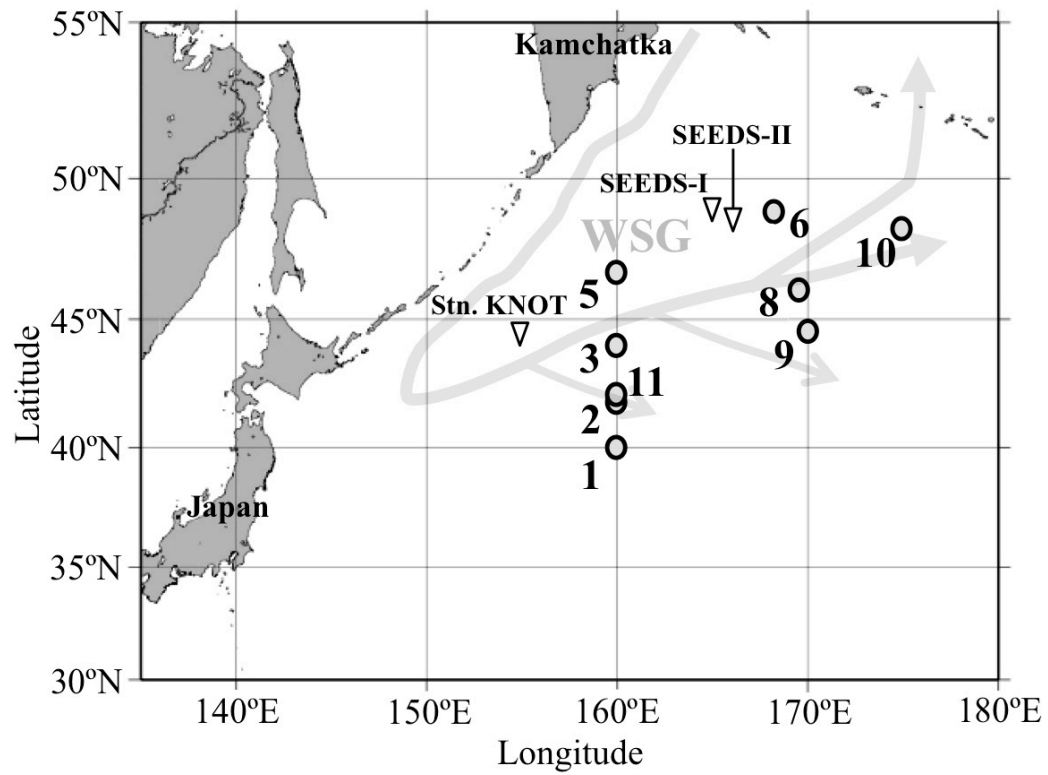


Fig. 2.1 Sampling stations during the KH08-2 cruise in the western subarctic Pacific. The locations of Stn KNOT, SEEDS-I and SEEDS-II are also indicated. The surface current is drawn with arrows following Yasuda (2003).

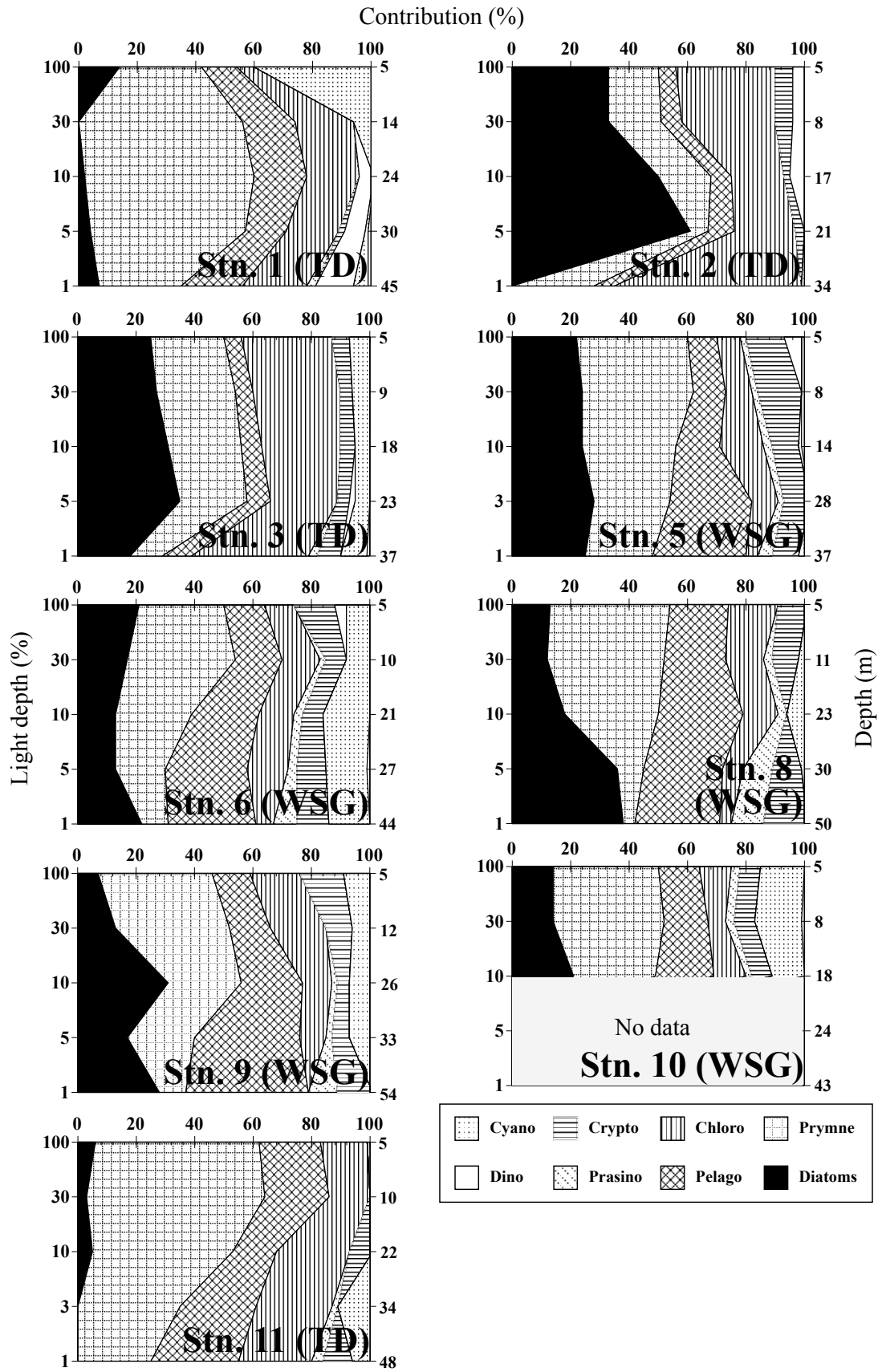


Fig. 2.2 Contributions of each phytoplankton group to the chlorophyll *a* biomass within the euphotic zone in the WSG (Stns 5, 6, 8, 9 and 10) and TD (Stns 1, 2, 3 and 11).

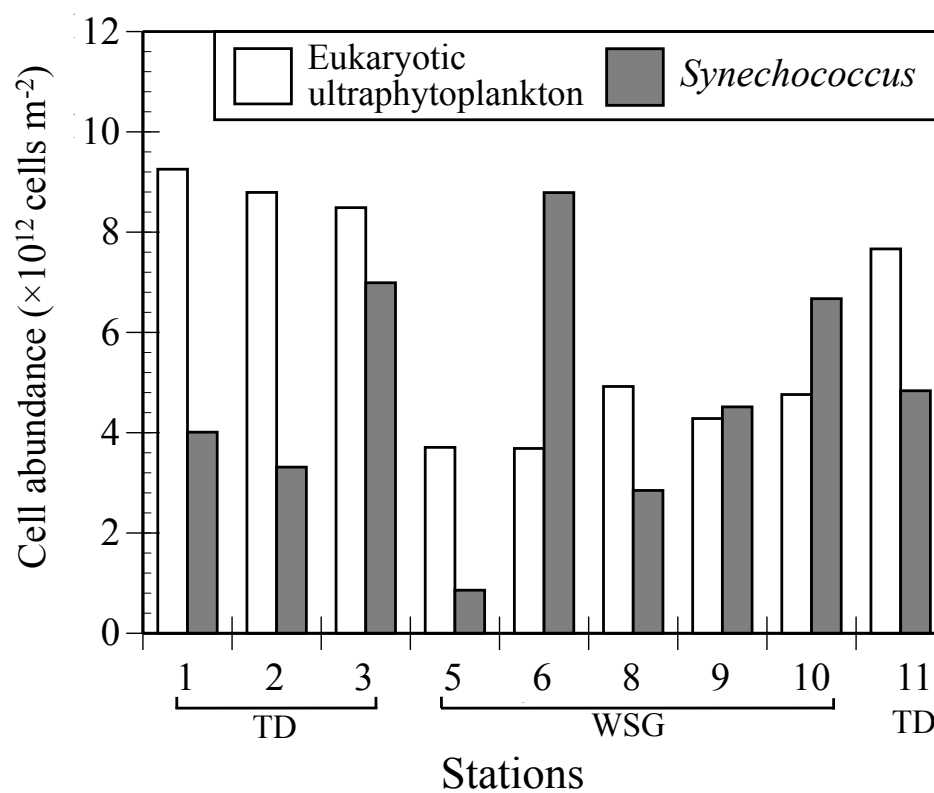


Fig. 2.3 Euphotic-depth-integrated cell abundances of eukaryotic ultraphytoplankton and *Synechococcus* in the WSG (Stns 5, 6, 8, 9 and 10) and TD (Stns 1, 2, 3 and 11).

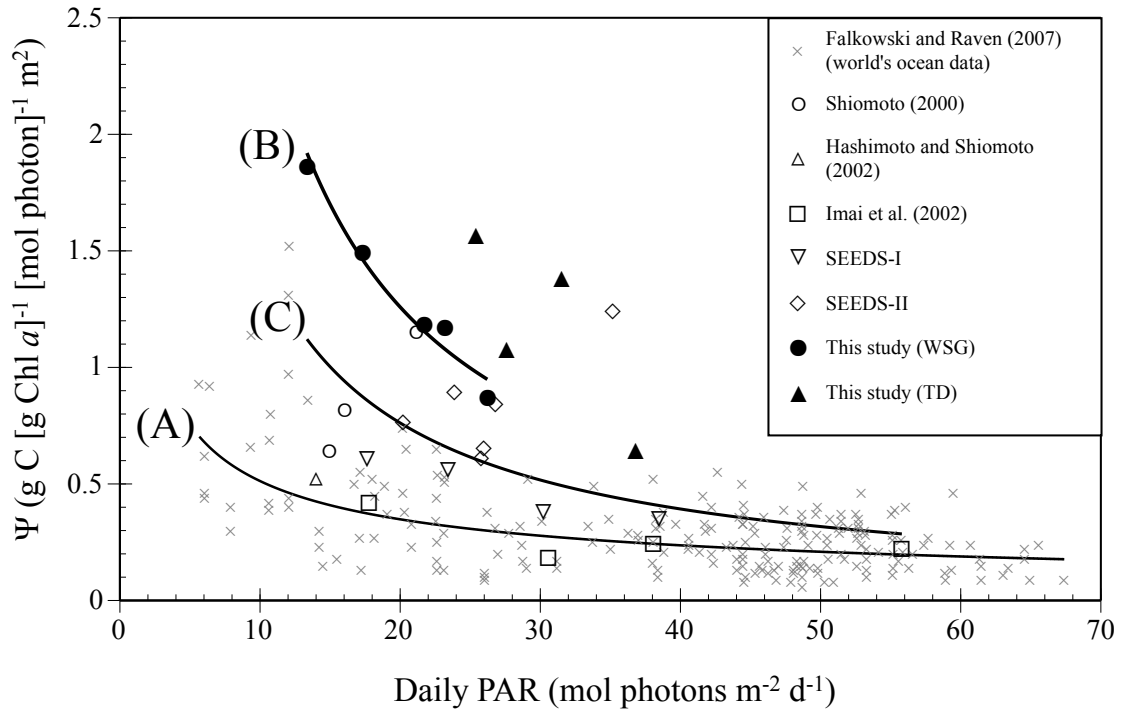


Fig. 2.4 Relationships between Ψ and daily PAR during the KH08-2 cruise (WSG and TD), other studies in the WSG, and the world's oceans. (A) The fitting curves obtained from the Falkowski and Raven (2007), (B) the fitting curve using the WSG data obtained from the KH08-2 cruise and (C) the fitting curve using all WSG data. The fitting curves (A), (B) and (C) correspond to equations (3), (4) and (5), respectively.

Chapter 3 – Dynamics of transparent exopolymer particles in the Oyashio region of the western subarctic Pacific during the spring diatom blooms

3.1 Introduction

In Chapter 2, I described that the water-column light utilization efficiency (Ψ) by phytoplankton was relatively high in the Western Subarctic Gyre during summer. Isada et al. (2009) also reported high Ψ values in the Oyashio region during the spring diatom blooms. The PAR level in latitude 40° at the meridian transit between January and May increases from ca. 1,000 to 2,000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Kirk, 2011). The increase of the PAR level from winter to spring lead to the water-column stratification (Kasai et al., 1997), and it may induce the massive spring diatom blooms. In this spring diatom blooms, the chlorophyll (Chl) *a* concentration and the primary production integrated within the water-column were reported to reach 40 mg Chl *a* m^{-3} (Kasai et al., 1998) and 3,200 mg C $\text{m}^{-2} \text{ d}^{-1}$ (Isada et al., 2010), respectively. The organic matters produced by phytoplankton were consumed by the predators such as zooplankton, fish and mammals and decomposed by bacteria. The consumption and decomposition may occur mainly in the surface ocean, because the photosynthesis depends on PAR level. The depth of the euphotic zone in the Oyashio region during the spring diatom booms were reported to be between 3 and 50 m (Kasai et al., 1998; Isada et al., 2010), and the depth may depend on the phytoplankton abundances (Saito et al., 2002). Whereas, a part of the organic matter produced by phytoplankton was exported from the euphotic zone to the deeper ocean. It is called the biological carbon pump. The biological carbon pump was defined as the biological transportation of the organic carbon from the euphotic zone to deeper ocean (Chisholm, 2000).

Takahashi et al. (2002) reported that the Oyashio region is one of the regions that the seasonal drawdown effect of $p\text{CO}_2$ by marine organisms is highest among oceans. This is caused by high primary production during the spring diatom blooms (Midorikawa et al., 2003; Ayers and Lozier, 2013). However, a large part of the organic matter fixed by phytoplankton is respired by bacteria, zooplankton, fish and marine mammals, and as a result, CO_2 is released to the seawater. In addition, even if the organic matter was transported from euphotic zone to deeper ocean, the settling organic matters can be decomposed by bacteria or consumed by zooplankton, fish and marine

mammals. Therefore, what important is the export of organic matters from the euphotic zone to deeper ocean. The setting velocity of the particles can be estimated from the Stokes' equation (Stokes, 1845). As an example obtained from a laboratory experiment of Iversen and Ploug (2010), the aggregate of 2.5-mm size produced by *Skeletonema costatum* (diatoms) had the setting velocity of 113 m d⁻¹. The average depth of ocean is about 3,700 m (Charette and Smith, 2010). Hence, if this aggregate was not decomposed or consumed, it could take 31 days from the sea surface to the floor. In fact, since the decomposition and consumption occur in the water column, it would be spent further time, and it is known that only about 0.1% of the export from the euphotic zone reaches to the sea floor (Honda, 2003; Williams and Follows, 2011). The refractory organic aggregates reached on the sea floor can be preserved for the geological time scale. On the other hand, the labile organic aggregates may be respired by the organisms in the deep ocean and in/on the floor, and after, the CO₂ released into the deep water can be fixed up to ca. 1,000 years in the ocean (Matsumoto, 2007). The mechanism of the carbon fixation into the ocean is important for the future high CO₂ world. Honda (2003) reported that the efficiency of biological carbon pump was relatively high in the Western Subarctic Gyre among the world's oceans. Kawakami et al. (2004) also showed that the POC fluxes and the ratios of POC export to primary production (e-ratios) in the western subarctic Pacific including the Oyashio region were much higher in spring than in winter.

The aggregate of particles may be facilitated by the existence of Transparent exopolymer particles (TEP), which are very sticky, exhibit the characteristics of gels, and particularly important for the aggregate of particles (e.g., Passow, 2002b, Wurl et al., 2011). TEP are defined as >0.4 µm transparent particles that consist of acid polysaccharides and are stainable with Alcian blue (Alldredge et al., 1993). In the marine system, many organisms, including phytoplankton and bacteria generate the extracellular polysaccharides (Passow, 2002b). Generally, the high TEP levels are known to associate with phytoplankton blooms, such as diatoms (e.g., Passow et al., 1995b), *phaeocystis* spp. (e.g., Hong et al., 1997), dinoflagellates (e.g., Berman and Viner-Mozzini, 2001), cryptomonads (e.g., Passow et al., 1995) and cyanobacteria (e.g., Grossart and Simon, 1997). Diatoms are especially well known for excreting large amount of polysaccharides during their actively growing and/or senescent conditions (Passow, 2002b). Therefore, TEP can increase the biological carbon pump efficiency

due to (I) TEP act as “bonding agent” and condense various particles existing in the ocean, and (II) the aggregated particles increase the sinking velocity in the water-column.

The TEP levels in the world’s oceans were highly variable (0–14,800 μg Xanthan gum equivalent L^{-1}) (Hong et al., 1997; Radić et al., 2005). The high TEP were also found within the hydrothermal plume area (Prieto and Cowen, 2007; Shackelford and Cowen, 2006). A survey of the TEP in the Western Subarctic Gyre of the North Pacific was carried out by Ramaiah et al. (2005), and the maximum value of the TEP levels was 190 μg Xanthan gum equiv. L^{-1} . In Japan, the TEP surveys were conducted in Isahaya Bay (Fukao et al, 2011), Sagami Bay (Sugimoto et al., 2007), Tokyo Bay (Ramaiah and Furuya, 2002) and Otsuchi Bay (Ramaiah et al., 2001). However, the TEP studies in Japan are limited in these bays.

As described above, the biological pump efficiency in the Oyashio region was higher in the spring (Kawakami et al., 2004). This is associated with the spring diatom blooms, and the diatoms may be the major source of the TEP. Hence, I studied the TEP levels in the Oyashio region during the spring diatom blooms. Simultaneously, I investigated the phytoplankton group, primary production, photosynthesis parameters and bacterial abundance, and so on. Since it was reported that the TEP were mainly formed from the DOC excreted by phytoplankton (Passow, 2002), I also estimated the production of dissolved organic carbon (DOC) by the phytoplankton, and this is the first study in the Oyashio region.

3.2 Materials and methods

3.2.1 Research cruises

The research cruises of the TEP in the Oyashio region during the spring diatom blooms were conducted from April 13 to 23, 2010, from June 7 to 16, 2010, and from May 5 to 13, 2011. The R/V *Wakataka-Maru* of the Tohoku national fisheries research institute, Japan was used for the two cruises of 2010, whereas the R/V *Tansei-Maru* of the Japan agency for marine-science and technology (JAMSTEC)/Atmospheric and Ocean Research Institute, the University of Tokyo was used for the 2011 cruise. The sampling stations in the April, June and May cruises were two (A1 and A2), four (J1, J2,

J3 and J4) and three (M1, M2 and M3), respectively (Fig. 3.1). In all cruises, incident PAR above the sea surface was continuously measured on deck with a PAR sensor (ML-020P, EKO Instruments Co., Ltd.) every 10 min on average, and the values were recorded with a data logger. Seawater sampling at all stations was accomplished using a CTD-CMS attached with Niskin bottles. Nutrients (nitrite plus nitrate—hereafter donated as nitrate, phosphate and silicate) were determined with a BRAN + LUEBBE auto-analyzer following the manufacturer's protocol.

3.2.2 *Phytoplankton pigments and CHEMTAX processing*

To obtain the size information of the phytoplankton in terms of Chl *a* concentration, Chl *a* concentrations of the <20, 2–10 and >10 μm fractions in the April and June cruises were determined using a Turner Design Model 10-AU fluorometer, according to the Welschmeyer method (1994). Water samples (500 mL) corrected from 5 m depth were size-fractionated using 47 mm Whatman GF/F filter and 47 mm Nuclepore filters (2 and 10 μm pore-size). Algal pigments were immediately extracted by soaking each filter in *N, N*-dimethylformamide (DMF) at -20°C for 24 hours and were analyzed on board.

For the analysis of phytoplankton pigments using high-performance liquid chromatography (HPLC), water samples (500 mL) collected from 5 to 300 m depths (12 layers) were filtered on the 25-mm Whatman GF/F filters under gentle vacuum (<100 mm Hg). The filter samples were folded, blotted with filter paper and stored in a deep-freezer (-80°C). Phytoplankton pigments were extracted with sonication in *N, N*-dimethylformamide (DMF) according to the protocol of Suzuki et al. (2005). HPLC pigment analysis was performed according to the method of Van Heukelem and Thomas (2001), except the flow rate of 1.2 mL min^{-1} . Before injection, 250 μL of algal extracts was mixed with 250- μL ion-pair solution (28 mM tetrabutyl ammonium acetate (TBAA), pH 6.5), and equilibrated for 5 min at 5°C . Two hundred fifty microliters of the mixture was injected into a Shimadzu HPLC (CLASS-VP) system incorporating an Agilent Eclipse XDB-C8 column (3.5- μm particle size, $4.6 \times 150\text{ mm}$). The HPLC solvent system was as follows: solvent A was 70% methanol and 30% 2.8 mM TBAA (pH 6.5) aqueous solution, and solvent B was methanol. A linear gradient with an isocratic eluent method was used: 0 min 95%A/5%B, 22min 5%A/95%B, 22–30 min 5%A/95%B.

To estimate the contributions of each algal group in the two layers (5–20 m and 30–50 m depths) to the total Chl *a* biomass, all data were interpreted by factorization using the CHEMTAX program (Mackey et al., 1996). Initial and final pigment ratios were calculated following the method of Latasa (2007) (Table 3.1), and the initial ratios were based on Suzuki et al. (2002, 2005, 2009) who estimated the community composition of phytoplankton in the western subarctic Pacific. Prymnesiophytes, pelagophytes and prasinophytes in our CHEMTAX analysis are synonymous with type 3 haptophytes, type 2 chrysophytes and type 3 prasinophytes of Mackey et al. (1996), respectively.

3.2.3 *Phytoplankton specific absorption coefficient ($\bar{a}_{ph}^*$)*

Duplicate water samples (each 500 mL) were collected at 5 m depth. The samples were filtered onto Whatman GF/F filters (25 mm diameter) under gentle vacuum (<100 mm Hg). The filters were immediately contained into Petri slide containers (Millipore), and covered with aluminum foil. The samples were stored in the deep-freezer (–80°C). On land, the absorption from 400 to 750 nm of the samples was measured with a spectrophotometer (MPS-2450, Shimadzu) equipped with an end-on-type photomultiplier tube. The measurements were carried out according to the glass-fiber filter technique of Kishino et al. (1985). The particulate matter on the filter was soaked in NaClO solution (1% final concentration) to bleach phytoplankton pigments. The bleached filter was measured with the spectrophotometer, and the absorption of detritus determined. The optical density of the phytoplankton was obtained by subtracting the optical density of the detritus from that of the total particles. The measured optical densities of particulate matter were corrected for the path-length amplification effect using the equation of Cleveland and Weidemann (Cleveland and Weidemann, 1993).

The mean Chl *a*-specific absorption coefficient of phytoplankton, $\bar{a}_{ph}^*$ (m² [mg Chl *a*]^{–1}) was weighted with the spectral irradiance of the incubator lamp from 400 to 700 nm to correct the spectral characteristics of the incubator lamp source according to Cota et al. (1994) (equation 3.1).

$$\bar{a}_{ph}^* = \int_{400}^{700} a_{ph}^*(\lambda) E(\lambda) d\lambda / \int_{400}^{700} E(\lambda) d\lambda \quad (3.1)$$

Where $E(\lambda)$ is the relative spectral irradiance of the incubator lamp, and the relative spectrum was obtained from the manufacturer.

3.2.4 Cell abundance of phytoplankton

Water samples (500 mL) were taken at 5 m depth for counting diatoms and coccolithophores and were fixed with buffered formalin (pH 7.8, 1% final concentration). An aliquot (3–11 mL) of the sample was filtered onto a Nuclepore membrane (1 μm pore size) filter set in a glass funnel (3 mm in diameter at the base) under vacuum (100–200 mm Hg). The filter membrane was rinsed with the Milli-Q water to remove salts and immediately dried for three hours in an oven at 60 °C. To count and identify the algal cells, the whole area of membrane (ca. 7 mm²) was examined with a scanning electron microscope (SEM, VE-8800, KEYENCE Corp.) at a magnification of approximately 2,000 $\times$. Species of phytoplankton were identified according to Fukuyo (1990), Thomas (1997), Young et al. (2003), Scott and Marchant (2005) and Round et al. (2007). In this study, the dominant species in the diatoms were defined as having more than 25% contributions to the total diatom abundances.

3.2.5 Flow cytometry

Quadruplicate water samples (each 2 mL) from 5 to 300 m depths were preserved with paraformaldehyde (0.2% final concentration) and stored in the deep-freezer (–80 °C) until analysis on land. An EPICS flow cytometer (XL ADC system, Beckman Coulter Inc.) equipped with a 15 mW air-cooled argon laser exciting at 488 nm and a standard filter setup was used to enumerate eukaryotic ultraphytoplankton (<5 μm in size, duplicate samples) and heterotrophic bacteria (duplicate samples). Prior to analysis, the samples were thawed and drawn through a 35- μm nylon-mesh-capped Falcon cell strainer (Becton-Dickinson) to remove larger cells. For the enumeration of eukaryotic ultraphytoplankton, a certain volume of Flow-count fluorospheres (Beckman Coulter) and 2.0 μm Fluoresbrite YG beads (Poly Sciences) were added to each sample. The details of flow cytometric analysis are described in Suzuki et al. (2005).

For the enumeration of heterotrophic bacteria, cells were stained with the nucleic acid stain SYBER Gold solution (Invitrogen). Stock SYBER Gold stain (10⁴-fold concentrations in the commercial solution) was diluted to 10-fold concentrations with the Milli-Q water. The 10-fold SYBER Gold stain of 25 μL was added to the bacteria sub-samples of 225 μL , and incubated in the dark at room temperature (25°C) for 30 min before analysis. The incubated samples were mixed with the Flow-Count

fluorospheres of 250 μ L, and then, analyzed.

3.2.6 Dissolved organic carbon (DOC) analysis

The samples of DOC were collected from 5 to 300 m depths (12 layers). In-line filter folders (PP-47, Advantec MFS Inc.) and tubes (TYGON R-3603, United States Plastic Corp.) were pre-cleaned by soaking in 1 M HCl and then rinsed with Milli-Q water. Before sampling, pre-combusted Whatman GF/F filters (47 mm) were set in the in-line filter folders, and the tubes were connected with the in-line filter folders. The water samples were taken by connects the tube with the spigot of Niskin bottle. At start of sampling, filtrates were well drained for prewashing of the tube, filter folder and filter, and after, triplicate samples were collected into pre-combusted 24-mL screw vials with acid-cleaned PTFE septum caps. The samples were immediately stored in the freezer (-20°C) until analysis on land. The frozen samples were thawed, and they were well shaken. The concentrations of DOC were determined with a total organic carbon analyzer (TOC-V CSH, Shimadzu).

3.2.7 Pulse amplitude modulation (PAM) fluorometer measurements

The water sample for the pulse amplitude modulation (PAM) fluorometer was collected into the 20-mL shading bottle at 5 m depth. The water sample was acclimated to dark for 30 min at the 5 m depth temperature. Three milliliter of the water sample was added into a quartz cuvette, and the photochemical quantum efficiency (F_v/F_m) of algal photosystem II (PSII) was measured with a Walz WATER-PAM fluorometer.

3.2.8 Particulate organic carbon (POC) production

Daily particulate organic carbon (POC) production was estimated using a simulated *in situ* incubation technique at 5 m depth, except Station M3 during the May cruise. The samples were dispensed into three 275-mL acid-cleaned polycarbonate bottles (two light and one dark) and were inoculated with a solution of $\text{NaH}^{13}\text{CO}_3$ (99% atom% ^{13}C), which was equivalent to ca. 10% of the total inorganic carbon (TIC) in the seawater. The TIC concentrations were measured with a total alkalinity analyzer (ATT-05, Kimoto Electric Co., Ltd.). All bottles were incubated for ca. 24 hours. The incubated samples were filtered onto pre-combusted Whatman GF/F filters (25 mm in diameter, 450°C for 5 hours) under a gentle vacuum (<100 mm Hg), and stored in the freezer ($-$

20°C) until analysis. On land, the samples were thawed in the room temperature, and exposed to HCl fumes in order to remove inorganic carbon, and completely dried in a desiccator under vacuum for ≥ 24 hours. The concentrations of POC on the filters and ^{13}C abundance were determined using a mass spectrometer (DELTA V, Thermo Fisher Scientific Inc.) with an on-line elemental analyzer (FlashEA1112, Thermo Fisher Scientific Inc.). The daily primary production was calculated according to equation (3.2) (Hama et al., 1983).

$$POC \text{ production rate} = \frac{(a_{is} - a_{ns})}{(a_{ic} - a_{ns})} \times \frac{[C]}{t} \times f \quad (3.2)$$

Where a_{is} is the ^{13}C atom% in an incubated sample, a_{ns} is the ^{13}C atom% in a natural (i.e., non-incubated) sample, a_{ic} is the ^{13}C atom% in inorganic carbon, $[C]$ is concentration of POC in the incubated sample, t is the incubation length (day) and f is the discrimination factor of ^{13}C (i.e. 1.025).

3.2.9 DOC production

Daily dissolved organic carbon (DOC) was estimated according to Hama and Yanagi (2001) in general. The filtered seawater samples of the incubated POC production were collected in pre-combusted 500-mL reagent bottles (450°C for 5 hours), and stored in the freezer (−20°C) until analysis. On land, the frozen samples were melted at room temperature (25°C), and the water samples were desalinated with an electro dialyzer (Micro Acilyzer S3, ASTOM Corp.) equipped AC-220-550 cartridge (CMX-SB/AMX-SB, ASTOM Corp.). The conductivity of the water samples decreased from ca. 53 to just 3.0 mS cm^{−1} ($\text{S} \equiv \Omega^{-1}$) for 2–3 hours. Before and after the desalination, DOC concentrations were examined in this study. The recovery percentage of DOC concentration ranged from 62 to 96%, whereas the conductivity decreased to 6% of the initial conductivity (Table 3.2). The desalinated seawater samples were concentrated to ca. 5 mL with a rotary evaporator at 45°C using pre-combusted 500-mL egg-plant shaped flask. HCl was added to the concentrated 5-mL samples to decrease the pH to 2. The low pH concentrates were purged with N₂ gas for ca. 9 min to remove the dissolved inorganic ^{13}C . Thereafter, the concentrates were re-concentrated to ca. 0.5–1 mL with the rotary evaporator at 45°C using a pre-combusted 10-mL pear shaped flask. Whatman GF/F filters were completely fragmented with a probe-type sonicator in

Milli-Q water, and the fragmented GF/F filter dried in an oven at 120°C. The dried GF/F filter (a lump of the GF/F filter) was disentangled with a metal scoopula, and collected in a 500-mL reagent bottle, and combusted in a muffle furnace (450 °C for 5 hours). A known weight of the GF/F filter fragments was added in tin capsules (Santis Analysis, 10 × 10 mm tin capsule) for the elemental analyzer, and after, the re-concentrated seawater samples of 500 µL was absorbed onto the GF/F fragments. The samples were completely dried in a desiccator under vacuum for a few days. The ¹³C abundances of the samples were determined using the mass spectrometer (DELTA V, Thermo Fisher Scientific Inc.) with the on-line elemental analyzer (FlashEA1112, Thermo Fisher Scientific Inc.). Daily primary production for DOC was calculated according to equation (3.3) (Hama and Yanagi, 2001).

$$DOC\ production\ rate = \frac{(a_{is} - a_{ns})}{(a_{ic} - a_{ns})} \times \frac{[C]}{t} \quad (3.3)$$

Where a_{is} is the ¹³C atom% in an incubated sample, a_{ns} is the ¹³C atom% in a natural (nonincubated) sample, a_{ic} is the ¹³C atom% in inorganic carbon, t is the incubation length (day), and $[C]$ is concentration of DOC estimated in the section 3.2.6 *DOC analysis*.

3.2.10 Photosynthesis-irradiance (P-E) curve experiments

Photosynthesis-irradiance (P-E) curve experiments were carried out according to Isada et al. (2009). The water samples collected at 5 m depth were dispensed into 275 mL acid-cleaned polystyrene bottles, inoculated with a solution of the NaH¹³CO₃ (99 atom% ¹³C), which was equivalent to ca. 10% of TIC in the seawater. The concentrations of TIC were measured with the total alkalinity analyzer (ATT-05, Kimoto Electric Co., Ltd.). Incubations were carried out for ca. 2 hours in a bench-top incubator, and equipped with a 150 W metal halide lamp (HQI-T 150W/WDL/UVS, Mitsubishi/Osram Co., Ltd.) as a light source. The incubator was cooled to the seawater temperature at 5 m depth with a temperature-controlled water circulator (CL-80R, TAITEC Corp.). The samples were exposed to irradiance levels from ca. 5 to 2400 µmol photons m⁻² s⁻¹. The irradiance levels at each bottle position were measured with a 4π PAR sensor (SQL-2101, Biospherical Instruments Inc.). After incubation, the samples were filtered onto pre-combusted Whatman GF/F filters (25 mm diameter)

under gentle vacuum (<100 mm Hg). Single water sample of 275 mL was also filtered in the same manner as before the incubation. The filter samples were stored in the freezer (−20°C) until analysis on land.

After the frozen filters were thawed, they were exposed to HCl fumes to remove inorganic carbon, and completely dried in a desiccator under a vacuum for more than 24 hours. The POC in the samples and ¹³C abundance were determined using the mass spectrometer (DELTA V, Thermo Fisher Scientific Inc.) with the on-line elemental analyzer (FlashEA1112, Thermo Fisher Scientific Inc.). Primary production per hour was calculated according to Hama et al. (1983). The primary production rate was normalized with the Chl *a* biomass. The Chl *a*-normalized primary production rate (mg C [Chl *a*]^{−1} h^{−1}) was fitted with the non-photoinhibition model of the Webb et al. (1974) (eq. 3.4) or with the photoinhibition model of Platt et al. (1980) (eq. 3.5).

$$P^* = P_{\max}^* \left(1 - \exp^{-\alpha^* E / P_{\max}^*} \right) \quad (3.4)$$

$$P^* = P_s^* \left(1 - \exp^{-\alpha^* E / P_s^*} \right) \times \left(1 - \exp^{-\beta^* E / P_s^*} \right) \quad (3.5)$$

In those model, $P_{\max}^*$ is the maximum photosynthesis rate (mg C [Chl *a*]^{−1} h^{−1}), α^* is the initial slope (mg C [Chl *a*]^{−1} h^{−1} [μmol photon m^{−2} s^{−1}]^{−1}) and/or β^* is the photoinhibition index (mg C [Chl *a*]^{−1} h^{−1} [μmol photon m^{−2} s^{−1}]^{−1}). For the photoinhibition model (eq. 3.5), $P_{\max}^*$ was estimated from equation (3.6).

$$P_{\max}^* = P_s^* \times \left(\frac{\alpha^*}{\alpha^* + \beta^*} \right) \times \left(\frac{\alpha^*}{\alpha^* + \beta^*} \right)^{\beta^* / \alpha^*} \quad (3.6)$$

The light saturation index (E_k) is defined as following equation (3.7).

$$E_k = P_{\max}^* / \alpha^* \quad (3.7)$$

Moreover, the maximum quantum yield of carbon fixation ($\Phi_{c \max}$, mol C [mol photon]^{−1}) was estimated from the following equation (3.8).

$$\Phi_{c \max} = 0.0231 \times \alpha^* / \bar{a}_{ph}^* \quad (3.8)$$

Where 0.0231 is factor to converts miligrams of carbon to moles, μmol photons to moles and hours to seconds, and $\bar{a}_{ph}^*$ is the phytoplankton specific absorption coefficient estimated in the equation (3.1).

3.2.11 TEP analysis

The water samples of TEP were taken from 5 to 300 m depths (12 layers). According to Passow and Alldredge (1995b), triplicate water samples were filtered onto Whatman 0.4- μm Nuclepore filters under gentle vacuum (<100 mm Hg), and TEP on the filter were stained for ca. 2 seconds with 1 mL of a 0.02% aqueous solution of Alcian blue (8GX, Sigma-Aldrich Inc. LLC) in 0.06% acetic acid (pH 2.5). The stained filter was immediately rinsed with Milli-Q water to remove excess dye. The filters were stored in the freezer (-20°C) until analysis on land.

For standard curve of TEP, I modified the method of the Claquin et al. (2008). Xanthan gum of 1 mg was put into a 15-mL centrifugal tube, and added the 10-mL Alcian blue stain. The tube was well shaken for about 1 hour. The tube was centrifuged ($3,200 \times g$, 20 min), and the supernatant liquid was removed using micro pipets. Ethanol (99.5%) was added into the tube, and the tube was centrifuged, and the supernatant liquid was also removed (ethanol precipitation method). The ethanol precipitation method was repeated until the solution becomes transparently (>4 times). The ethanol in the tube was removed as much as possible. To dry the blue-colored xanthan gum, N_2 gas was gently sprayed into the tube. Subsequently, the xanthan gum was completely dried in a desiccator under a vacuum for more than 24 hours. The blue colored xanthan gum was extracted with 6-mL 80% H_2SO_4 , and dilution series for the TEP standard curves were made to determine the TEP concentrations of the natural samples. Absorption at 787 nm was measured with a Shimadzu spectrophotometer (MPS-2400) in 1-cm cuvettes with reference to Milli-Q water. The slopes of the TEP standard curves were examined with those of previously studies. The slopes in this study ranged within those of previous studies (Table 3.3). Passow and Alldredge (1995) also reported that the slopes vary according to the batch of staining solution.

For filter blanks, Passow and Alldredge (1995) reported that average absorption of

the filter blanks with 0.4- μm polycarbonate filters ranged between 0.07 and 0.09. In this study, the filter blank (average $\pm$ standard deviation) was 0.081 ± 0.003 ($n = 4$).

The natural filter samples were transferred into 20-mL vials. Six milliliters of 80% H_2SO_4 were added to the vials, and the filters soaked for 2 hours. The vials were gently agitated 5 times over this period. Absorption at 787 nm was measured with the spectrophotometer in the 1-cm cuvettes against Milli-Q water as reference. TEP levels (μg Xanthan gum equivalent L^{-1}) were determined following the equation (3.9; Passow and Alldredge, 1995).

$$\text{TEP level} = (E_{787} - C_{787}) \times \frac{1}{(V_f)} \times f_x \quad (3.9)$$

where E_{787} is the absorption at 787 nm of the sample, C_{787} is the absorption of the blank (i.e. 0.081), V_f is the filtered volume (L), and f_x is the calibration factor in micrograms estimated from the TEP standard curve.

The carbon content (TEP-C) in the TEP were estimated according to the equation (3.10; Engel and Passow, 2001).

$$\text{TEP-C} = \text{TEP level} \times 0.75 \quad (3.10)$$

Where 0.75 is a conversion factor from the TEP levels to TEP-C. Engel and Passow (2001) estimated the carbon content in the TEP using a variety of diatom strains, and showed that the carbon content the TEP ranged between 0.53 and 0.88 $\mu\text{g C}$ [μg Xanthan gum equiv.] $^{-1}$. The 0.75 is the average value of the conversion factors obtained in the study of Engel and Passow (2001).

3.3 Results

3.3.1 Hydrography

Hydrographic conditions at 5 m depth are summarized with Chl *a*, POC, POC/Chl *a* ratio and diatom abundances in Table 3.4. Although those surveys were carried out on April and June in 2010 and May in 2011, the temperature increased from April to June. The salinity (average $\pm$ standard deviation) also little changed throughout April, May and June (33.0 ± 0.14). Generally, the nutrient levels of nitrate, silicate and phosphate

did not deplete during those surveys.

3.3.2 *Phytoplankton pigments and community composition as estimated by CHEMTAX program*

Chlorophyll *a* levels decreased from April to June (Fig. 3.2). The highest concentration at 5 m depth was shown at the A1 station of April (8.8 mg m^{-3}), whereas the lowest concentration was found at the J2 station of June (0.4 mg m^{-3}) (Table 3.4). In general, the Chl *a* levels decreased with depth. The averaged percentages of the Chl *a* in the $>10 \text{ }\mu\text{m}$ fraction were relatively high in April ($98 \pm 3\%$), and lower in June ($18 \pm 12\%$).

The final ratio matrix in the 5–20 m depths estimated by the CHEMTAX program (Table 3.1B) was within the range of Mackey et al. (1996), except alloxanthin:Chl *a* ratio of the cryptophytes. The ratio (0.25) for cryptophytes was slightly higher than that of the maximum value (0.23) of Mackey et al. (1996). The final ratio matrix in the 30–50 m depths (Table 3.1D) was within the range of Mackey et al. (1996). Average compositions of each phytoplankton group to the Chl *a* biomass from 5 to 50 m depths are shown in Fig. 3.3. Diatoms dominated during April ($>90\%$) and May ($>75\%$). In contrast, contribution of diatoms to the Chl *a* biomass in June was lower than those in April and May ($<46\%$), and the other phytoplankton groups such as cryptophytes (12–37%), prasinophytes (13–26%) and prymnesiophytes (6–16%) appeared. The high Chl *a* levels and diatom compositions indicated that this study was conducted from the diatom bloom to the post-bloom phases.

3.3.3 *Cell abundances and compositions of diatoms and coccolithophores by estimated SEM*

Scanning electron microscopy (SEM) identified 59 centric diatom species, 14 pennate diatom species, and 3 coccolithophore species including a holococcolith (HOL) species (Table 3.5). The cell abundances of diatoms at 5 m depth were highest at April ($335 \pm 67 \times 10^3 \text{ cells L}^{-1}$) and lowest at June ($5 \pm 4 \times 10^3 \text{ cells L}^{-1}$), and May was highly variable ($150 \pm 115 \times 10^3 \text{ cells L}^{-1}$) (Table 3.4). Dominant species in April and May were *Thalassiosira nordenskiöldii* and *Chaetoceros* sp. 1 (Photo 3.1A) and, *Ch. atlanticus* and *Chaetoceros* sp. 6 (Photo 3.1B), respectively. *Fragilariopsis pseudonana* and *Neodenticula seminae* were predominant in June. I also found that the dominant

diatom species changed from centrics between April-May to pennates in June (see Table 3.5). The cell abundances of coccolithophores were low throughout those surveys ($0\text{--}3.6 \times 10^3$ cells L^{-1}).

The CHEMTAX analysis revealed that the Chl *a* concentrations in April were principally derived from diatoms.

3.3.4 Cell abundances of eukaryotic ultraphytoplankton and *Synechococcus* estimated by flow cytometry

The cell abundances of eukaryotic ultraphytoplankton from 5 to 300 m depths ranged between 1.1×10^2 cells mL^{-1} at 300 m depth of the M1 station and 5.2×10^4 cells mL^{-1} at 20 m depth of the J4 station (Figs. 3.4A, B). In the subsurface layer (30–75 m depths) during the May and June surveys, dramatic changes in the cell abundances occurred, but that was not found in April. The average cell abundances in April, May and June at 5 m depth were $5.3 \pm 2.2 \times 10^3$ cells mL^{-1} , $2.1 \pm 1.5 \times 10^3$ cells mL^{-1} and $2.0 \pm 1.3 \times 10^4$ cells mL^{-1} , respectively.

The cell abundances of *Synechococcus* in the 5–300 m depths ranged between 58 cells mL^{-1} at 300 m depth of the A2 station and 2.7×10^3 cells mL^{-1} at 30 m depth of the J4 station (Figs. 3.4C, D). Generally, the cell abundances of *Synechococcus* were lower than one order of magnitude in those of the eukaryotic ultraphytoplankton. Although the changes in the subsurface layer (30–100 m depths) were found at some stations in May and June, they little changed, compared to those of the eukaryotic ultraphytoplankton. The average cell abundances at 5 m depth were $9.7 \pm 6.6 \times 10^2$ cells mL^{-1} in April, $9.5 \pm 3.9 \times 10^2$ cells mL^{-1} in May and $5.2 \pm 2.9 \times 10^2$ cells mL^{-1} .

3.3.5 Bacteria abundance estimated by flow cytometry

The vertical profiles of bacterial abundances were shown in Figs. 3.4E and F. The cell abundances ranged between 5.4×10^4 cells mL^{-1} at 300 m depth of the A2 station and 5.7×10^5 cells mL^{-1} at 150 m depth in the J2 station. The vertical profile of subsurface (5–50 m depths) at the M1 station was slightly different with those in other stations. The vertical profiles of bacteria in 100–300 m depths were different between April-May and June, and the integrated cell abundances of 100–300 m depths in June ($5.7 \pm 2.7 \times 10^7$ cells cm^{-2}) were significantly higher than those in April ($1.8 \pm 0.5 \times 10^7$ cells cm^{-2}) and May ($1.8 \pm 0.3 \times 10^7$ cells cm^{-2}) (Wilcoxon rank sum test, June vs.

April-May: $p < 0.05$, $n = 9$). In contrast, the bacterial abundances at 5 m depth little changed from April to June ($4.8 \pm 0.8 \times 10^5$ cells mL⁻¹ in April, $3.8 \pm 1.9 \times 10^5$ cells mL⁻¹ in May and $3.4 \pm 0.8 \times 10^5$ cells mL⁻¹ in June).

3.3.6 POC concentration

The concentrations of POC at 5 m depth decreased from April to June, as well as the Chl *a* levels (April: 454 ± 70 µg L⁻¹; May: 203 ± 37 µg L⁻¹; June: 143 ± 8 µg L⁻¹) (Table 3.4). In contrast, the ratio of POC/Chl *a* level was increased from April to June (April: 77 ± 27 ; May: 134 ± 25 ; June: 237 ± 58).

3.3.7 DOC concentration

The concentrations of DOC generally decreased from 5 to 300 m depths (Fig. 3.5A, B). The highest concentration (64 µM) was found at 10 or 20 m depths in the J2 station and at 10 m depth in the J3 station, and the lowest concentration (45 µM) was found at 300 m depth of the M2, J3 and J4 stations. The DOC concentrations at 5 m depth were 58 ± 0.9 µM in April, 62 ± 0.9 µM in May and 62 ± 1.3 µM in June. The integrated concentrations of DOC were slightly higher in April (16.3 ± 0.4 mol m⁻²) than in May (15.0 ± 0.3 mol m⁻²) and June (15.3 ± 0.3 mol m⁻²).

3.3.8 Maximum photochemical quantum efficiency (F_v/F_m) of photosystem II for phytoplankton

The highest value (0.65 ± 0.02) of F_v/F_m was found at the A1 station in April, and the lowest value (0.27 ± 0.03) was observed at the J1 station in June (Table 3.6). The average values in April, May and June were 0.59 ± 0.07 , 0.46 ± 0.13 and 0.32 ± 0.04 , respectively, and significant differences were found between April and June, and between May and June (Steel-Dwass test, April vs. June: $p < 0.01$, $n = 18$; May vs. June: $p < 0.01$, $n = 21$).

3.3.9 POC and DOC production

Production of POC at 5 m depth was ranged between 25 mg m⁻³ d⁻¹ at the J1 station and 290 mg m⁻³ d⁻¹ at the A1 station (Table 3.6). The average productivities in April, May and April decreased from April to June (195 ± 135 mg m⁻³ d⁻¹ in April, 72 ± 41 mg m⁻³ d⁻¹ in May and 33 ± 8 mg m⁻³ d⁻¹ in June). Significant differences were found

between April and June, and between May and June, as well the F_v/F_m ratios (Steel-Dwass test, April vs. June: $p < 0.05$, $n = 12$; May vs. June: $p < 0.05$, $n = 12$).

The averaged values of DOC productivity at 5 m depth in April, May and June was $2.9 \pm 1.9 \text{ mg m}^{-3} \text{ d}^{-1}$, $3.2 \pm 1.3 \text{ mg m}^{-3} \text{ d}^{-1}$ and $1.9 \pm 0.5 \text{ mg m}^{-3} \text{ d}^{-1}$, respectively (Table 3.6). No significant differences were found between months (Steel-Dwass test, April vs. May, May vs. June and April vs. June: $p > 0.05$).

The ratios (PER) of the DOC production/total (DOC plus POC) production were estimated. The average ratios slightly increased from April to June ($2.3 \pm 2.5\%$ in April, $4.6 \pm 0.8\%$ in May and $5.5 \pm 1.5\%$ in June) (Table 3.6).

3.3.10 Phytoplankton specific absorption coefficient ($\bar{a}_{ph}^*$)

The values of phytoplankton specific absorption coefficient ($\bar{a}_{ph}^*$) at 5 m depth were shown in Table 3.7. I found that the values in June were significantly high than those in April and May (Steel-Dwass test, April vs. June: $p < 0.05$, $n = 12$; May vs. June: $p < 0.05$, $n = 14$).

3.3.11 P-E parameters and the maximum quantum yield ($\Phi_{c \max}$) of carbon fixation for photosynthesis

Photosynthesis-Irradiance (P-E) curves were drawn, and the calculated parameters for P-E curves were summarized in Table 3.7. The maximum photosynthesis rates ($P_{\max}^*$) in April, May and June were 2.7–3.3 (average 3.0), 2.2–3.6 (2.7) and 2.5–3.4 (2.9) $\text{mg C [Chl } a]^{-1} \text{ h}^{-1}$, respectively. Similarly, the values of initial slope (α^*) in April, May and June were 0.031–0.048 (0.039), 0.020–0.035 (0.026) and 0.026–0.045 (0.034) $\text{mg C [Chl } a]^{-1} \text{ h}^{-1} [\mu\text{mol photon m}^{-2} \text{ s}^{-1}]^{-1}$, respectively. The photoinhibition indexes (β^*) in the April (A2 station), May and June were 0.0010, 0.0014–0.0026 (0.0021) and 0.0007–0.0014 (0.0011) $\text{mg C [Chl } a]^{-1} \text{ h}^{-1} [\mu\text{mol photon m}^{-2} \text{ s}^{-1}]^{-1}$, respectively. The values of light saturation index (E_k) in April, May and June were 70–89 (80), 68–182 (116) and 68–128 (92) $\mu\text{mol photons m}^{-2} \text{ d}^{-1}$ in June. The all parameters of $P_{\max}^*$, α^* , β^* and E_k were not significantly different between April-May and June (Wilcoxon rank sum test, $P_{\max}^*$, α^* and E_k : $p > 0.7$, $n = 9$, Wilcoxon singled-rank test, β^* : $p = 0.34$, $n = 4$), and between May and June (Wilcoxon rank sum test, $P_{\max}^*$, α^* , β^* and E_k : $p > 0.2$, $n = 7$).

The values of maximum quantum yield of carbon fixation ($\Phi_{c \max}$) ranged from

0.024 mol C [mol photon]⁻¹ at the M3 station in May to 0.074 mol C [mol photon]⁻¹ at the A1 station in April (Table 3.7). The average values decreased from April to June (0.072 ± 0.004 mol C [mol photon]⁻¹ in April, 0.041 ± 0.019 mol C [mol photon]⁻¹ in May and 0.037 ± 0.011 mol C [mol photon]⁻¹ in June). The values of April were significantly higher than those in May, and those in June (Steel-Dwass test, April vs. May: $p < 0.05$, $n = 10$; April vs. June: $p < 0.05$, $n = 12$).

3.3.12 TEP levels

The vertical profiles of TEP levels in each month were shown in Fig. 3.6. The minimum and maximum values of the TEP levels were found at the A1 station, and those values were <10 µg Xanthan gum equiv. L⁻¹ and 171 µg Xanthan gum equiv. L⁻¹, respectively. Although the TEP levels at the J2 station were higher in the deeper layer (50–300 m) than in subsurface layer (5–40 m), the TEP levels of the subsurface layer in each month were significantly higher than in the deeper layer (Wilcoxon rank sum test, April: $p < 0.0001$, $n = 51$; May: $p < 0.0001$, $n = 101$; June: $p < 0.0001$, $n = 144$). The averaged TEP levels at 5 m depth decreased from April to June (109 ± 21 µg Xanthan gum equiv. L⁻¹ in April, 80 ± 48 µg Xanthan gum equiv. L⁻¹ in May and 57 ± 18 µg Xanthan gum equiv. L⁻¹), and the TEP levels in April were significantly higher than those in June (Table 3.8) (Steel-Dwass test, $p < 0.01$, $n = 18$). I tested the relationships between TEP levels and other parameters at 5 m depth. As a result, the TEP levels were correlated with the parameters of the Chl *a*, POC/Chl *a*, bacteria abundance and POC production (Table 3.9). The average levels of the integrated TEP values from 5 and 300 m depths in April, May and June were 9.7 ± 0.2 g Xanthan gum equiv. m⁻², 14.4 ± 0.3 g Xanthan gum equiv. m⁻² and 12.0 ± 2.5 g Xanthan gum equiv. m⁻², respectively (Table 3.8).

Interestingly, the ratios of TEP/Chl *a* (hereafter TEP*) at 5 m depth significantly increased from April to June (Table 3.8; Steel-Dwass test, April vs. May: $p < 0.05$, $n = 15$; May vs. June: $p < 0.01$, $n = 21$). In contrast, the ratios of the TEP/POC in April were significantly lower than those in June (Steel-Dwass test, $p < 0.05$, $n = 18$). Similarly, the ratios of the TEP-carbon content (TEP-C)/POC were also significantly lower in April than in June (Steel-Dwass test, $p < 0.05$, $n = 18$). The average values of the TEP/POC and TEP-C/POC ratios during this study were 36 ± 12% and 27 ± 9%, respectively. I also investigated the relationships between TEP* levels and other parameters at 5 m

depth, except the Chl *a*-related parameters. As a result, the TEP* levels correlated with the parameters of the water temperature, salinity, POC, DOC, diatom abundances and F_v/F_m (Table 3.9).

3.4 Discussion

3.4.1 Comparisons of TEP levels between the Oyashio region and other oceans

To compare the TEP levels (units: μg Xanthan gum equivalent L^{-1}) obtained in this study, I summarized the values of the TEP range and maximum level *in situ* in the world's oceans including estuary, polar and hydrothermal plume regions from 1995 to early 2013 (Fig. 3.7, Table. 3.10). The comparisons revealed that the TEP levels (<10–171; No. 60 in the Table 3.10) in the spring diatom bloom of the Oyashio region were relatively low. On the other hand, my results were the first report on oceanic TEP levels off Japan, because the TEP studies in Japan (No. 56–59 in the Table 3.10) were only carried out in the bays (Isahaya Bay, Sagami Bay, Tokyo Bay and Otsuchi Bay; No. 56–59 in the Table 3.10). The Western Subarctic Gyre (WSG) in the upstream region of the Oyashio region has been recognized as a high-nitrate, low-chlorophyll (HNLC) waters. The HNLC phenomenon in the WSG is mainly attributed to low iron availability and zooplankton grazing (Tsuda et al. 2003; 2007). In the HNLC region, the *in situ* experiment of the iron fertilization called Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study I (SEEDS-I) was carried out in summer, 2001. TEP observations during SEEDS-I were conducted in both inside the iron-patch (Fe-in) and outside the iron-patch (Fe-out) (Ramaiah et al., 2005). The TEP levels in Fe-in increased with Chl *a* concentrations (ca. from 40 to 190 μg Xanthan gum equiv. L^{-1} , No. 62 in the Table 3.10), whereas those in Fe-out almost little changed (40–60 μg Xanthan gum equiv. L^{-1} , No. 61 in the Table 3.10). The TEP levels in the Fe-in were similar to TEP levels in this study, and *Chaetoceros debilis* during the massive bloom in the Fe-in of SEEDS-I dominated (Tsuda et al., 2003). Ramaiah et al. (2005) discussed that *Ch. debilis* dominated during the SEEDS-I might be a low TEP producer, because the TEP levels of >1,000 μg Xanthan gum equiv. L^{-1} were reported in the diatom blooms (Passow, 2002b). Radić et al. (2005) also reported the high TEP levels of >2,000 μg Xanthan gum equiv. L^{-1} when the three diatoms of *Chaetoceros* sp., *Skeletonema costatum* and *Pseudonitzschia delicatissima* dominated. Although the centric diatoms of *Chaetoceros* sp. 1 and *Thalassiosira nordenskioeldii* dominated in April when the

diatom bloom occurred in this study (Table 3.5), the TEP levels were clearly lower than those observed previously. The results suggest that these diatom species did not produce high amount of TEP or its precursors.

The most intensive observations were conducted in the Santa Barbara Channel (No. 7–14 in Table 3.10), where Dr. Uta Passow (Marine Science Institute, University of California, Santa Barbara) energetically investigated. Unfortunately, the cross-sectional observation of TEP in the Pacific or Atlantic has not been yet reported, and a lot of surveys were carried out at near shore waters (Fig. 3.7). The highest level of 14,800 μg Xanthan gum equiv. L^{-1} was observed in the Adriatic Sea of the Mediterranean Sea (No. 39 in Table 3.10), and such massive TEP was taken to a photograph (Photo 3.2; Kaiser et al., 2011). As the reason for the highest TEP level, the authors noted that a shallow basin under direct influence of Po River nutrient inputs contributed (Radić et al., 2005). In contrast, zero (No. 1 in Table 3.10) or the undetectable TEP levels (No. 19, 21–23 and 34 in the Table 3.10) were reported in the Antarctic regions and also Mediterranean Sea. Generally, the high TEP levels of $>1,000 \mu\text{g}$ Xanthan gum equiv. L^{-1} were found in the semi-closed regions such as estuary, bay and sea ice regions. A few studies also have conducted within the hydrothermal plume area of the deep ocean (No. 15 in the Table 3.10: Prieto and Cowen, 2007; Shackelford and Cowen, 2006 for TEP abundances). Interestingly, high TEP levels (up to 6,451 μg Xanthan gum equiv. L^{-1}) were observed in the hydrothermal plume areas of the deep ocean, and it has been discussed that bacteria in the hydrothermal plume area were associated with the high TEP levels. Then, the TEP levels can increase not only the euphotic zone but also the deep ocean where close to the hydrothermal plume vent.

3.4.2 TEP level in the Oyashio region during the spring diatom blooms

Generally, the high TEP levels are known to associate with phytoplankton blooms, such as diatoms (e.g., Passow et al., 1995b), *phaeocystis* spp. (e.g., Hong et al., 1997), dinoflagellates (e.g., Berman and Viner-Mozzini, 2001), cryptomonads (e.g., Passow et al., 1995) and cyanobacteria (e.g., Grossart and Simon, 1997). In this study, the high TEP levels at 5 m depth were observed in April and May rather than in June (Table 3.8), and diatoms particularly dominated in April and May (Table 3.4 and Fig. 3.3). Even if bacterial abundances in this study were well correlated positively with the TEP levels (Table 3.9), it is suggested that the organic matters produced by the phytoplankton

changed bacterial abundance and/or composition as reported by Mari and Kiørboe (1996) and Tada et al. (2011). Bacteria are also known not only to attach with TEP, to decompose and consume the TEP (e.g., Passow et al., 2001), and to increase the growth rate (Smith et al., 1995), but also to form the TEP from the TEP precursors (e.g., Yamada et al., 2013). These previous results support the significant relationship between bacterial abundances and TEP levels observed in this study. However, the interactions between TEP and bacteria are presumably complex due to the combinations of various factors as reviewed by Passow (2002b).

On the other hand, phytoplankton clearly contributed to TEP levels at 5 m depth, because a significant relationship was found between the TEP and Chl *a* concentrations (Table. 3.9), and similar results have also been reported previously (e.g., Passow et al., 1995; Ramaiah and Furuya, 2002; Wurl et al., 2011). The positive relationship also suggested that, in the Oyashio region, the TEP levels would be the highest during the spring diatom booms throughout the year. Subsequently, to estimate the TEP levels within the surface mixed layer, I examined the relationship between TEP and Chl *a* concentrations within the mixed layer. As a result, the significant relationship was found in Fig. 3.8. Also, the below equation (eq. 3.11) was proposed to estimate the TEP level ($TEP_{\text{mixed layer}}$) from the Chl *a* concentration within the surface mixed layer in the Oyashio region during the spring blooms.

$$TEP_{\text{mixed layer}} = 61 \times Chl\ a^{0.32} \quad (3.11)$$

Where, *Chl a* is the Chl *a* concentration within the mixed layer. This equation can be used during the spring bloom in the Oyashio region from April to June, provided that the Chl *a* concentration ranged from 0.4 to 8.8 $\mu\text{g L}^{-1}$. In the review on TEP by Passow (2002b), the relationship between TEP and Chl *a* concentrations was also shown with the power function of Eq. (3.11), and the slope and intercept values were 3.63 and 1 in the Ross Sea (Hong et al., 1997), 0.45 and 176 in the East Sound (Kiørboe, 1996; Passow, 2002a), 0.33 and 282 in the Baltic Sea (Engel, 1998) and 0.46–1.06 and 106–221 in the laboratory experiments using diatom strains (Passow, 2002a), respectively. In this study, those values within the mixed layer were 0.32 and 60 (see Eq. 3.11), respectively. Assumed that all TEP production was derived from phytoplankton, the TEP productivity by the Oyashio diatoms in particularly April was relatively low (see

Fig. 3.9), compared with other regions reported by Passow (2002b).

3.4.3 Contribution of TEP to biological carbon pump in the Oyashio region during the spring blooms

The TEP-carbon contents (TEP-C) at 5 m depth were estimated assuming the conversion factor of 0.75 from TEP level (μg Xanthan gum equivalent L^{-1}) to TEP-C ($\mu\text{g C L}^{-1}$) (Engel and Passow, 2001). The TEP-C/POC ratios in this study were shown in the Table 3.8, and the average value was $27 \pm 9\%$. I summarized the average values of the TEP-C/POC ratio obtained from various regions (Table 3.11). Surprisingly, the ratios ranged from 1–103%. For the reason of the $>100\%$ (No. 4, 24 and 25 in the Table 3.11), the difference ($0.4 \mu\text{m}$ for TEP and ca. $0.8 \mu\text{m}$ for POC) of the pore size between the TEP and POC filters could cause some errors (Beauvais et al., 2003; Bar-Zeev et al., 2011). The ratios roughly obtained from the sea surface of the world's oceans (No. 1–3, 5, 7, 9 and 25 in the Table 3.11) were averaged for the comparison with my data, and the value was $33 \pm 20\%$. The percentage of TEP-C to the POC pool in the sea surface little differed between the Oyashio region during spring diatom bloom and the other regions. Nevertheless, TEP have a high impact on the POC pool in the surface (e.g., Engel and Passow, 2001; Engel et al., 2002). In addition, because the ratio was reported to increase from the surface to deeper layer (No. 10–13 and No. 23–25 in the Table 3.11; Ramaiah et al., 2005; Bar-Zeev et al., 2011), the TEP itself must be also important for the biological carbon pump. On the other hand, an evaluation of the stickiness of the TEP was not carried out in this study. The stickiness of the TEP excreted from the diatoms was showed to vary between species (e.g., Kiørboe and Hansen, 1993). If the stickiness of the TEP during the Oyashio spring diatom blooms was higher than those in the other regions, the synergistic effect of the high abundance of the large-diatom and the high stickiness of the TEP would increase the efficiency of the biological carbon pump.

3.4.4 TEP production during the spring diatom blooms in the Oyashio region

Baines and Pace (1991) reviewed that the ratio of DOC production to total production (PER) obtained from 16 studies including lacustrine, marine and estuarine were average ca. 13%. In the eutrophic regions, the PER values were found on average 5% in Hakata Bay, Kyusyhu, Japan (Hama and Yanagi, 2001) and 19% in Riá de Bigo

(42°14'N, 8° 47'W), Spain (Marañón et al., 2004). In contrast, the higher values were reported in the oligotrophic regions such as the Mediterranean Sea (32% reported by Fernández et al., 1994; up to 45% reported by Alonso-Sáez et al., 2008; average 37% reported by Lopez-Sandval et al., 2011) and Sargasso Sea (44% reported by Thomas, 1971). The increment of the surface to volume ratio of phytoplankton may increase the PER value, because the increment can facilitate the passive diffusion of the biological materials from the inside to outside the cell (Bjørnsen, 1988; Kiørboe, 1993). In this study, the fraction of the large-sized phytoplankton (>10 µm) obtained from Chl *a* concentration at 5 m depth was clearly high in April (average $98 \pm 3\%$), and in contrast, that of June became relatively low (average $18 \pm 12\%$). Although the statistical analysis was incapable due to the small sample size, this difference might contribute to the PER in this study (Table 3.6), and eventually, the PER might influence the formation of TEP.

Previous studies reported that TEP are mainly formed from dissolved acid polysaccharides excreted from phytoplankton and/or bacteria (e.g., Alldredge et al., 1993; Passow et al., 1994; Passow, 2000). This study focused on the relationship between the TEP levels in the seawater and the DOC production by phytoplankton, because the TEP production of bacteria was presumably complex, and did not investigate in detail. Unexpectedly, it was not found that the TEP level was significantly correlated with the DOC production (Table 3.9). However, the relationship between TEP and Chl *a* concentrations indicate that the TEP level correlated with phytoplankton. Interestingly, the TEP* values significantly increased from April to June (Table 3.8). Assuming that all TEP production derived phytoplankton, algal cellular TEP production might increase toward the decline of the diatom blooms. This also suggests that the TEP production by the diatoms was relatively low, compared with the other phytoplankton groups appeared in the post-bloom. In fact, the TEP* levels were significantly negative correlated with the diatom abundances (Table 3.9). I also tested the relationships between TEP* and other parameters, and found that TEP* was significantly correlated with the temperature, salinity, POC, diatom cell abundances F_v/F_m (Table 3.9). However, TEP* levels were not significantly correlated with DOC production (Table 3.9). The TEP or its precursors produced by phytoplankton in the Oyashio region during the spring bloom could be significantly affected by the formation, decomposition, and consumption of bacteria (e.g., Engel et al. 2004b; Wurl et al., 2011; Yamada et al., 2013), and/or those of zooplankton (e.g., Shimeta, 1993; Ling and

Allredge, 2003).

Table 3.1 Final accessory pigment:chlorophyll *a* ratio matrices obtained by CHEMTAX: (A) Initial ratio matrix in the 5–20 m depths; (B) Final ratio matrix obtained by CHEMTAX in the 5–20 m depths; (C) Initial ratio matrix in the 30–50 m depths; (D) Final ratio matrix obtained by CHEMTAX in the 30–50 m depths.

	Fucox	19'-But	19'-Hex	Peri	Diadinox	Allox	Violax	Prasinox	Chl <i>b</i>	Zeax	Chl <i>a</i>
(A)											
Diatoms	0.43	0	0	0	0.06	0	0	0	0	0	1
Prymne	0	0	0.73	0	0.14	0	0	0	0	0	1
Pelago	0.56	0.72	0	0	0.34	0	0	0	0	0	1
Chloro	0	0	0	0	0	0	0.015	0	0.38	0.049	1
Prasino	0	0	0	0	0	0	0.08	0.37	0.97	0	1
Crypto	0	0	0	0	0	0.25	0	0	0	0	1
Dino	0	0	0	0.59	0	0	0	0	0	0	1
Cyano	0	0	0	0	0	0	0	0	0	0.33	1
(B)											
Diatoms	0.43	0	0	0	0.06	0	0	0	0	0	1
Prymne	0	0	0.72	0	0.14	0	0	0	0	0	1
Pelago	0.56	0.71	0	0	0.34	0	0	0	0	0	1
Chloro	0	0	0	0	0	0	0.015	0	0.39	0.049	1
Prasino	0	0	0	0	0	0	0.17	0.35	0.91	0	1
Crypto	0	0	0	0	0	0.25	0	0	0	0	1
Dino	0	0	0	0.59	0	0	0	0	0	0	1
Cyano	0	0	0	0	0	0	0	0	0	0.33	1
(C)											
Diatoms	0.53	0	0	0	0.03	0	0	0	0	0	1
Prymne	0	0	0.73	0	0.14	0	0	0	0	0	1
Pelago	0.56	0.78	0	0	0.24	0	0	0	0	0	1
Chloro	0	0	0	0	0	0	0.035	0	0.37	0.042	1
Prasino	0	0	0	0	0	0	0.06	0.30	0.90	0	1
Crypto	0	0	0	0	0	0.13	0	0	0	0	1
Dino	0	0	0	0.70	0	0	0	0	0	0	1
Cyano	0	0	0	0	0	0	0	0	0	0.33	1
(D)											
Diatoms	0.53	0	0	0	0.03	0	0	0	0	0	1
Prymne	0	0	0.73	0	0.14	0	0	0	0	0	1
Pelago	0.56	0.79	0	0	0.24	0	0	0	0	0	1
Chloro	0	0	0	0	0	0	0.035	0	0.37	0.042	1
Prasino	0	0	0	0	0	0	0.07	0.30	0.84	0	1
Crypto	0	0	0	0	0	0.13	0	0	0	0	1
Dino	0	0	0	0.70	0	0	0	0	0	0	1
Cyano	0	0	0	0	0	0	0	0	0	0.33	1

Abbreviations: Prymne, prymnesiophytes; Pelago, pelagophytes; Chloro, chlorophytes; Prasino, prasinophytes; Crypto, cryptophytes; Dino, dinoflagellates; Cyano, cyanobacteria; Fucox, fucoxanthin; 19'-But, 19'-butanoyloxyfucoxanthin; 19'-Hex, 19'-hexanoyloxyfucoxanthin; Peri, peridinin; Diadinox, diadinoxanthin; Allox, alloxanthin; Violax, violaxanthin; Prasinox, prasinoxanthin; Chl *b*, chlorophyll *b*; Zeax, zeaxanthin; Chl *a*, chlorophyll *a*.

Table 3.2 Conductivity and DOC concentrations at before (original) and after (desalted) of the desalination. The parentheses show the percentages between the before and after. The conductivity decreased to ca. 6% of the initial conductivity, whereas the recovery percentages of DOC concentration ranged from 62 to 96%.

Sample No.	Conductivity (mS cm ⁻¹)		DOC concentration (µg C L ⁻¹)	
	Original	Desalted	Original	Desalted
1	53.3 (100)	3.0 (5.6)	704 (100)	438 (62.2)
2	53.1 (100)	3.0 (5.6)	704 (100)	447 (63.5)
3	53.3 (100)	3.0 (5.6)	688 (100)	506 (73.6)
4	52.8 (100)	3.0 (5.7)	715 (100)	687 (96.2)

Table 3.3 Comparisons of the TEP standard curves between this study and previous studies. The slopes (calibration factor) were shown for an inverse number (f^{-1}) of the regressions of Alcian blue absorbance vs. xanthan gum level. The slopes in this study ranged within those of previous studies. It is reported that the slopes vary according to the batch of staining solution (Passow and Alldredge, 1995).

Reference	Slope (calibration factor)	r^2	Batch of Alcian blue stain
This study	120	0.99	Previous experiment
This study	120	0.99	Previous experiment
This study	124	0.99	April and June, 2010 cruises
This study	129	0.99	April and June, 2010 cruises
This study	105	0.99	May, 2011 cruise
This study	108	0.99	May, 2011 cruise
Passow and Alldredge (1995)	88	0.99*	
Passow and Alldredge (1995)	139	0.98*	
Claquin et al. (2008)	100	0.97	
Claquin et al. (2008)	111	0.99	

*Correlation coefficient

Table 3.4 Hydrographic conditions, Chl *a*, POC, POC/Chl *a* ratio and diatom abundances. They were shown in order to the Chl *a* concentrations, that is alignment sequence of April, 2010, May, 2011 and June, 2010 cruises.

Cruise	Station	Temperature (°C)	Salinity	Nitrate (µM)	Silicate (µM)	Phosphate (µM)	Chl <i>a</i> (µg L ⁻¹)	POC (µg L ⁻¹)	POC / Chl <i>a</i> ratio	Diatom abundances (×10 ³ cells L ⁻¹)
April, 2010	A1	3.1	32.9	8	20	0.9	8.8	503	57	382
April, 2010	A2	4.5	33.3	11	24	1.0	4.2	404	96	288
May, 2011	M1	5.0	33.1	13	11	1.1	1.0	163	155	23
May, 2011	M2	4.3	33.0	9	6	0.7	2.0	213	106	247
May, 2011	M3	4.4	32.9	7	2	0.6	1.7	235	142	320
June, 2010	J1	7.0	32.9	8	8	1.0	0.6	143	233	11
June, 2010	J2	7.0	32.9	8	8	1.0	0.4	137	319	4
June, 2010	J3	9.0	32.8	4	9	0.8	0.7	139	191	1
June, 2010	J4	7.8	32.9	8	13	1.0	0.8	154	204	3

Table 3.5 List of the phytoplankton species identified. Genus and species names are arranged alphabetically, not systematically. Dominant species in the April, May and June showed as red, purple and blue colors, respectively.

Centric diatoms	Pennate diatoms	Coccolithophores
<i>Actinocyclus curvatulus</i>	<i>Fragilariopsis atlantica</i>	<i>Coccolithus pelagicus</i> ssp. <i>Pelagicus</i>
<i>Actinocyclus</i> sp. 1	<i>Fragilariopsis cylindriciformis</i>	<i>Emiliana huxleyi</i>
<i>Actinocyclus</i> sp. 2	<i>Fragilariopsis cylindrus</i>	
<i>Asteromphalus hyalinus</i>	<i>Fragilariopsis oceanica</i>	<i>Coccolithus pelagicus</i> ssp. <i>Pelagicus</i> HOL*
<i>Chaetoceros atlanticus</i>	<i>Fragilariopsis pseudonana</i>	
<i>Chaetoceros concavicornis</i>	<i>Fragilariopsis</i> sp. 1	
<i>Chaetoceros convolutus</i>	<i>Fragilariopsis</i> sp. 2	
<i>Chaetoceros decipience</i>	<i>Navicula directa</i>	
<i>Chaetoceros debilis</i>	<i>Navicula</i> sp. 1	
<i>Chaetoceros diadema</i>	<i>Neodenticula seminae</i>	
<i>Chaetoceros neglectus</i>	<i>Nitzschia</i> sp. 1	
<i>Chaetoceros pseudocurvisetus</i>	<i>Pseudonitzschia</i> sp. 1	
<i>Chaetoceros radicans</i>	<i>Thalassionema nitzschioides</i>	
<i>Chaetoceros</i> sp. 1	<i>Thalassiothrix longissima</i>	
<i>Chaetoceros</i> sp. 2		
<i>Chaetoceros</i> sp. 3		
<i>Chaetoceros</i> sp. 4		
<i>Chaetoceros</i> sp. 5		
<i>Chaetoceros</i> sp. 6		
<i>Chaetoceros</i> sp. 7		
<i>Corethron criophilum</i>		
<i>Corethron inerme</i>		
<i>Coscinodiscus</i> sp. 1		
<i>Odontella aurita</i>		
<i>Rhizosolenia</i> sp. 1		
<i>Rhizosolenia</i> sp. 2		
<i>Rhizosolenia</i> sp. 3		
<i>Rhizosolenia</i> sp. 4		
<i>Stephanopyxis turris</i>		
<i>Thalassiosira allenii</i>		
<i>Thalassiosira angulata</i>		
<i>Thalassiosira eccentrica</i>		
<i>Thalassiosira gracilis</i>		
<i>Thalassiosira hyalina</i>		
<i>Thalassiosira lineata</i>		
<i>Thalassiosira lineoides</i>		
<i>Thalassiosira mala</i>		
<i>Thalassiosira nordenskiöldii</i>		
<i>Thalassiosira oceanica</i>		
<i>Thalassiosira oestrupii</i>		
<i>Thalassiosira pacifica</i>		
<i>Thalassiosira leptopus</i>		
<i>Thalassiosira trifulta</i>		
<i>Thalassiosira</i> sp. 1		
<i>Thalassiosira</i> sp. 2		
<i>Thalassiosira</i> sp. 3		
<i>Thalassiosira</i> sp. 4		
<i>Thalassiosira</i> sp. 5		
<i>Thalassiosira</i> sp. 6		
<i>Thalassiosira</i> sp. 7		
<i>Thalassiosira</i> sp. 8		
<i>Thalassiosira</i> sp. 9		
<i>Thalassiosira</i> sp. 10		
<i>Thalassiosira</i> sp. 11		
<i>Thalassiosira</i> sp. 12		
<i>Thalassiosira</i> sp. 13		
<i>Thalassiosira</i> sp. 14		
<i>Thalassiosira</i> sp. 15		
<i>Thalassiosira</i> sp. 16		

* Abbreviation: HOL, holococcolith

Table 3.6 Maximum photochemical quantum efficiency (F_v/F_m) of photosystem II for phytoplankton, POC production and DOC production. PER was the percentage of DOC production/(DOC plus POC production). They were shown in order to the Chl *a* concentrations, that is alignment sequence of April, 2010, May, 2011 and June, 2010 cruises.

Cruise	Station	$F_v/F_m \pm \text{S.D.}$	POC production $\pm \text{S.D.}$ ($\text{mg m}^{-3} \text{ d}^{-1}$)	DOC production $\pm \text{S.D.}$ ($\text{mg m}^{-3} \text{ d}^{-1}$)	PER (%)
April, 2010	A1	0.65 ± 0.02	290 ± 17	1.6 ± 0.04	0.5
April, 2010	A2	0.53 ± 0.02	100 ± 1	4.2 ± 0.1	4.1
May, 2011	M1	0.41 ± 0.03	43 ± 0.4	2.3 ± 0.9	5.1
May, 2011	M2	0.63 ± 0.03	100 ± 5	4.2 ± 0.4	4.0
May, 2011	M3	0.36 ± 0.03			
June, 2010	J1	0.27 ± 0.03	25 ± 1	1.9 ± 0.02	7.1
June, 2010	J2	0.37 ± 0.03	28 ± 0.3	1.7 ± 0.4	5.8
June, 2010	J3	0.34 ± 0.01	43 ± 2	2.6 ± 0.2	5.7
June, 2010	J4	0.30 ± 0.01	36 ± 1	1.3 ± 0.02	3.5

Table 3.7 Summary of phytoplankton specific absorption coefficient ($\bar{a}_{\text{ph}}^*$) (m^2 [mg Chl a] $^{-1}$), the maximum photosynthesis rate of P - E curve (P_{max}^*) (mg C [Chl a] $^{-1}$ h^{-1}), the initial slope (α^*) (mg C [Chl a] $^{-1}$ h^{-1} [$\mu\text{mol photon m}^{-2} \text{s}^{-1}$] $^{-1}$), the photoinhibition index (β^*) (mg C [Chl a] $^{-1}$ h^{-1} [$\mu\text{mol photon m}^{-2} \text{s}^{-1}$] $^{-1}$), the light saturation index (E_k) ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$), the coefficient of determination for the P - E fitting curve (r^2) and the maximum quantum yield of carbon fixation ($\Phi_{\text{c max}}$) (mol C [mol photon] $^{-1}$) at 5 m depth. They were shown in order to the Chl a concentrations, that is alignment sequence of April, 2010, May, 2011 and June, 2010 cruises.

Cruise	Station	$\bar{a}_{\text{ph}}^* \pm \text{S.D.}$	P_{max}^*	α^*	β^*	E_k	r^2	$\Phi_{\text{c max}} \pm \text{S.D.}$
April, 2010	A1	0.010 ± 0.0001	2.7	0.031		89	0.99	0.074 ± 0.0040
April, 2010	A2	0.016 ± 0.0003	3.3	0.048	0.0010	70	0.99	0.069 ± 0.0011
May, 2011	M1	0.013 ± 0.0015	2.2	0.022	0.0014	99	0.98	0.038 ± 0.0030
May, 2011	M2	0.013 ± 0.0001	2.4	0.035	0.0023	68	0.90	0.061 ± 0.0004
May, 2011	M3	0.019 ± 0.0011	3.6	0.020	0.0026	182	0.99	0.024 ± 0.0009
June, 2010	J1	0.019 ± 0.0001	3.4	0.026	0.0011	128	0.97	0.030 ± 0.0002
June, 2010	J2	0.026 ± 0.0006	3.1	0.045	0.0014	68	0.92	0.040 ± 0.0009
June, 2010	J3	0.017 ± 0.0008	2.9	0.037	0.0013	76	0.89	0.051 ± 0.0025
June, 2010	J4	0.021 ± 0.0008	2.5	0.026	0.0007	96	0.91	0.028 ± 0.0010

Table 3.8 The levels of TEP, and the ratios of TEP/Chl *a*, TEP/POC and TEP-C/POC at 5 m depth, and the integrated levels from 5 to 300 m depths. They were shown in order to the Chl *a* concentrations, that is alignment sequence of April, 2010, May, 2011 and June, 2010 cruises.

Cruise	Station	5 m depth				5 - 300 m integrated
		TEP level $\pm$ S.D. (μg Xanthan gum equiv. L^{-1})	TEP / Chl <i>a</i> ratio $\pm$ S.D.	TEP / POC ratio $\pm$ S.D.	TEP-C / POC ratio $\pm$ S.D.	TEP level (g Xanthan gum equiv. m^{-2})
April, 2010	A1	110 $\pm$ 22	13 $\pm$ 3	0.22 $\pm$ 0.04	0.16 $\pm$ 0.03	9.9
April, 2010	A2	109 $\pm$ 26	26 $\pm$ 6	0.27 $\pm$ 0.06	0.20 $\pm$ 0.05	9.5
May, 2011	M1	33 $\pm$ 6	32 $\pm$ 5	0.21 $\pm$ 0.03	0.15 $\pm$ 0.03	14.1
May, 2011	M2	121 $\pm$ 34	59 $\pm$ 16	0.57 $\pm$ 0.16	0.43 $\pm$ 0.12	14.7
May, 2011	M3	87 $\pm$ 48	52 $\pm$ 29	0.37 $\pm$ 0.20	0.28 $\pm$ 0.15	14.5
June, 2010	J1	52 $\pm$ 5	84 $\pm$ 9	0.36 $\pm$ 0.20	0.27 $\pm$ 0.03	9.6
June, 2010	J2	34 $\pm$ 15	80 $\pm$ 36	0.25 $\pm$ 0.11	0.19 $\pm$ 0.08	15.3
June, 2010	J3	71 $\pm$ 12	98 $\pm$ 16	0.51 $\pm$ 0.09	0.38 $\pm$ 0.06	10.8
June, 2010	J4	69 $\pm$ 11	92 $\pm$ 14	0.45 $\pm$ 0.07	0.34 $\pm$ 0.05	12.4

Table 3.9 Relationships between TEP and other parameters, and between TEP* and other parameters. A significant relationship showed by boldface.

Parameters	TEP			TEP*		
	ρ	p	n	ρ	p	n
Temperature	-0.65	0.07	9	0.83	0.01	9
Salinity	0.35	0.36	9	-0.80	0.01	9
Nitrate	-0.05	0.91	9	-0.42	0.27	9
Silicate	0.05	0.91	9	-0.33	0.39	9
Phosphate	-0.55	0.13	9	-0.15	0.71	9
Chl <i>a</i>	0.77	0.02	9	-	-	-
POC	0.68	0.05	9	-0.83	0.01	9
POC/Chl <i>a</i>	-0.78	0.02	9	-	-	-
DOC	-0.11	0.78	9	0.85	0.003	9
CHEMRAX Diatom%	0.57	0.12	9	-	-	-
Diatom derived-Chl <i>a</i>	0.68	0.05	9	-	-	-
Diatom cell abundance	0.58	0.11	9	-0.73	0.03	9
Eukaryotic ultraphytoplankton	-0.30	0.44	9	0.58	0.11	9
<i>Synechococcus</i> abundance	-0.17	0.68	9	-0.53	0.15	9
Bacteria abundances	0.98	0.0004	9	-0.26	0.54	9
F_v/F_m	0.57	0.12	9	-0.80	0.01	9
POC production	0.75	0.03	8	-0.69	0.06	8
DOC production	0.34	0.42	8	-0.14	0.73	8
PER	-0.62	0.12	8	0.45	0.27	8
$\bar{a}^*_{ph}$	-0.54	0.14	9	-	-	-
P^*_{max}	0.03	0.95	9	-	-	-
α^*	0.26	0.50	9	-	-	-
β^*	0.13	0.76	8	-	-	-
E_k	-0.37	0.34	9	-	-	-
$\Phi_{c\ max}$	0.55	0.13	9	-	-	-

– Blank page –

Table 3.10 Summary of the TEP surveys from 1995 to early 2013. This summary was only listed the TEP levels reported for the photometric (i.e., unit: Xanthan equivalent).

No.	Sampling area	Latitude, Longitude	Sampling Month, year	Sampling depth	TEP level (μg Xanthan gum equiv. L^{-1})		Reference
					Range	Average $\pm$ S.D. (Maximum)	
1	Ross Sea, Antarctic	76°30'S, 175°20'W - 73°30'S, 168°30'E	November - December, 1994	Surface	0 - 2800	308	Hong et al. (1997)
2	Station ALOHA (off Hawaii), USA	22°45'N, 158°00'W	December, 1999	within mixed layer	86 - 468	-	Prieto et al. (2006, unpub.), Passow (2002b)
3	Station ALOHA (off Hawaii), USA	22°45'N, 158°00'W	December, 1999	below mixed layer	63 - 477	-	Prieto et al. (2006, unpub.), Passow (2002b)
4	East Sound, USA	48°40'N, 122°54'W	April, 1994	< 20 m		83 (159)	Kiorboe et al. (1996), Passow (2002b)
5	Monterey Bay, USA	36°50'N, 121°55'W	July, 1993	1 - 12 m	50 - 310		Passow and Alldredge (1995b)
6	Monterey Bay, USA	36°50'N, 121°55'W	July, 1993	10 - 50 m	46 - 63	59 $\pm$ 12	Passow and Alldredge (1995b)
7	Santa Barbara Channel, USA	34°N, 120°W	1 year, 1994 - 1995	< 20 m		89 (461)	Passow (2002b)
8	Santa Barbara Channel, USA	34°N, 120°W	2 years, 1995 - 1997	< 20 m		213 (1042)	Passow et al. (2001), Passow (2002b)
9	Santa Barbara Channel, USA	34°N, 120°W	June - July, 1993	1 - 10 m	85 - 252	147 $\pm$ 63	Passow and Alldredge (1995b)
10	Santa Barbara Channel, USA	34°N, 120°W	June - July, 1993	50 - 500 m	14 - 44	24 $\pm$ 8	Passow and Alldredge (1995b)
11	Santa Barbara Channel, USA	34°N, 120°W	January - February, 1994	0 - 75 m	29 - 68	47 $\pm$ 12	Passow and Alldredge (1995b)
12	Santa Barbara Channel, USA	34°20'N, 119°50'W	April & May, 1997	< 20 m		207 $\pm$ 61 (290)	Azetsu-Scott (2004)
13	Santa Barbara Channel, USA		Spring, 1997	< 75 m		183	Dunne et al. (2003; pers. com.), Passow (2002b)
14	Santa Barbara Channel, USA		June, 1995	10 m		72 (74)	Passow (2000), Passow (2002b)
15	Guaymas Basin, USA	around 27°01'N, 111°24'W	April - May, 2002	Deep (1515 - 2012 m)	8 - 6451		Prieto and Cowen (2007)
16	Neuse River estuary, USA	35°04'N, 76°33'W - 35°08'N, 77°03'W	May, 2007 - April, 2008		850 - ca. 3500		Wetzel et al. (2009)
17	Chesapeake Bay, USA	around 39°20'N, 76°30'W	January - October, 2007 & 2008	< ca. 23 m	37 - 2820		Malpezzi et al. (2013)
18	Delaware Bay, USA	39°00'N, 75°08'W	Spring	-	653 - 1034		B. Logan & D. Kirchmann, pers. com., Passow (2002b)
19	Bellingshausen Sea, Antarctic	around 66°S, 70°W and 65°S, 66°W	February, 2005	5 - 100 m	Detection limit - 34	14 $\pm$ 10	Ortega-Retuerta et al. (2009)
20	near Anvers Island, Antarctic	64°46'S, 64°04'W	November, 1994 - February, 1995	2 - 6 m	15 - ca. 500	207	Passow et al. (1995), Passow (2002b)
21	Bransfield Strait, Antarctic	around 63°S, 60°W	February, 2005	5 - 150 m	Detection limit - 36	16 $\pm$ 9	Ortega-Retuerta et al. (2009)
22	Bransfield Strait, Antarctic	around 63°S, 59°W	December, 1999 - January, 2000	5 - 100 m	Detection limit - 346	57	Corzo et al. (2005)
23	Northwest Weddell Sea (Water), Antarctic	around 64°S, 57°W	February, 2005	5 - 200 m	Detection limit - 49	16 $\pm$ 13	Ortega-Retuerta et al. (2009)
24	Northwest Weddell Sea (Ice core), Antarctic	around 68°S, 55°W	November, 2004 - January, 2005	Ice core (0 - 0.90 m)	3 - 3071		Dumont et al. (2009)
25	Estuarine lagoon of Canaëia-Iguape, Brazil	25°03'S, 47°55'W & 24°40'S, 47°26'W	July, 2001 & January, 2002	-	13 - 1199	(124)	Barreira-Alba et al. (2012)
26	North-South transect of Northeast Atlantic	ca. 59°N, 20°W - ca. 43°N, 20°W	June & July, 1996	10 - 70 m		53	Engel (2004)
27	North-South transect of Northeast Atlantic	40°N, 20°W - 60°N, 20°W	June & July, 1996	Surface	30 - 300	29 $\pm$ 10	Engel et al. (1997), Passow (2002b)
28	Northeast Atlantic	around 47°N, 20°W	September & October, 1996	< 50 m		36 $\pm$ 13	Engel (2004)
29	Northeast Atlantic	47°N	Autumn 1996	Surface		100 (600)	Engel and Passow (2001), Passow (2002b)
30	Gulf of Cádiz, Portugal & Spain	around 36°30'N, 7°50'W	June - July, 1997	5 - 100 m			García et al. (2002), Passow (2002b)
31	Gulf of Cádiz, Portugal & Spain	around 36°30'N, 7°50'W	May, 2001	10 - 200 m	24 - 205		Prieto et al. (2006)
32	Strait of Gibraltar, Spain & Morocco	around 35°56'N, 5°35'W	June - July, 1997	< 75 m	27 - 354		Prieto et al. (2006)
33	Strait of Gibraltar, Spain & Morocco	around 35°56'N, 5°35'W	February, 1999	< 820 m	25 - 93		Prieto et al. (2006)
34	Alboran Sea, Mediterranean Sea	around 36°10'N, 4°50'W	June - July, 1997	< 75 m	Detection limit - 560		Prieto et al. (2006)

35	Longreville-sur-Mer, France	48°56'N, 1°36'W	Spring and Autumn, 2006 - 2009	1 m	26 - 3605	452	-	Klein et al. (2011)
36	Baie des Veys, France	49°25'N, 1°07'W	Spring and Autumn, 2006 - 2009	1 m	37 - 1735	281	-	Klein et al. (2011)
37	Western-Eastern transect of Mediterranean Sea	40°N, 02°E - 31°N, 30°E	May, 2007	5 - 200 m	5 - 94	21	123	Ortega-Retuerta et al. (2010)
38	Kieler Bucht, Baltic Sea	off Kiel (54°19'N, 10°07'E)	Spring, 1996	-	50 - 200	-	-	Kraus (1997), Passow (2002b)
39	Northern Adriatic Sea, Mediterranean Sea	around 45°N, 13°E	3 years, 1999 - 2002	<35 m	4 - 14800	-	-	Radić et al. (2005)
40	Northern Adriatic Sea, Mediterranean Sea	around 45°00'N, 13.01°E	May - July, 2007	<31 m	-	(1752)	32	Najdek et al. (2011)
41	Northern Adriatic Sea, Mediterranean Sea	-	April, 1996	1 m	1600 - 11000	-	-	Engel, pers. com., Passow (2002b)
42	Transect of Central Baltic Sea	55°27'N, 16°20'E - 59°25'N, 20°10'E	June, 1999	4 - 20 m	145 - 322	-	-	Engel et al. (2002)
43	Baltic Sea	-	Summer, 1999	-	-	241 ± 66	-	Engel and Passow (2001), Passow (2002b)
44	Balsfjord, Norway	69°21'N, 19°06'E	May, 1992	<18 m	100 - 255	190 ± 53	8	Passow and Alldredge (1995a)
45	Balsfjord, Norway	69°21'N, 19°06'E	May, 1992	21 - 63 m	125 - 250	191 ± 45	8	Passow and Alldredge (1995a)
46	Balsfjord, Norway	69°22'N, 19°07'E	March & May, 1992	<36 m	-	193 (258)	16	Riebesell et al. (1995), Passow (2002b)
47	Balsfjord, Norway	69°37'N, 19°12'E	1 year, 1996	<175 m	-	(1415)	-	Reigstad and Wassmann (2007), Passow (2002b)
48	Transect of Levantine basin, Mediterranean Sea	34°N, 25°E - 33°N, 34°E, & around 35°N, 29°E	February - March, May - June, & September, 2008, & July, 2009	<1000 m	19 - 600	-	72	Bar-Zeev et al. (2011)
49	Northern Gulf of Aqaba, Red Sea	29°28'N, 34°55'E	April, 2008	5 - 300 m	23 - 228	-	15	Bar-Zeev et al. (2009)
50	Transect in Arabian Sea, Indian Sea	15°N, 64°E - 21°N, 64°E	August, 1996	<1000 m	ca. 100 - 1020	-	-	Kumar et al. (1998)
51	Dona Paula bay, Indian Sea	15°27'N, 73°48'E	June, 1998 - July, 2000	1 m	1 - 149	-	-	Blaskar and Bhosle (2006)
52	Transect in Bay of Bengal, Indian Sea	6°N, 90°E - 18°N, 90°E	September, 1996	<1000 m	70 - 130	-	-	Kumar et al. (1998)
53	Australian sector of Antrantic	64°S, 112°E - 65°S, 119°E	October, 2003	<30 m	133 - 853	-	-	Dumont et al. (2009)
54	Australian sector of Antarctic	64°S, 112°E - 65°S, 119°E	October, 2003	Ice core (0 - 0.81 m)	20 - 2703	-	15	Dumont et al. (2009)
55	Pearl River estuary, China	23°05'N 113°25'E - 21°58', 113°43'E	August, 2009 & January, 2010	< ca. 10 m	89 - 1727	-	32	Sun et al. (2012)
56	Isahaya Bay, Japan	around 32°54'N, 130°13'E	March - May, 2007, February - May, & September - October, 2008	1 m	10 - 3490	495	99	Fukao et al. (2011, Japanese; abstract in English)
57	Sagami Bay, Japan	35°09'N, 139°09'E	December, 2004 - April, 2005	Surface	50 - 250	-	5	Sugimoto et al. (2007)
58	Tokyo Bay, Japan	around 35°30'N, 139°45'E	December, 1997 - November, 1998	> 10 m or <20 m	14 - 1774	-	170	Ramaiah and Furuya (2002)
59	Otsuchi Bay, Japan	39°20'N, 141°56'E	January - April, 1998	<15 m	136 - 2321	1344	51	Ramaiah et al. (2001)
60	Oyashio region, Japan	around 41°30'N, 144°20'E	April & June, 2010, & May, 2011	5 - 300 m	<10 - 171	54 ± 28	293	This study
61	Western subarctic Pacific (outside of Fe patch)	around 49°N, 165°E	July - August, 2001	5 - 70 m	40 - 60	-	18	Ramaiah et al. (2005)
62	Western subarctic Pacific (inside of Fe patch)	around 49°N, 165°E	July - August, 2001	5 - 70 m	40-190	-	36	Ramaiah et al. (2005)
63	Great Barrier Reef, Australia	16°S - 18°S, around 146°E	December, 1999 and January, 2000	5 m	152 - 791	291	72	Fabrizius et al. (2003)
64	Great Barrier Reef, Australia	-	December, 1999 - February, 2000	5 m	23 - 791	-	41	Passow (2002b)

Table 3.11 Summary of TEP-C/POC ratios from 2001 to early 2013.

No.	Sampling area	Sampling month, year	Sampling layer	TEP/POC ratio (average $\pm$ SD)	Reference
1	Mesocosm experiment; Bergen, Norway	June - July, 1995	2 m	35 $\pm$ 2%	Engel et al. (2004b)
2	Santa Barbara Channel, USA, Pacific	January - December, 1996	0 - 10 m	50%	Engel and Passow (2001)
3	Coastal Baltic Sea	March - April, 1996	0 - 10 m	22%	Engel and Passow (2001)
4	Northern Adriatic Sea	April, 1996	0 - 30 m	103%	Engel and Passow (2001)
5	Central Baltic Sea	June, 1999	0 - 9 m	ca. 40%	Engel et al. (2002)
6	North East Atlantic	June - July, 1996	0 - 30 m	17%	Engel and Passow (2001)
7	North East Atlantic	June - November, 1996	5 m	ca 18%	Engel (2004)
8	North East Atlantic	September - October, 1996	Chlorophyll maximum	23%	Engel and Passow (2001)
9	Bay of Bengal (Dona Paula bay), the West Coast of India, Indian Sea	June, 1998 - July, 2000	1 m	7 $\pm$ 6%	Bhaskar and Bhosle (2006)
10	The Gyre of Western Subarctic Pacific (outside the iron patch)	July - August, 2001	5 - 30 m	23 $\pm$ 6%	Ramaiah et al. (2005)
11	The Gyre of Western Subarctic Pacific (outside the iron patch)	July - August, 2001	50 and 70 m	42 $\pm$ 7%	Ramaiah et al. (2005)
12	The Gyre of Western Subarctic Pacific (inside the iron patch)	July - August, 2001	5 - 30 m	24 $\pm$ 12%	Ramaiah et al. (2005)
13	The Gyre of Western Subarctic Pacific (inside the iron patch)	July - August, 2001	50 and 70 m	49 $\pm$ 13%	Ramaiah et al. (2005)
14	Mesocosm Experiment; Bergen, Norway	June - July, 2002		maximum 50%	Pedrotti et al. (2010)
15	Australian Sector and Western Weddell Sea, Antarctic	September - November, 2003 Australian sector, and November, 2004 - January, 2005 Western Weddell Sea	Sea ice	26 $\pm$ 19%	Dumont et al. (2009)
16	Southwest Lagoon of New Caledonia	November, 2004	5 m	18 - 60%	Mari et al. (2007)
17	Northern Bay of Biscay, North Eastern Atlantic	May - June, 2006	Water column <150 m	2 - 68%	Harlay et al. (2009)
18	Northern Bay of Biscay, North Eastern Atlantic	May - June, 2006	<60 m	5 $\pm$ 1%	Engel et al. (2012)
19	Chesapeake Bay (estuary), USA, Atlantic	2007 - 2008	< ca. 22 m	32 $\pm$ 16%	Malpezzi et al. (2013)
20	Northern Bay of Biscay, North Eastern Atlantic	June, 2007	<40 m	26 $\pm$ 4%	Harlay et al. (2010)
21	North Carolina's Neuse River Estuary, North Carolina, USA, Atlantic	May - September, 2007 and October, 2007 - April, 2008	Sea surface	16%	Wetz et al. (2009)
22	North Bay of Biscay, North Eastern Atlantic	May, 2007	<60 m	15 $\pm$ 3%	Engel et al. (2011)
23	Eastern Mediterranean Sea	January - November, 2008, July, 2009, and September, 2009	Near-surface	63 $\pm$ 3%	Bar-Zeev et al. (2011)
24	Eastern Mediterranean Sea		Chlorophyll maximum	83 $\pm$ 37%	Bar-Zeev et al. (2011)
25	Eastern Mediterranean Sea		Deep (>300 m)	>100%	Bar-Zeev et al. (2011)
26	Oyashio Region, Western Subarctic Pacific	April and June, 2010, and May, 2011	5 m	27 $\pm$ 9%	This study

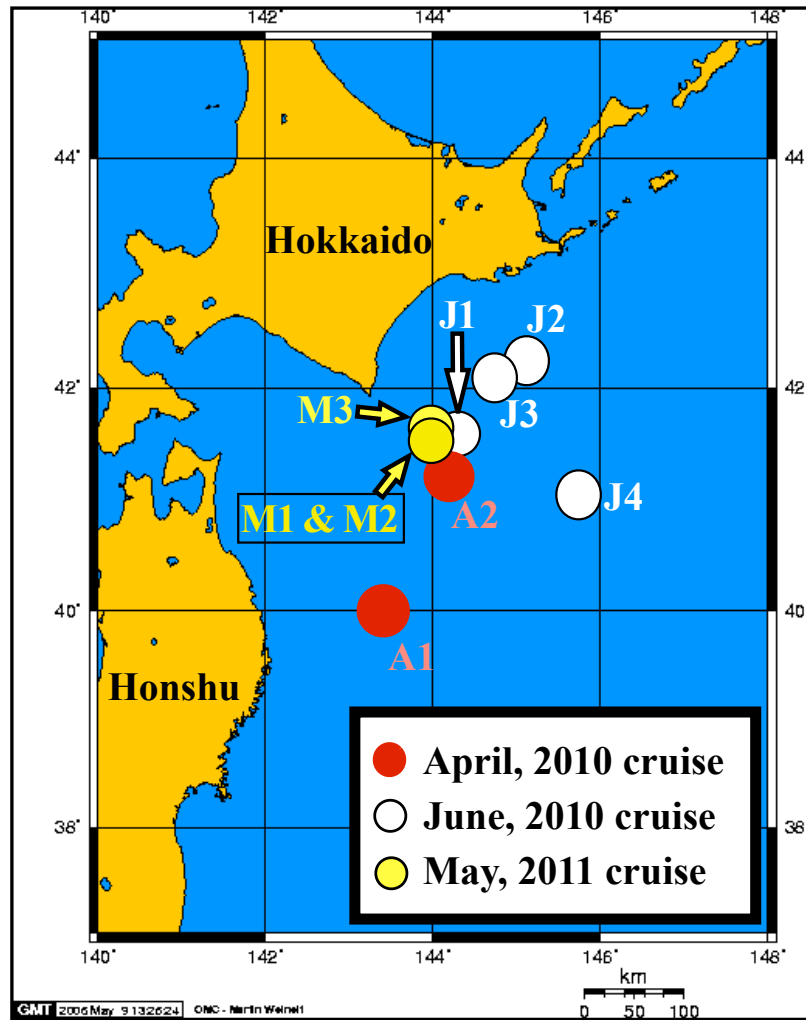


Fig. 3.1 Sampling locations in the TEP survey cruises during the Oyashio spring diatom blooms. The stations in April and June, 2010 were shown with red color (A1 and A2) and white color (J1, J2, J3 and J4), respectively. The stations in May, 2011 were also shown with yellow color (M1, M2 and M3).

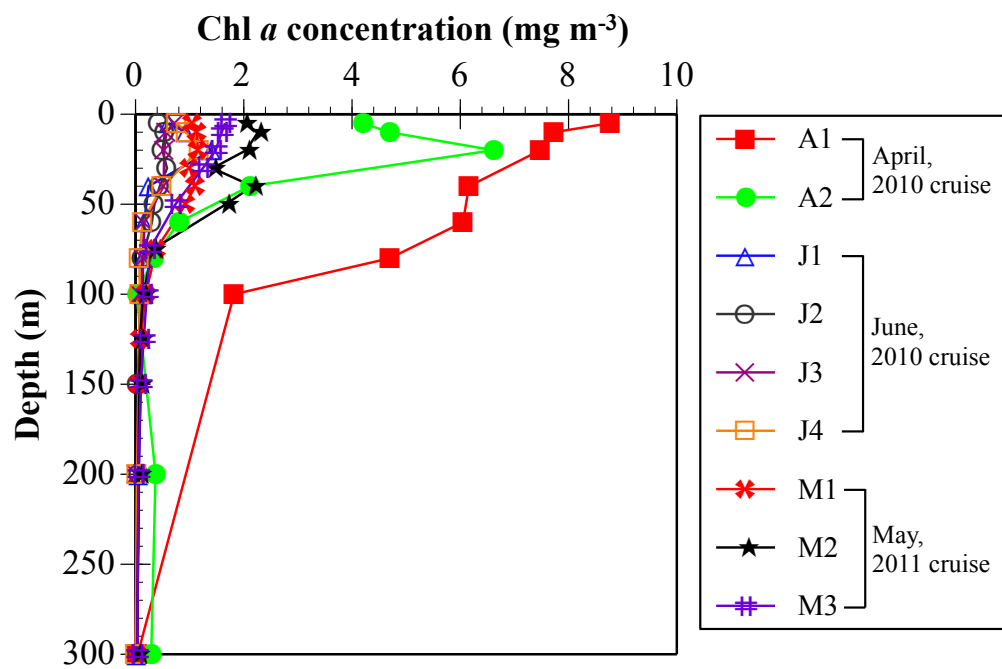


Fig. 3.2 Chlorophyll *a* vertical profile in the Oyashio spring phytoplankton blooms.

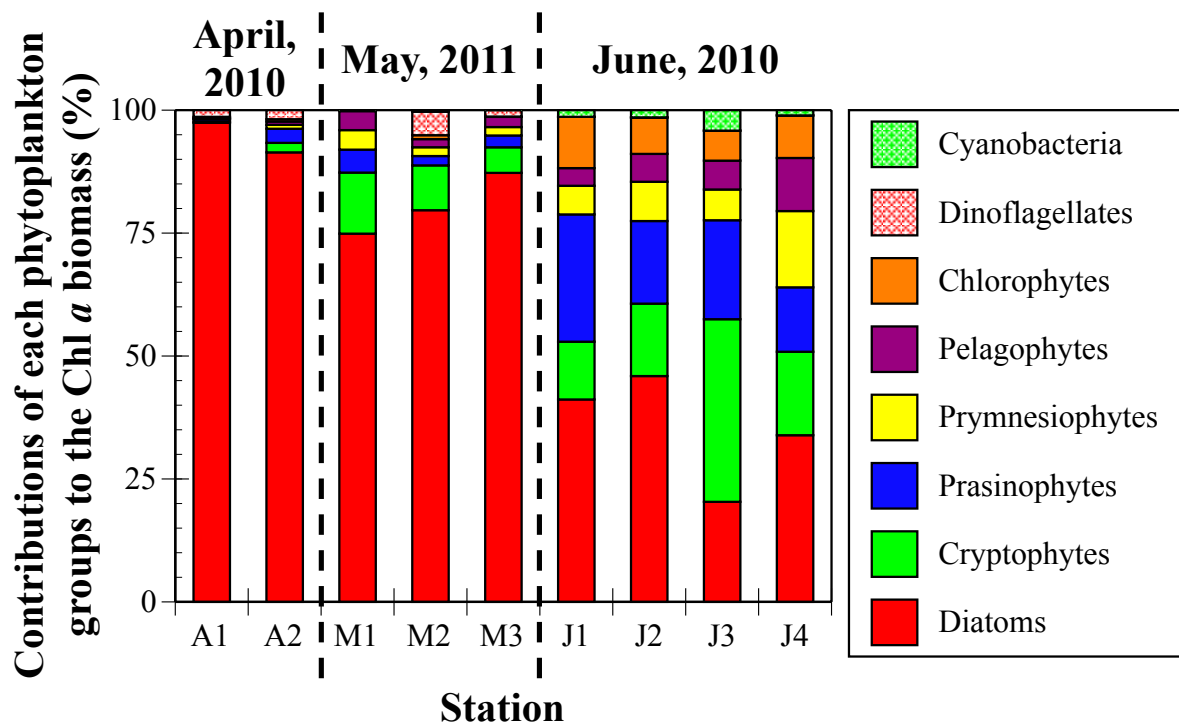


Fig. 3.3 Average contributions of each phytoplankton group to the Chl *a* biomass within 5–50 m depths. They were shown in order of the Chl *a* concentrations, that is alignment sequence of April, 2010, May, 2011 and June, 2010 cruises.

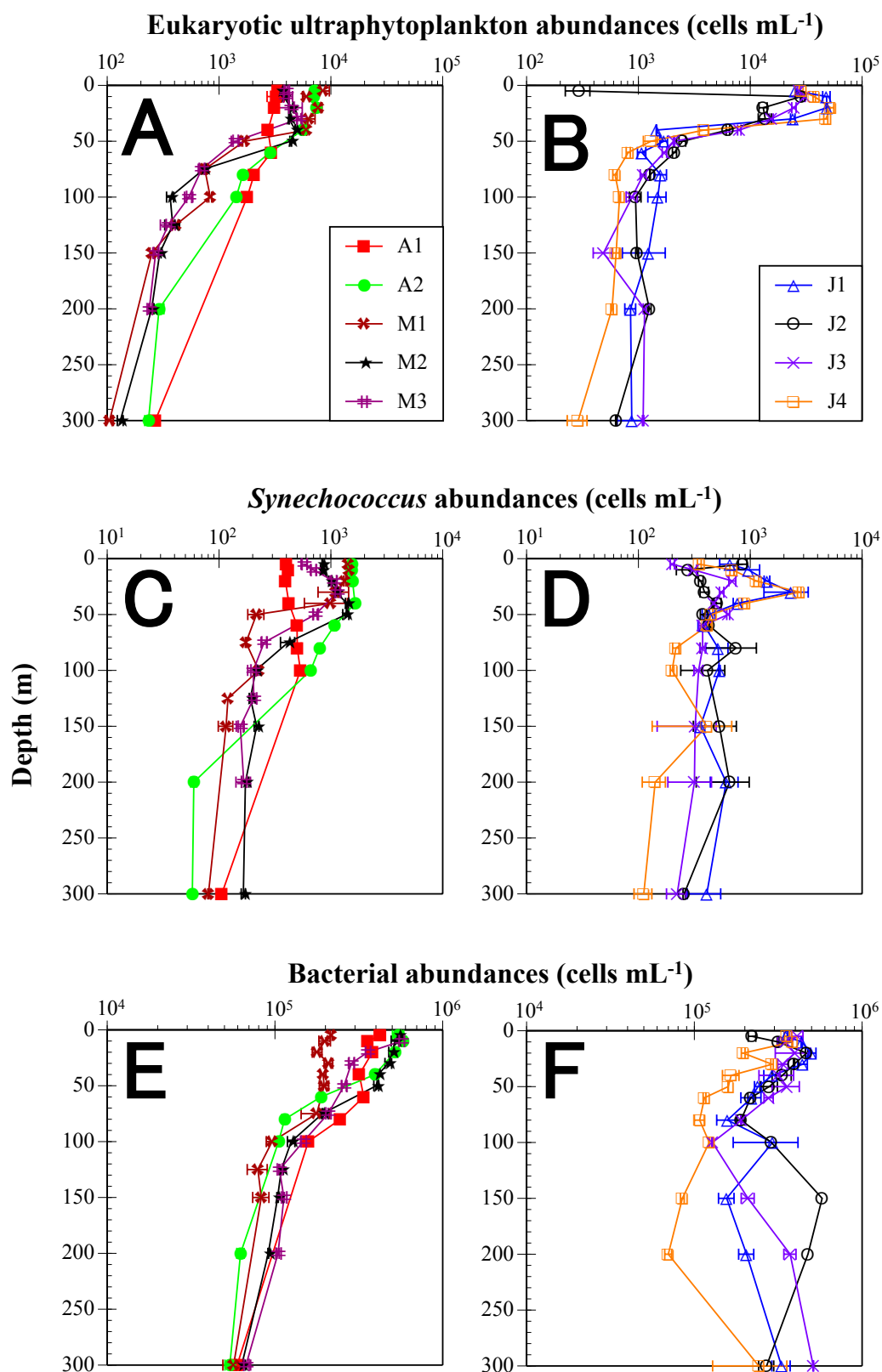


Fig 3.4 Vertical distributions of eukaryotes (A, B), *Synechococcus* (C, D) and bacteria (E, F).

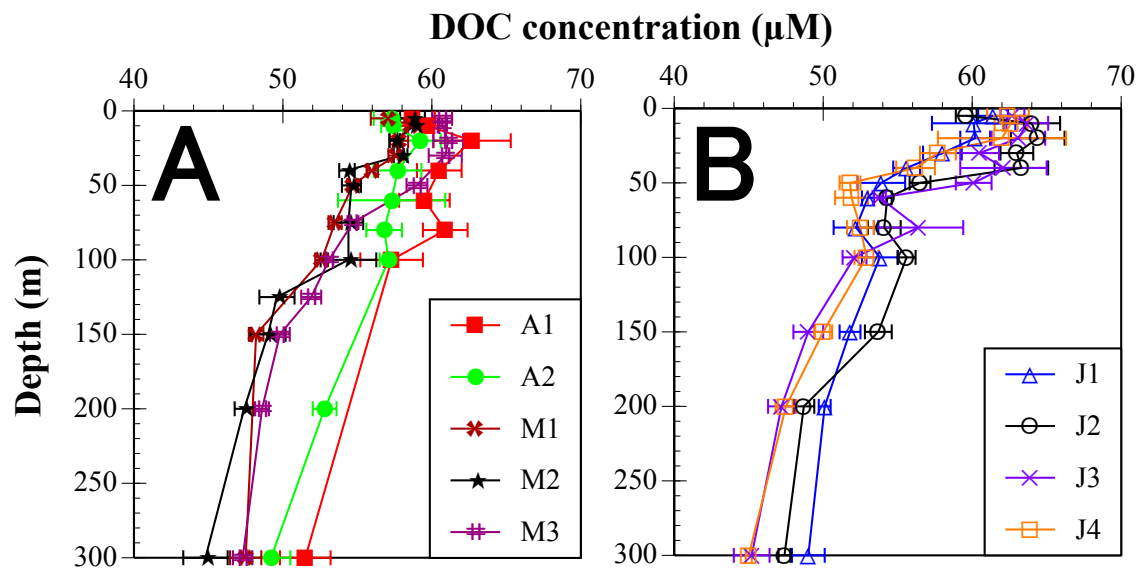


Fig. 3.5 Vertical profiles of dissolved organic carbon (DOC) concentrations in April and May cruises (A), and June cruise (B).

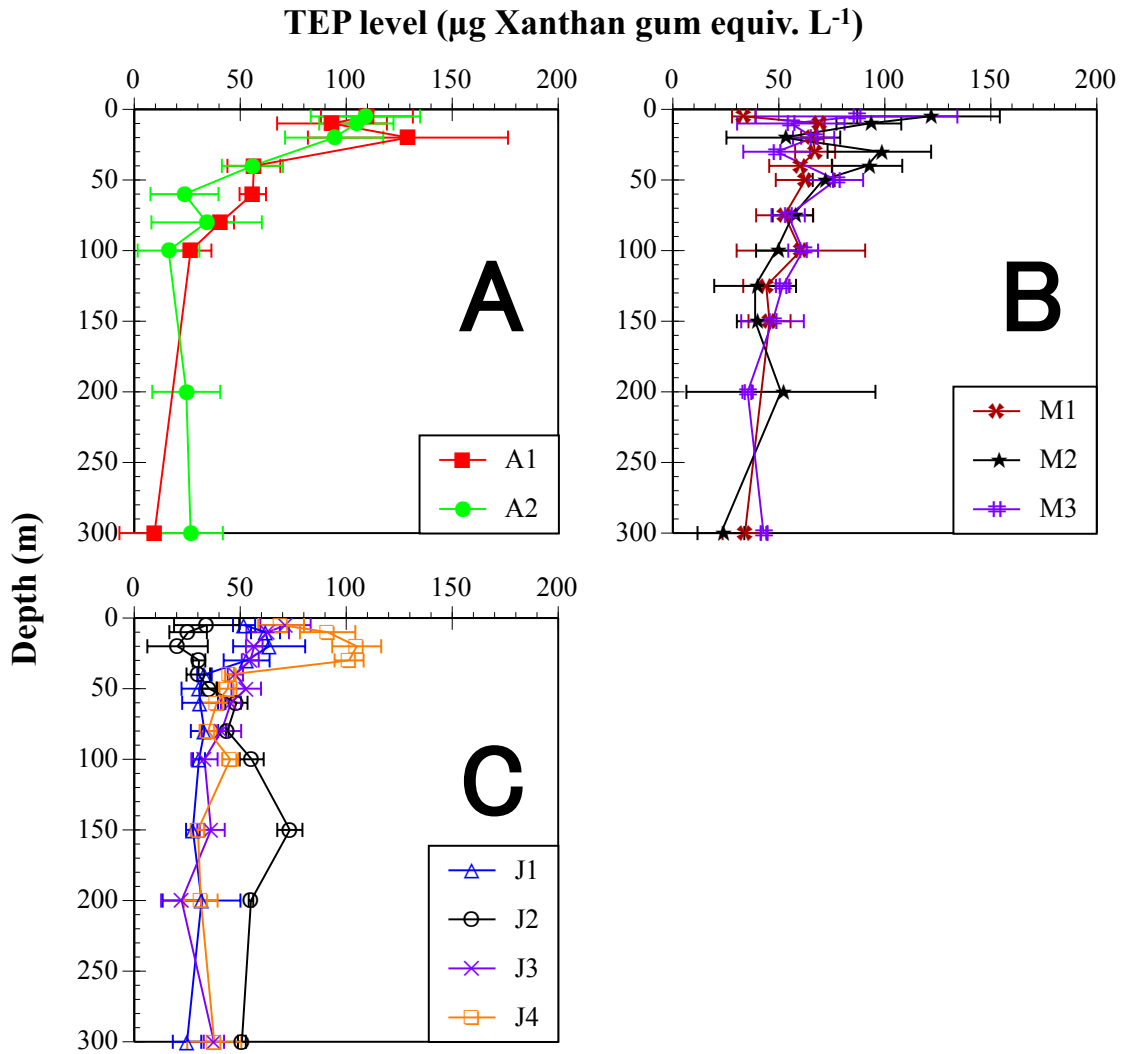


Fig. 3.6 Vertical profiles of TEP levels in April (A), May (B) and June (C), and of TEP/Chl *a* ratios in April and May (D) and June (E).

– Blank page –

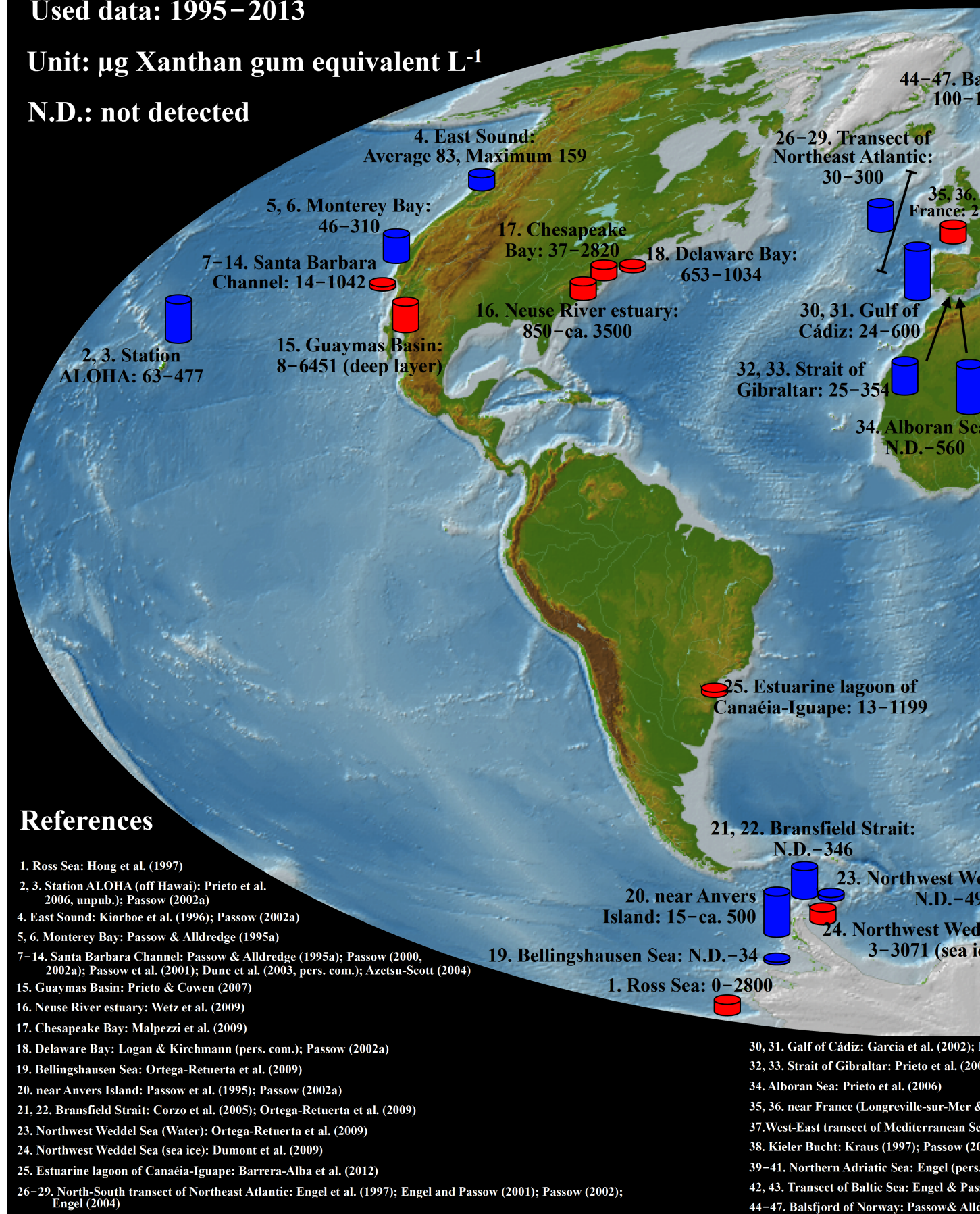
Fig. 3.7

TEP II

Used data: 1995–2013

Unit: μg Xanthan gum equivalent L^{-1}

N.D.: not detected

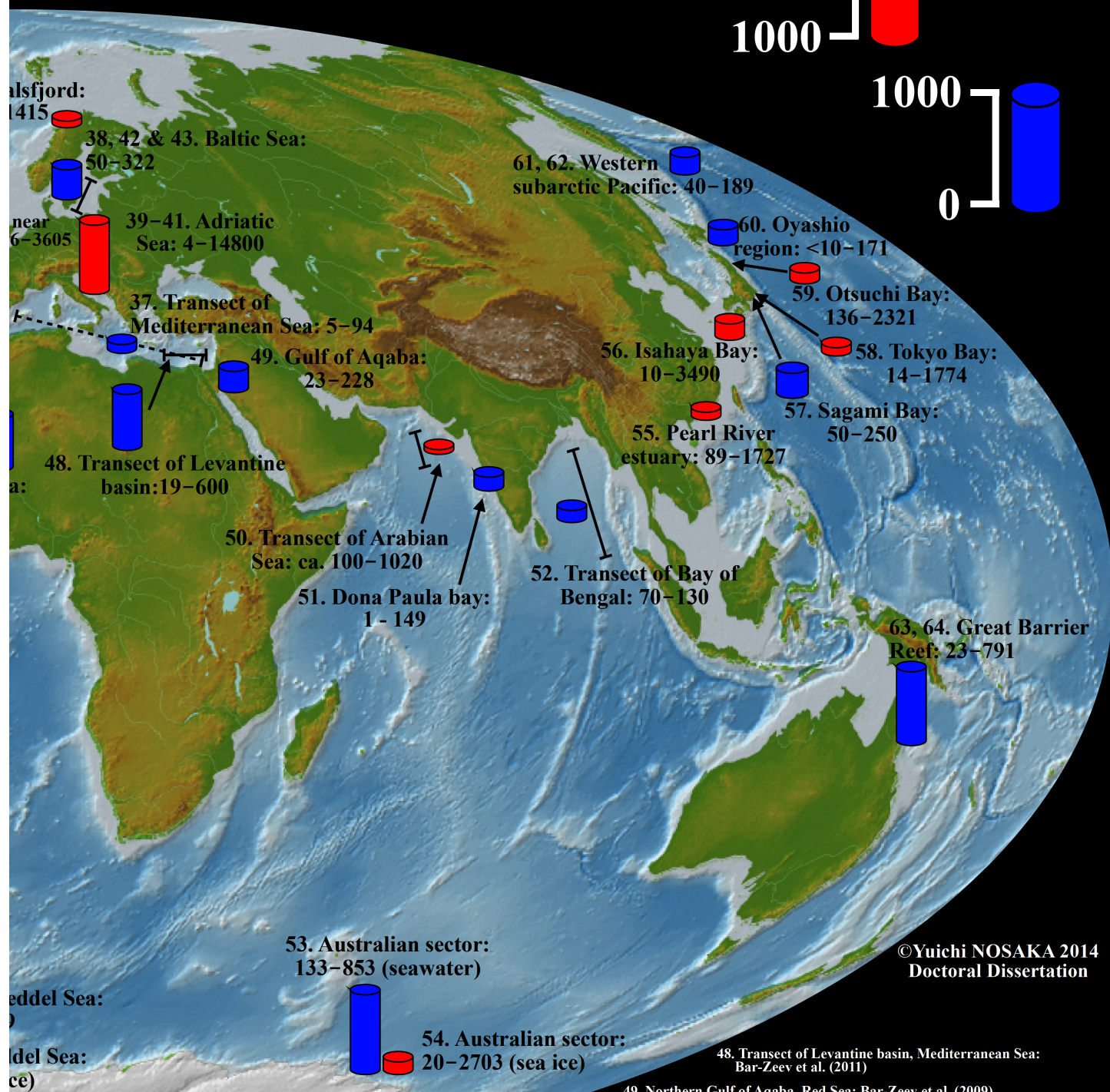
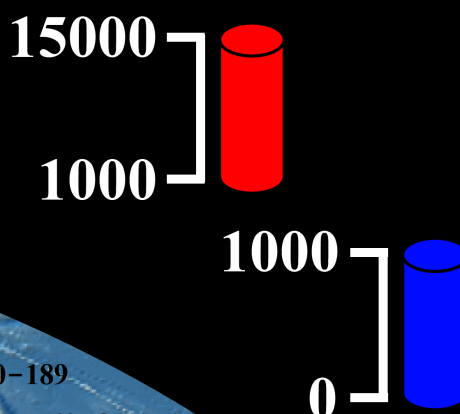


References

1. Ross Sea: Hong et al. (1997)
- 2, 3. Station ALOHA (off Hawaii): Prieto et al. 2006, unpub.; Passow (2002a)
4. East Sound: Kiorboe et al. (1996); Passow (2002a)
- 5, 6. Monterey Bay: Passow & Alldredge (1995a)
- 7–14. Santa Barbara Channel: Passow & Alldredge (1995a); Passow (2000, 2002a); Passow et al. (2001); Dune et al. (2003, pers. com.); Azetsu-Scott (2004)
15. Guaymas Basin: Prieto & Cowen (2007)
16. Neuse River estuary: Wetz et al. (2009)
17. Chesapeake Bay: Malpezzi et al. (2009)
18. Delaware Bay: Logan & Kirchmann (pers. com.); Passow (2002a)
19. Bellingshausen Sea: Ortega-Retuerta et al. (2009)
20. near Anvers Island: Passow et al. (1995); Passow (2002a)
- 21, 22. Bransfield Strait: Corzo et al. (2005); Ortega-Retuerta et al. (2009)
23. Northwest Weddell Sea (Water): Ortega-Retuerta et al. (2009)
24. Northwest Weddell Sea (sea ice): Dumont et al. (2009)
25. Estuarine lagoon of Canaëia-Iguape: Barrera-Alba et al. (2012)
- 26–29. North-South transect of Northeast Atlantic: Engel et al. (1997); Engel and Passow (2001); Passow (2002); Engel (2004)

Levels

Maximum TEP levels



©Yuichi NOSAKA 2014
Doctoral Dissertation

eddel Sea:
del Sea:
ce)

Passow (2002a); Prieto et al. (2006)
06)

& Baie des Beys); Klein et al. (2011)
ea: Ortega-Retuerta et al. (2010)
002a)

. com.); Passow (2002a); Radić et al. (2005); Najdek et al. (2011)
sow (2001); Engel et al. (2002b); Passow (2002a)

ledge (1995a); Riebbessel et al. (1995); Passow (2002a); Reigstad & Wassmann (2007)

- 48. Transect of Levantine basin, Mediterranean Sea: Bar-Zeev et al. (2011)
- 49. Northern Gulf of Aqaba, Red Sea: Bar-Zeev et al. (2009)
- 50. Transect of Arabian Sea: Kumar et al. (1998)
- 51. Bay of Bengal (Dona Paula bay), Indian Sea: Bhaskar and Bhosle (2006)
- 52. Transect in Bay of Bengal: Kumar et al. (1998)
- 53, 54. Australian sector of Antarctic: Dumont et al. (2009)
- 55. Pearl River estuary: Sun et al. (2007)
- 56. Isahaya Bay: Fukao et al. (2011, in Japanese; abstract in English)
- 57. Sagami Bay: Sugimoto et al. (2007)
- 58. Tokyo Bay: Ramaiah & Furuya (2002)
- 59. Otsuchi Bay: Ramaiah et al. (2001)
- 60. Oyashio region: Nosaka (2014, This doctoral dissertation)
- 61, 62. Western Subarctic Pacific (outside & inside of Fe patch): Ramaiah et al. (2005)
- 63, 64. Great Barrier Reef: Passow (2002); Fabricius et al. (2003)

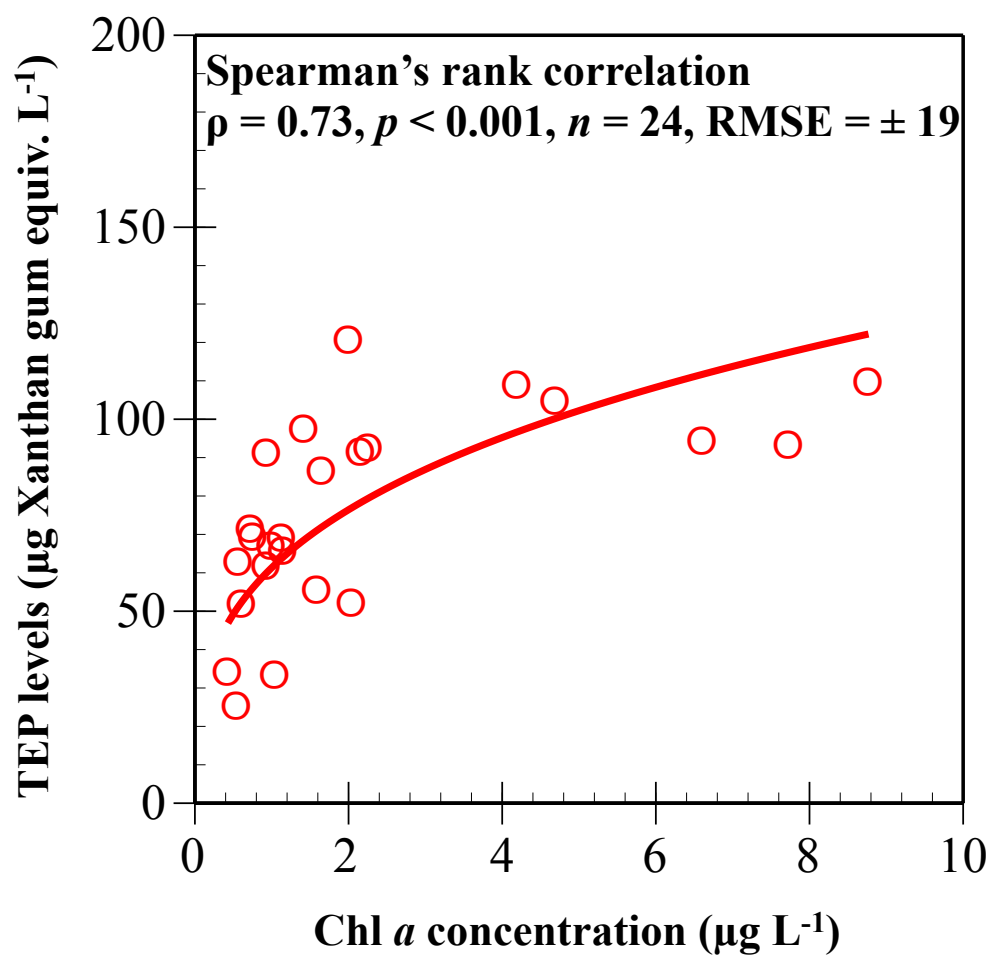


Fig. 3.8 Relationship between TEP and Chl *a* concentrations within the mixed layer.

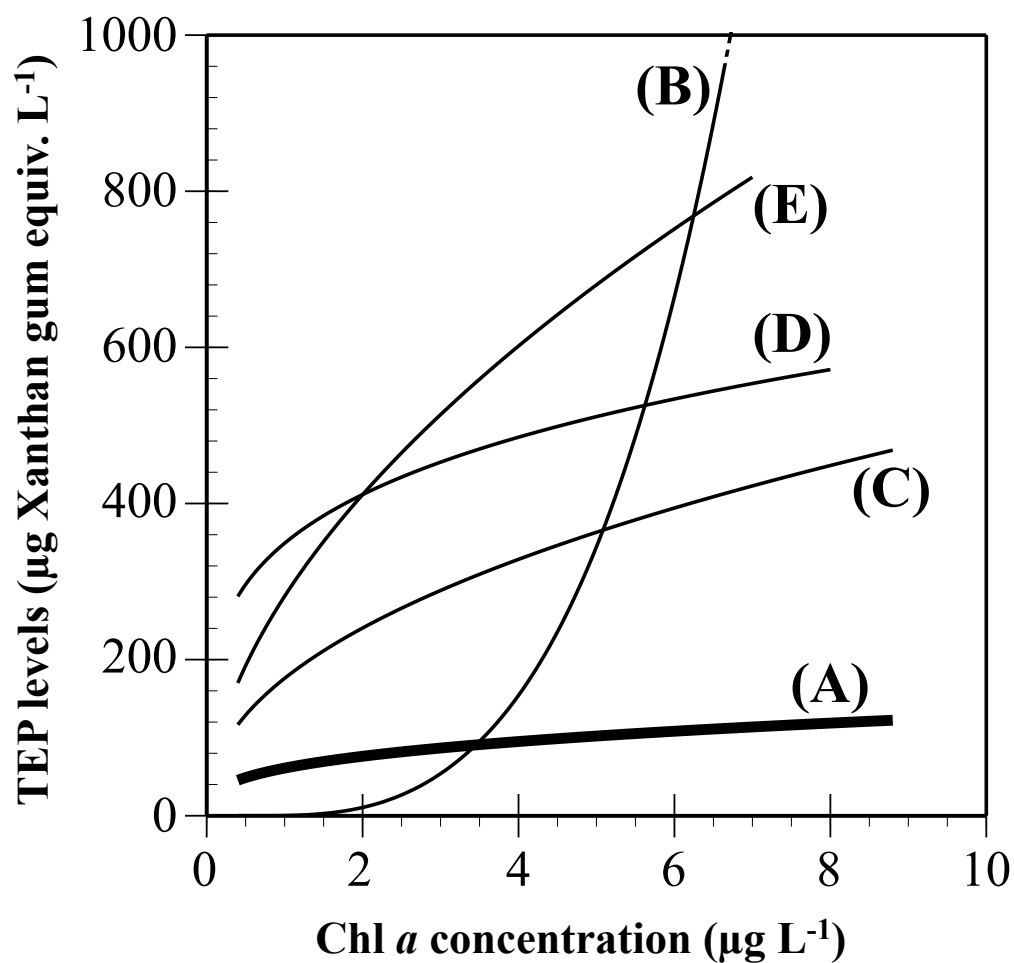
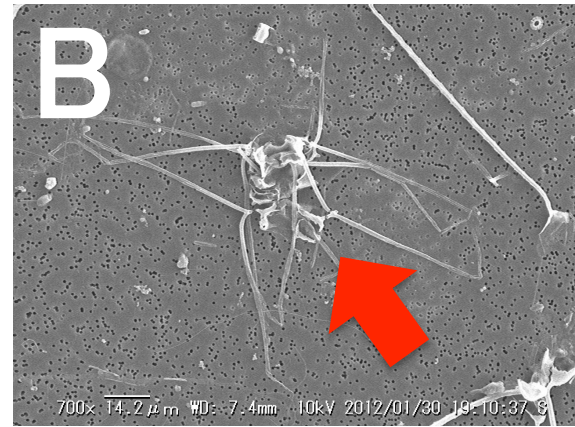


Fig. 3.9 Relationships between TEP and Chl *a* concentrations obtained in the various region. This study (A), Hong et al. (1997) (B), Kiørboe (1996) and Passow (2002a) (C), Engel (1998) (D), Average of the diatom strains (Passow, 2002a) (E).



***Chaetoceros* sp. 1**



***Chaetoceros* sp. 6**

Photo 3.1 Photos of *Chaetoceros* sp.1 (A) and *Chaetoceros* sp. 6 (B).



Photo 3.2 A Photo of massive TEP (marine “snowflake”) in the Adriatic Sea (Kaiser et al., 2011).

Chapter 4 – Formation of transparent exopolymer particles from the diatom *Thalassiosira nordenskioeldii* strain

4.1 Introduction

In Chapter 3, I investigated the dynamics of the transparent exopolymer particles (TEP) in the Oyashio region during the spring diatom boom. The levels of TEP at 5 m were highest in April, and generally decreased from April to June. Chlorophyll (Chl) *a* concentration significantly correlated with the TEP levels. It has been reported that the centric diatom *Thalassiosira nordenskioeldii* dominated in the Oyashio spring boom (Chliba et al., 2004; Ichinomiya et al., 2010; Suzuki et al., 2011). Indeed, *T. nordenskioeldii* also predominant in the phytoplankton community in April 2010 (see Chapter 3). Hence, in this chapter I examined the TEP productivity of *T. nordenskioeldii* more in depth using the batch cultivation technique.

The precursors of TEP have been considered as dissolved acid polysaccharides (e.g., Engel, 2004; Thornton et al., 2007; Wurl et al., 2011), and they might be mainly derived from the dissolved organic carbon (DOC) excreted by phytoplankton (Passow, 2002b). The release of the polysaccharides by phytoplankton depends on species, individual physiological state and environmental growth conditions (Passow, 2002b). Myklestad (1974) reported that the ratios of the polysaccharide/carbohydrate excreted by nine diatoms (*Chaetoceros affinis*, *Ch. curvisetus*, *Ch. decipiens*, *Ch. debilis*, *Ch. socialis*, *Corethron hystrix*, *Skeletonema costatum*, *T. gravida* and *T. fluviatilis*) varied from 1–125%. Other study of Myklestad using *Ch. affinis* also revealed that carbohydrates, especially polysaccharides, reached to 80% of the total DOC production (Myklestad, 1989). The investigations of the DOC and POC productivities by phytoplankton have been conducted intensively (e.g., Hellebust, 1965; Fogg, 1983; López-Sandoval et al., 2011). Hellebust (1965) reported that the ratio (PER: percentage of extracellular release) of the DOC productivity/total organic carbon (TOC) productivity (DOC + POC productivities) in four diatoms (*Phaeodactylum tricornutum*, *S. costatum* and *T. fluviatilis*) were higher in the high light intensity (ca. 1,800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) than the low or middle light intensities (ca. 50 and 400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). This was mainly explained by the damages of diatom cells with photooxidation. The relationship between the PER and light intensity was also studied by Zlotnik and Dubinsky (1989)

and Marañón et al. (2004). In contrast to Hellebust (1965), those studies showed that the PER in the high light intensity (ca. 1,900–3,000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) was not higher than the low or middle light intensity. On the contrary, the PER became higher in the low or middle light intensities (ca. <400 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). These results revealed that the behaviors of the PER, and POC and DOC productivities to the light intensity differed between species. The values of the PER can be influenced by the growth phase of phytoplankton. The absolute DOC productivity of *Ch. affinis* was higher in the exponential growth phase (exponential phase) than in the stationary growth phase (stationary phase), whereas the PER became 5-fold higher in the stationary phase than in the exponential phase (Mykkestad, 1995). Some previous papers (Mykkestad, 1974; Mykkestad et al., 1989; Obernosterer and Herndl, 1995) also showed similar results with Mykkestad (1995).

The TEP were known to protect phytoplankton cells from the attachment of bacteria (Azam and Smith, 1991; Smith et al., 1995). Hence, the existence of bacteria may increase the TEP productivity by phytoplankton. On the other hand, it has recently been reported that bacteria promoted the aggregation of polysaccharides (Yamada et al., 2013). Moreover, it was reported that extracellular polymeric substances (EPS), which are TEP precursors, may also act as a salinity barrier and a cryoprotectant (Krembs and Engel, 2001; Tamaru et al., 2005; Underwood et al., 2010). Therefore, phytoplankton possibly excrete the TEP or TEP precursors to moderate ambient environment changes.

Claquin et al. (2008) and Fukao et al. (2012) reported the TEP productivity of diatoms. The four diatoms (*Ichochrysis* aff. *galbana*, *Pseudo-nitzschia fraudulenta*, *S. marinoi* and *T. pseudonana*) ranged between ca. 0 and 50 $\mu\text{g Xanthan gum equiv.} [\mu\text{g Chl } a]^{-1} \text{ d}^{-1}$ under the continuously light conditions of 130 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Claquin et al., 2008). *Coscinodiscus granii* had the cellular TEP productivities between 3.2–8.2 ng Xanthan gum equiv. $[\text{cell}]^{-1} \text{ d}^{-1}$ (light:dark cycle = 14 hours:10 hours, light intensity: 150–200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) (Fukao et al., 2012). However, the TEP productivity of *T. nordenskiöldii* dominated in the Oyashio spring bloom have not ever been reported to date. Hence, I investigated not only the TEP productivity, but also the relationship between the DOC and TEP productivity using *T. nordenskiöldii* isolated from the Oyashio spring bloom. Moreover, to examine between the DOC productivity and the light intensity or between the DOC productivity and the growth phase, the photosynthesis–irradiance (*P–E*) curve experiment was also carried out in the

exponential and stationary phases.

4.2 Materials and Methods

4.2.1 Design of laboratory culture experiment

4.2.1.1 Isolation, sterilization and acclimation of *Thalassiosira nordenskioeldii*

A single cell of *Thalassiosira nordenskioeldii* (diatom, photo 4.1) was isolated from the seawater collected in the Oyashio cruise of May 2011. The isolated *T. nordenskioeldii* were incubated in f/2 medium (Guillard and Ryther, 1962; Guillard, 1975) at 10°C before the onset of the laboratory culture experiment. According to Sugimoto et al. (2007), *T. nordenskioeldii* were sterilized with the antibiotics of penicillin (final concentration: 25 U mL⁻¹) and streptomycin (final concentration: 25 µg mL⁻¹) (catalog no. 17-603E, Penicillin/Streptomycin stock 5K/5K, Lonza Group Ltd.).

The oligotrophic surface water was collected from the subtropical North Pacific at 23°N, 180° on December 2011 (temperature: 26.2°C, salinity: 35.3, nitrate: <0.05 µM, ammonium: 0.02 µM, phosphate: 0.02 µM, silicate: 1.1 µM) was used as the base seawater of this culture experiment. One liter of the oligotrophic seawater was filtered through Whatman GF/F filter (47 mm in diameter) and corrected into a 2-L polycarbonate bottle. The salinity was adjusted at 33.0 by adding Milli-Q water to the filtered seawater. The stock solutions for the f/2 medium (Guillard and Ryther, 1962; Guillard, 1975) were added to the filtered seawater in the bottle, and the nutrient concentrations were coordinated with those at the before of the spring diatom blooms in the Oyashio region (nitrate: ca. 20 µM; silicate: ca. 35 µM; phosphate: ca. 2.0) (Saito et al., 2002; Kono and Saito, 2010). Subsequently, 50 µL of the trace metal and 25 µL of vitamin stock solutions for the f/2 medium were added to 1 L of the filtered seawater, (final concentrations: FeCl₃ · 6H₂O at 600 nM, vitamin B₁₂ at 15 nM). The Oyashio-simulated medium was autoclaved (121°C, 20 min; TOMY BS-305, TOMY SEIKO Co., Ltd.), and the medium was cooled in a clean bench. Thereafter, the medium was transported into an incubator (MIR-554, SANYO Electric Co., Ltd.) for the laboratory culture experiment (temperature at 5°C). Six fluorescent lamps (FL20SS · BRN/18, TOSHIBA × 6) were mounted to the upper part in the incubator, and photosynthetic available radiation (PAR) of ca. 100 µmol photons m⁻² s⁻¹ at the base of the bottle was exposed with the light-dark cycle of each 12 hour. The axenic *Thalassiosira nordenskioeldii* were acclimated to the above condition for ca.

30-generations in the exponential phase before onset of the culture experiment.

4.2.1.2 Preparation of the culture experiment

Two polycarbonate culture vessels (volume: ca. 20 L, P/N 2600-0012 culture vessel, Thermo Scientific Nalge Nunc International) with four ports were used. Similarly to the section 4.2.1.1, the oligotrophic surface water was collected from the subtropical North Pacific at 23°N, 180° on December 2011 was filtered through the 47-mm Whatman GF/F filter. To conduct the aseptic culture experiment of the final volume 17-L per the 20-L vessel, and because autoclaving the medium of the large-volume was some problems, I divided the filtered seawater into two vessels. Fourteen and 3 liters of the filtered seawater were corrected in the 20-L culture vessel and a 6-L polycarbonate bottle, respectively. According to the section 4.2.11, the Oyashio-simulated mediums were made, and autoclaved. The autoclaved 3-L medium was added into the 20-L polycarbonate culture vessel; therefore, the total medium volume of the culture vessel was ca. 17 liters.

To aseptic sampling from the 20-L culture vessels, the tubes, bents and three-way cock pre-cleaned by soaking for >24 hours in 1 M HCl were connected with the ports of the 20-L culture vessel. The 20-L culture vessels were covered with plastic bags, and once, they were cooled in a refrigerator (ca. 3°C). After, the 20-L culture vessels were transported in the same incubator (temperature: 5°C, MIR-554, SANYO Electric Co., Ltd.).

4.2.1.3 Start of the culture experiment and sampling

To start the culture experiment, the axenic *T. nordenskioeldii* were added into the 20-L culture vessels in the clean bench, and immediately, the 20-L culture vessels were restored in the incubator (Fig. 4.1). The medium in the 20-L culture vessels was well stirred with turn round and round the bottles, every morning (ca. 6:30 AM). After stirred, sampling was carried out every other day (samples: nutrients, cell size and count, and TEP levels) or once in four days (samples: particulate organic carbon (POC), particulate nitrogen (PN), dissolved organic carbon (DOC), and pigments). Moreover, the photosynthesis–irradiance (*P–E*) curve experiments to investigate the photosynthesis physiology of *T. nordenskioeldii* were conducted in the exponential and stationary phases (days 18 and 20 for the exponential phase, days 34 and 36 for the

stationary phase).

The sampling system mentioned above is shown with some photos (see Fig.4.2). The sampling tubing pre-cleaned by the HCl was jointed to both the three-way cock and a pre-cleaned sampling bottle, and the three-way cock was twisted from the atmosphere opening thorough the disposable inline filter (0.2 μm ; ADVANTEC DISMIC[®]-25AS, Toyo Roshi Kaisha Ltd.) to the sampling bottle. The air pressure in the sampling bottle was lowered with an aspirator (ADVANTEC PSA152AB, Toyo Roshi Kaisha Ltd.). Therefore, the water sample was transported from the 20-L culture vessel to the sampling bottle. A part of the first water sample was discarded for prewashing the tubing and sampling bottle. After sampling, the three-way cock was twisted for atmosphere opening through the 0.2- μm inline filter, and then the sampling tubing was removed.

4.2.2 Samples of every other day

4.2.2.1 Nutrients

Duplicate water samples per the 20-L culture vessel were corrected into acrylic tubes with screw cap (11-D, SANPLATEC Corp.), and the samples were immediately stored in the freezer (-20°C). The frozen samples were thawed in the room temperature (25°C) and they were well shaken. Because high abundance of cells in the sample hampers nutrient analyses, the samples were centrifuged, and the supernatant was transferred into the new tubes. Nutrients (nitrate, nitrite, ammonium, phosphate and silicate) were determined with an auto-analyzer (QuAAtro 2-HR, BLTEC Corp.) for nutrient analyses following the manufacturer's protocol.

4.2.2.2 Cell size and count

The water sample of 10–50 mL was transferred into 15-mL or 50-mL polypropylene centrifuge tubes (part no. 339650 or 339652, Nunc[™] 15mL or 50mL Conical Sterile Polypropylene Centrifuge Tubes, Thermo Fisher Scientific inc.). During days 0–14, *T. nordenkioeldii* were filtered onto 25 mm Whatman Nuclepore membrane filters with 1.0 μm pore-size, and were immediately re-suspended in the non-concentrated water samples (concentration factor: 5–10 times). One point five milliliters of the concentrated water sample (days 0–14) or the non-concentrated water sample (days 16–40) was transferred to a micro slide glass chamber of thickness 1.1

mm (S109502, MATSUNAMI GLASS Ind., Ltd.), and covered with a cover glass. To estimate the cell size and abundance, duplicate samples from the 20-L culture vessels were observed with an epifluorescence microscope (BZ-9000, KEYENCE Corp.) equipped with a GFP optical filter (excitation wavelength: 480 ± 15 nm, emission wavelength: >510 nm, OP-66835 BZ filter GFP, KEYENCE Corp.). In the light field, the cell diameter and perivalvar axis (length between valve and valve) were observed 11 cells during days 0–8, and 21 cells during days 10–40, and those lengths were determined with assistance of an image analysis software (KEYENCE Corp.). The area ($Cell_a$: mm^2) and volume ($Cell_v$: mm^3) of the cell were estimated from the equations (4.1) and (4.2), respectively. Then, the cell shape of *T. nordenskioeldii* in this experiment is assumed as a cylinder.

$$Cell_a = 2\pi r^2 + 2\pi rL \quad (4.1)$$

$$Cell_v = 2\pi r^2 L \quad (4.2)$$

Where π is the circular constant, r is the cell radius and L is the length of the perivalvar axis.

For the cell abundance, the Chl *a*-derived fluorescence in *T. nordenskioeldii* were automatic photographed with 28 fields in magnification $4\times$, and the fluorescence were summed with the image analysis software above. The specific growth rate per day (μ : d^{-1}) during the culture experiment was estimated with the method of Guillard (1973) as the following eq. (4.3).

$$\mu = (\ln N_t - \ln N_0) / (t_t - t_0) \quad (4.3)$$

where N_0 is the initial cell abundance (cells mL^{-1}), N_t is the cell abundance after t days, t_0 is the initial time (d^{-1}), and t_t is the time after t days. Subsequently, the growth rates per day (M : division d^{-1}) were calculated from eq. (4.4).

$$M = \ln(2) / \mu \quad (4.4)$$

where $\ln(2)$ is 0.693.

4.2.2.3 Transparent exopolymer particle (TEP) levels

According to Passow and Alldredge (1995b), triplicate water samples (1–20 mL) were filtered onto Whatman 0.4- μm Nuclepore membrane filters under gentle vacuum (<100 mm Hg), and TEP on the filter were stained for ca. 2 seconds with 1 mL of a 0.02% aqueous solution of Alcian blue (8GX, Sigma-Aldrich Inc. LLC) in 0.06% acetic acid (pH 2.5). The stained filter was immediately rinsed with Milli-Q water to remove the excess dye. The filters were stored in a freezer (-20°C) until analysis.

For standard curve of TEP, I modified the method of the Claquin et al. (2008), and the analytical procedure was described in the section 3.2.11.

The TEP filter samples were transferred into 20-mL vials. Six milliliters of 80% H_2SO_4 were added to the vials, and the filters soaked for 2 hours. The vials were gently agitated 5 times over this period. Absorption at 787 nm was measured with the spectrophotometer in the 1-cm cuvettes against Milli-Q water as reference. TEP levels (μg Xanthan gum equivalent L^{-1}) were determined according eq. (4.5) (Passow and Alldredge, 1995b).

$$\text{TEP level} = (E_{787} - C_{787}) \times \frac{1}{(V_f)} \times f_x \quad (4.5)$$

Where E_{787} is the absorption at 787 nm of the sample, C_{787} is the absorption of the blank (i.e. 0.081), V_f is the filtered volume (L), and f_x is the calibration factor in micrograms estimated from the TEP standard curve.

The carbon content (TEP-C) in the colorimetric TEP (unit: Xanthan gum equivalent) was estimated according to the eq. (4.6; Engel and Passow, 2001).

$$\text{TEP-C} = \text{TEP level} \times 0.75 \quad (4.6)$$

Where 0.75 is a conversion factor from the Xanthan gum TEP levels to TEP-C. Engel and Passow (2001) estimated the carbon content in the TEP using a variety of diatom strains, and showed that the carbon content the TEP ranged between 0.53 and 0.88 $\mu\text{g C}$ [μg Xanthan gum equiv.] $^{-1}$. The 0.75 is the average value of the conversion factors obtained from the study of Engel and Passow (2001).

4.2.2.4 TEP productivity

Based on the TEP levels in the vessels, the cell-normalized (cellular) TEP productivities (pg Xanthan gum equivalent [cell]⁻¹ d⁻¹) were estimated according to the eq. (4.7; Zhang et al., 1996; Fukao et al., 2012).

$$TEP_{prod}^{cell} = \mu \times \frac{(TEP_t - TEP_0)}{(N_t - N_0)} \quad (4.7)$$

where μ is the specific growth rates estimated in the eq. (4.3) (day⁻¹), TEP_0 is TEP levels in the initial time (μ g Xanthan gum equiv. L⁻¹), TEP_t is the TEP levels after t days, N_0 is the initial cell abundance (cells L⁻¹), and N_t is the cell abundance after t days.

4.2.3 Samples of once in four days

4.2.3.1 Particulate organic carbon (POC) and particulate nitrogen (PN)

The water samples of 10–50 mL were filtered onto pre-combusted Whatman GF/F filters (25 mm in diameter, 450°C for 5 hours) under a gentle vacuum (<100 mm Hg), and stored in the freezer (–20°C) until analysis. The samples were thawed in the room temperature, and completely dried in a desiccator under vacuum for more than 24 hours. The concentrations of POC and PN on the filters were determined with element analyzer (FlashEA1112, Thermo Fisher Scientific Inc.).

4.2.3.2 Dissolved organic carbon (DOC)

A filter funnel (25 mm in diameter, PN 4203, PALL Corp.) was pre-cleaned by soaking in 1 M HCl and then rinsed with Milli-Q water. A pre-combusted Whatman GF/F filters (25 mm) was set in the filter funnel, and the funnel was equipped with a suction vessel (VT-500, Toyo Roshi Kaisha Ltd.). The filtration of the water sample was carried out under a gentle vacuum (<100 mm Hg). At start of sampling, a few milliliters of the filtrates were drained for prewashing of the GF/F filter and the filter funnel, and after, duplicate samples were collected into pre-combusted 24-mL screw vials with acid-cleaned PTFE septum caps. The samples were immediately stored in the freezer (–20°C) until analysis. The frozen samples were thawed, and they were well shaken. The concentrations of DOC were determined with a total organic carbon analyzer (TOC-V CSH, Shimadzu).

4.2.3.3 DOC productivity estimated from DOC concentration

Based on the DOC concentrations in the culture vessels, the cellular DOC productivity ($\mu\text{g C [cell]}^{-1} \text{d}^{-1}$) was estimated (eq. 4.8; Zhang et al., 1996).

$$DOC_{prod}^{cell} = \mu \times \frac{(DOC_t - DOC_0)}{(N_t - N_0)} \quad (4.8)$$

Where, μ is the specific growth rates estimated in the eq. (4.3) (day^{-1}), DOC_0 is DOC concentration in the initial time ($\mu\text{g C L}^{-1}$), DOC_t is the DOC concentration after t days, N_0 is the initial cell abundance (cells L^{-1}), and N_t is the cell abundance after t days.

4.2.3.4 Pigments

For the analysis of phytoplankton pigments using high-performance liquid chromatography (HPLC), the water samples (10–50 mL) were filtered on the 25-mm Whatman GF/F filters under gentle vacuum ($<100 \text{ mm Hg}$). The filter samples were folded, blotted with filter paper and stored in a deep-freezer (-80°C). Phytoplankton pigments were extracted with sonication in N, N -dimethylformamide (DMF) according to the protocol of Suzuki et al. (2005). HPLC pigment analysis was performed as described in Chapter 3.

4.2.4 Photosynthesis-irradiance (P-E) curve experiments in the exponential and stationary phases

4.2.4.1 Phytoplankton specific absorption coefficients ($\bar{a}_{ph}^*$ and $\bar{a}_{ph}^{cell}$)

In the $P-E$ curve experiments of exponential and stationary phases, samplings of the phytoplankton specific absorption coefficients ($\bar{a}_{ph}^*$ and $\bar{a}_{ph}^{cell}$) were carried out. Duplicate water samples (20–300 mL) were filtered onto Whatman GF/F filters (25 mm diameter) under gentle vacuum ($<100 \text{ mm Hg}$). Once, the filters were immediately contained into Petri slide containers (Millipore), and covered with aluminum foil. The samples were stored in the deep-freezer (-80°C). Within 24 hours, the absorption from 400 to 750 nm of the samples was measured with a spectrophotometer (MPS-2450, Shimadzu) equipped with an end-on-type photomultiplier tube. The measurements were described in Chapter 3.

The mean Chl *a*-specific absorption coefficient of phytoplankton, c_{ph}^* ($m^2 [mg \text{ Chl } a]^{-1}$) was weighted with the spectral irradiance of the incubator lamp from 400 to 700 nm to correct the spectral characteristics of the incubator lamp source according to Cota et al. (1994) (eq. 4.9).

$$\bar{a}_{ph}^* = \int_{400}^{700} a_{ph}^*(\lambda) E(\lambda) d\lambda / \int_{400}^{700} E(\lambda) d\lambda \quad (4.9)$$

Where $E(\lambda)$ is the relative spectral irradiance of the incubator lamp, and the relative spectrum was obtained from the manufacturer.

Similarly, $\bar{a}_{ph}^{cell}$ ($m^2 [cell]^{-1}$) was also estimated from eq. (4.10).

$$\bar{a}_{ph}^{cell} = \int_{400}^{700} a_{ph}^{cell}(\lambda) E(\lambda) d\lambda / \int_{400}^{700} E(\lambda) d\lambda \quad (4.10)$$

4.2.4.2 *P–E curve experiment for POC and DOC*

In the exponential phase (days 20 for the vessel 1, days 18 for the vessel 2) and stationary phase (days 36 for the vessel 1, days 34 for the vessel 2), photosynthesis–irradiance (*P–E*) curve experiments were conducted to investigate the relationships between POC and DOC productivities and the light intensity. The water samples were dispensed into 275 mL acid-cleaned polystyrene bottles, inoculated with a solution of the $NaH^{13}CO_3$ (99 atom% ^{13}C), which was equivalent to ca. 10% of TIC in the seawater. The concentrations of TIC were measured with the total alkalinity analyzer (ATT-05, Kimoto Electric Co., Ltd.). Incubations were carried out for ca. 5 hours in a bench-top incubator, and equipped with a 150 W metal halide lamp (HQI-T 150W/WDL/UVS, Mitsubishi/Osram Co., Ltd.) as a light source. The water temperature in the incubator was maintained to the 5°C with a temperature-controlled water circulator (CL-80R, TAITEC Corp.). The samples were exposed to irradiance levels from ca. 5 to 2300 $\mu mol \text{ photons } m^{-2} s^{-1}$. The irradiance levels at each bottle position were measured with a 4 π PAR sensor (SQL-2101, Biospherical Instruments Inc.). After incubation, the POC samples were filtered onto pre-combusted Whatman GF/F filters (25 mm diameter) under gentle vacuum (<100 mm Hg). Single water sample of 275 mL was also filtered in the same manner as before the incubation. The filter samples were stored in the freezer (–20°C) until analysis. For the DOC samples, the filtrates of the POC samples

were corrected within pre-combusted 500-mL reagent bottles (450°C for 5 hours), and stored in the freezer (−20°C) until analysis.

After the frozen filter samples for the POC were thawed, they were exposed to HCl fumes to remove inorganic carbon, and completely dried in a desiccator under a vacuum for more than 24 hours. The POC in the samples and ¹³C abundance were determined using the mass spectrometer (DELTA V, Thermo Fisher Scientific Inc.) with the on-line elemental analyzer (FlashEA1112, Thermo Fisher Scientific Inc.). Primary productivity per hour was calculated according to Hama et al. (1983). The primary productivity was normalized with the Chl *a* biomass or cell abundance. The Chl *a*-normalized primary productivity (μg C [Chl *a*]^{−1} h^{−1}) was fitted with the photoinhibition model of Platt et al. (1980) (eq. 4.11). Similarly, the cellular primary productivity (pg C [cell]^{−1} h^{−1}) was estimated from the eq. (4.12).

$$P^* = P_s^* \left(1 - \exp^{-\alpha^* E/P_s^*}\right) \times \left(1 - \exp^{-\beta^* E/P_s^*}\right) \quad (4.11)$$

$$P^{cell} = P_s^{cell} \left(1 - \exp^{-\alpha^{cell} E/P_s^{cell}}\right) \times \left(1 - \exp^{-\beta^{cell} E/P_s^{cell}}\right) \quad (4.12)$$

In the model of the eq. (4.11), α^* is the initial slope (mg C [Chl *a*]^{−1} h^{−1} [μmol photon m^{−2} s^{−1}]^{−1}) and β^* is the photoinhibition index (mg C [Chl *a*]^{−1} h^{−1} [μmol photon m^{−2} s^{−1}]^{−1}). In the model of the eq. (4.12), α^{cell} is the initial slope (pg C [cell]^{−1} h^{−1} [μmol photon m^{−2} s^{−1}]^{−1}) and β^{cell} is the photoinhibition index (pg C [cell]^{−1} h^{−1} [μmol photon m^{−2} s^{−1}]^{−1}). Subsequently, the parameters of $P_{\max}^*$ and $P_{\max}^{cell}$ were estimated from eq. (4.13) and (4.14), respectively.

$$P_{\max}^* = P_s^* \times \left(\frac{\alpha^*}{\alpha^* + \beta^*}\right) \times \left(\frac{\alpha^*}{\alpha^* + \beta^*}\right)^{\beta^*/\alpha^*} \quad (4.13)$$

$$P_{\max}^{cell} = P_s^{cell} \times \left(\frac{\alpha^{cell}}{\alpha^{cell} + \beta^{cell}}\right) \times \left(\frac{\alpha^{cell}}{\alpha^{cell} + \beta^{cell}}\right)^{\beta^{cell}/\alpha^{cell}} \quad (4.14)$$

The Chl *a*- or cell-normalized light saturation index (E_k) was defined as following eq. (4.15).

$$E_k = P_{\max}^{* \text{ or } cell} / \alpha^{* \text{ or } cell} \quad (4.15)$$

Where, $P_{\max}^{* \text{ or } cell}$ is $P_{\max}^*$ or $P_{\max}^{cell}$, and $\alpha^{* \text{ or } cell}$ is α^* or α^{cell} . Moreover, the maximum quantum yields of the Chl *a*-normalized carbon fixation ($\Phi_{\text{Chl-a-c max}}$, mol C [mol photon]⁻¹) or the cellular carbon fixation ($\Phi_{\text{cell-c max}}$, mol C [mol photon]⁻¹) were estimated from the following eq. (4.16) or (4.17), respectively.

$$\Phi_{\text{Chl-a-c max}} = 0.0231 \times \alpha^* / \bar{a}_{\text{ph}}^* \quad (4.16)$$

$$\Phi_{\text{cell-c max}} = 0.0231 \times \alpha^{cell} / \bar{a}_{\text{ph}}^{cell} \quad (4.17)$$

Where 0.0231 is factor to converts miligrams of carbon to moles, $\mu\text{mol photons}$ to moles and hours to seconds, and $\bar{a}_{\text{ph}}^*$ and $\bar{a}_{\text{ph}}^{cell}$ are the phytoplankton specific absorption coefficients estimated in the equations (4.9) and (4.10), respectively.

The valuation of the DOC productivity were performed as described in 3.2.9 of Chapter 3.. The estimated DOC productivity per hour ($\mu\text{g C L}^{-1} \text{ h}^{-1}$) was normalized with the Chl *a* concentration ($\mu\text{g L}^{-1}$) or cell abundance (cells L^{-1}). Finally, the Chl *a*-normalized ($\mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1}$) or cellular ($\text{pg C } [\text{cell}]^{-1} \text{ h}^{-1}$) productivity was plotted to the exposed PAR levels.

The ratio (PER) of the DOC productivity to the total organic carbon (TOC) productivity was defined as following eq. (4.18).

$$\text{PER} = \frac{DOC_{\text{prod}}}{(DOC_{\text{prod}} + POC_{\text{prod}})} \times 100 \quad (4.18)$$

Where, DOC_{prod} and POC_{prod} are the DOC productivity and POC productivity, respectively.

4.3 Results and Discussion

4.3.1 Cell abundance and condition during the incubation

Cell abundance in the culture vessel 1 increased from 2 cells mL⁻¹ at day 0 to 2.5×10^4 cells mL⁻¹ at day 40 (Fig. 4.4). The cell abundance exponentially increased between

days 0 and 28, whereas that almost did not increase between days 30–40. These results suggest that exponential and stationary phases were days 0–28 and days 30–40, respectively. In the vessel 1, the decline phase was not observed. In the vessel 2, the cell abundances from day 0 to days 40 increased from 2 cells mL⁻¹ to 2.2×10^4 cells mL⁻¹ (Fig. 4.4). The cell abundance exponentially increased from days 0 to 26, whereas those during days 28–40 little increased. Therefore, the exponential and stationary phases corresponded to days 0–26 and days 28–40, respectively. The decline phase was not found in the vessel 2 as well.

The specific growth rates (μ : d⁻¹) and the division rates per day (M : division d⁻¹) in the exponential and stationary phases of the vessels 1 and 2 were showed in Table 4.1. The averaged μ of the vessels 1 and 2 were 0.31 ± 0.02 d⁻¹ during the exponential phase and 0.03 ± 0.02 d⁻¹ during the stationary phase. Similarly, the averaged M values were 0.44 ± 0.03 division d⁻¹ during the exponential phase and 0.05 ± 0.02 division d⁻¹ during the stationary phase. The μ and M values during the exponential phase were ca. 10-fold higher than those during the stationary phase.

In the culture vessel 1, nitrate plus nitrite (NO₃ + NO₂) and silicate (Si(OH)₄) concentrations at day 0 were 20.2 μ M and 37.4 μ M, respectively (Fig. 4.3 vessel 1). The NO₂ concentration were <0.09 μ M throughout the experiment. NO₃ + NO₂ concentrations between days 26 and 28 rapidly decreased from 11.5 μ M to 0.9 μ M, and that at day 30 became <0.05 μ M. The Si(OH)₄ concentration also decreased between days 28 (20.0 μ M) and 30 (3.0 μ M). The averaged concentration (average $\pm$ standard deviation) between days 30 and 40 was 3.3 ± 0.8 μ M. Phosphate (PO₄) concentration was 2.07 μ M at day 0, and the lowest concentration was found at day 30 (0.33 μ M). In the culture vessel 2, NO₃ + NO₂ and SiO₂ concentrations at day 0 were 18.4 μ M and 33.9 μ M, respectively (Fig. 4.3 vessel 2). The NO₂ concentration was <0.06 μ M throughout this experiment. NO₃ + NO₂ concentrations between days 22 and 24 decreased from 7.6 μ M to 0.5 μ M, and that at day 26 became <0.05 μ M. Si(OH)₄ concentration also decreased from days 24 (19.0 μ M) to 26 (7.3 μ M). The averaged concentration of silicate between days 26 and 40 was 3.4 ± 1.7 μ M. PO₄ concentration was 1.84 μ M at day 0, and the lowest concentration was found at day 38 (0.29 μ M). On day 30 for vessel 1 and day 26 for vessel 2, the nitrate concentration became the detection limit (<0.05 μ M), whereas silicate concentrations were >3.0 μ M. The commonly observed half-saturation constants of the nitrate and silicate uptakes by

diatoms were approximately 1.6 μM and 3.9 μM , respectively (Sarhou et al., 2005). Hence, the results of the nutrients in the laboratory experiment indicated that the growth of *T. nordenskiöldii* in both the vessels 1 and 2 was limited by nitrate rather than silicate availability (Fig. 4.4).

In both of the vessels 1 and 2, the cell diameters tended to decrease from day 0 ($15.3 \pm 0.7 \mu\text{m}$ for the vessel 1, $15.6 \pm 0.7 \mu\text{m}$ for the vessel 2) to day 40 ($14.0 \pm 1.1 \mu\text{m}$ for the vessel 1, $13.3 \pm 1.4 \mu\text{m}$ for the vessel 2) (Fig. 4.5A). The decrease in the cell diameter from day 0 to day 40 might contribute to the asexual division (e.g., Lalli and Parsons, 1997). The average cell lengths of pervalvar axis in the vessels 1 and 2 could be higher during days ca. 6–16 than those during days ca. 30–40 (Fig. 4.5A). The difference for the length of pervalvar axis was unknown in this study. However, the elongation of the pervalvar axis could be caused by the active cell division during the exponential phase, because *T. nordenskiöldii* elongated to a direction of the pervalvar axis, and thereafter the cell division occurred. Overall, the changes in cell size during this experiment were higher in the length than the diameter. Therefore, the changes in the area and volume depended on the length rather than the diameter (Fig 4.5B).

4.3.2 Pigments

The samplings of the pigment were generally carried out in the term between the *P–E* curve experiments of the exponential and stationary phase (i.e., days 20–36 for the vessel 1, days 18–38 for the vessel 2). The six pigments of Chl *a*, fucoxanthin, Chl *c*₂, Chl *c*₁, diadinoxanthin, diatoxanthin, and carotenes were detected. The concentrations of the all pigments were significantly linear correlated with the cell abundance (Spearman's rank correlation, $\rho = 0.87$, $p < 0.0001$, $n = 15$ for the all pigments) (Fig. 4.10). The chlorophyll *a* concentration ranged between 6.0 and 42.6 $\mu\text{g L}^{-1}$.

The ratios of the accessory pigments to Chl *a* were calculated through the exponential to stationary phases. Those ratios (average $\pm$ standard deviation [coefficient of variation]) were 0.446 ± 0.040 [9%] for fucoxanthin, 0.118 ± 0.017 [14%] for Chl *c*₂ + *c*₁, 0.093 ± 0.035 [38%] for diadinoxanthin, 0.023 ± 0.011 [49%] for diatoxanthin, and 0.020 ± 0.005 [24%] for carotenes. The pigment composition of *T. nordenskiöldii* could little change between the exponential and stationary phases. Also, those pigment ratios were within the pigment ratios of Bacilliarophyceae obtained from the various diatoms (Mackey et al., 1996).

The pigment concentrations per cell in the exponential and stationary phases were 11.6 ± 4.1 and 2.1 ± 0.1 pg cell^{-1} for Chl *a*, 5.7 ± 2.1 and 0.85 ± 0.06 pg cell^{-1} for fucoxanthin, 1.5 ± 0.6 and 0.22 ± 0.01 pg cell^{-1} for Chl $c_2 + c_1$, 0.98 ± 0.53 and 0.23 ± 0.07 pg cell^{-1} for diadinoxanthin, 0.20 ± 0.14 and 0.06 ± 0.01 pg cell^{-1} for diatoxanthin, and 0.21 ± 0.09 and 0.05 ± 0.01 pg cell^{-1} , respectively. Those concentrations were significantly difference between the exponential and stationary phases (Wilcoxon rank sum test, $p < 0.001$, $n = 15$ for Chl *a*, fucoxanthin, Chl $c_2 + c_1$, carotenes; $p < 0.05$, $n = 15$ for diadinoxanthin), except for the diatoxanthin (Wilcoxon singled-rank test, $p = 0.08$, $n = 8$). The diadinoxanthin and diatoxanthin were known to be the photoprotective pigments, referred to as the xanthophyll cycle (Dermers et al., 1991; Moisan et al., 1998; Fujiki et al., 2003). The ratio of the pigments can change with the light irradiance to protect the photosynthetic apparatus against high-irradiance conditions (Young et al., 1997; Falkowski and Raven, 2007). Dermers et al. (1991) reported that the change time occurred within spite of the short periods between 30 and 60 minutes. Hence, to precisely analyze those pigments is needed to immediately extract the filtrated sample (e.g., Fujiki et al., 2003) or immediately placed in liquid nitrogen (e.g., Moisan et al., 1998). In this study, although the samples were stored in a deep-freezer (-80°C) as soon as possible, the operations were carried out. Therefore, the concentrations of the diadinoxanthin and diatoxanthin were roughly concentrations, compared with the detected other pigments.

The relationship between Chl *a* concentration and cell abundance was shown as following eq. (4.19).

$$\text{Chl } a \text{ concentration} = 1.75 \times 10^{-6} \times \text{Cells} + 6.06 \quad (4.19)$$

where the unit of Chl *a* concentration is $\mu\text{g L}^{-1}$, and *Cells* is cell abundance in the unit of cells L^{-1} . The coefficient of determination (r^2) was 0.99, and the root mean square error (RMSE) in this equation was $\pm 1.6 \mu\text{g L}^{-1}$. This equation is valid within days 20–36 for the vessel 1 and days 18–38 for the vessel 2.

The chlorophyll *a* in the *P–E* curve experiments were shown in Table 4.2. The Chl *a* concentrations of the vessels 1 at the day 20 and day 36 were $6.1 \pm 0.1 \mu\text{g L}^{-1}$ and $42.5 \pm 0.3 \mu\text{g L}^{-1}$, respectively. In the vessel 2, the Chl *a* concentrations of the day 18 and day 34 were $7.1 \pm 0.1 \mu\text{g L}^{-1}$ and $42.6 \pm 1.5 \mu\text{g L}^{-1}$, respectively.

4.3.3 TEP levels and TEP productivities

The TEP levels at day 0 were not measurable ($<10 \mu\text{g}$ Xanthan gum equiv. L^{-1}) in the vessel 1 and $16 \pm 21 \mu\text{g}$ Xanthan gum equiv. L^{-1} in the vessel 2 (Fig. 4.6). The TEP levels in the vessel 1 at day 36 were $918 \pm 247 \mu\text{g}$ Xanthan gum equiv. L^{-1} , and unfortunately, the data between days 38 and 40 were lost by a mistake during the sampling process. The TEP levels in the vessel 2 in days 40 were $1,107 \pm 195 \mu\text{g}$ Xanthan gum equiv. L^{-1} . I calculated the ratio of TEP/Chl *a* (TEP^*). The averaged TEP^* ratios in the exponential and stationary phases were $39 \pm 10 \mu\text{g}$ Xanthan gum equiv. $[\mu\text{g Chl } a]^{-1}$ and $22 \pm 4 \mu\text{g}$ Xanthan gum equiv. $[\mu\text{g Chl } a]^{-1}$. The ratio in the exponential phase was significantly higher than that in the stationary phase (Wilcoxon singed-rank test, $p < 0.0005$, $n = 10$). This would indicate that the TEP^* ratio of *T. nordenskioldii* was useful as an indicator of the TEP productivity.

Subsequently, the levels of cellular TEP productivity during the exponential and stationary phases were also estimated. Generally, the values decreased exponentially from day 0 to day 40, and those values ranged between 0.2 and 1,870 pg Xanthan gum equiv. $[\text{cell}]^{-1} \text{d}^{-1}$. Furthermore, the averaged cellular TEP productivity was 12-fold higher in the exponential phase ($25.1 \pm 0.2 \text{ pg}$ Xanthan gum equiv. $[\text{cell}]^{-1} \text{d}^{-1}$) than in the stationary phase ($2.0 \pm 0.5 \text{ pg}$ Xanthan gum equiv. $[\text{cell}]^{-1} \text{d}^{-1}$) (Table 4.1). These results indicated that the TEP productivity of *T. nordenskioldii* were highly variable. Fukao et al. (2012) showed that the values of cellular TEP productivity for *Co. granii* were ca. 3,200–8,200 pg Xanthan gum equiv. $[\text{cell}]^{-1} \text{d}^{-1}$. However, it should be noted that the incubation conditions differed between their and my experiments: the light to dark cycle (14 h:10 h), light intensity ($150\text{--}200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and temperature (10, 15, 20 and 25°C) in Fukao et al. (2012). Even if the light conditions of *Co. granii* were better than those (light:dark cycle = 12 hours:12 hours, and light intensity = ca. $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) of *T. nordenskioldii* in my laboratory experiment, the averaged TEP productivity of *T. nordenskioldii* were lower than *Co. granii*. On the other hand, the maximal potential ($1,870 \text{ pg}$ Xanthan gum equiv. $[\text{cell}]^{-1} \text{h}^{-1}$) of the TEP production in *T. nordenskioldii* could be comparable with the TEP productivity of *Co. granii*.

The chlorophyll *a*-normalized TEP productivities between days 20 and 36 for the vessel 1, and between days 18 and 38 for the vessel 2 were estimated from eq. (4.19).

The averaged values of the Chl *a*-normalized TEP productivities during the exponential and stationary phases were $6.3 \pm 3.4 \mu\text{g Xanthan gum equiv. } [\mu\text{g Chl } a]^{-1} \text{ d}^{-1}$ and $0.9 \pm 0.3 \mu\text{g Xanthan gum equiv. } [\mu\text{g Chl } a]^{-1} \text{ d}^{-1}$, respectively. The TEP productivities were also higher in the exponential phase than in the stationary phase, as well as the cellular TEP productivities. Therefore, it was considered that the high diatom biomass and TEP productivity during the Oyashio spring bloom might have contributed to high TEP levels in the seawater.

4.3.4 Relationship between DOC and TEP productivities

Dissolved organic carbon (DOC) concentrations increased from day 0 to day 40 (Fig. 4.9). The DOC concentrations in the vessels 1 and 2 at day 0 were $1,209 \mu\text{g C L}^{-1}$ and $1,086 \mu\text{g C L}^{-1}$, respectively. Unfortunately, the data at day 40 in the vessel 1 were lost by a mistake during the sampling process, and the DOC concentration at day 36 was $1,637 \mu\text{g C L}^{-1}$. The DOC concentration in the vessel 2 at day 40 was $1,818 \mu\text{g C L}^{-1}$.

The levels of cellular DOC productivity during the exponential and stationary phases were calculated. As a result, the productivity decreased exponentially from day 0 to day 40, and those values ranged between 1.1 and $1,799 \text{ pg C } [\text{cell}]^{-1} \text{ d}^{-1}$. The precursors of TEP are known to be dissolved acid polysaccharides (Engel, 2004; Thornton et al., 2007; Wurl et al., 2011). Since the TEP levels at day 0 in this experiment were almost zero, an origin of TEP should contribute to dissolved polysaccharides excreted by *T. nordenskiöldii*. However, unfortunately, the dissolved polysaccharides were not analyzed in this experiment. On the other hand, the DOC concentration was measured once per four days. Therefore, the relationship between the cellular TEP and DOC productivity was examined. In the exponential phase, the cellular TEP productivity significantly correlated with the cellular DOC productivity (Spearman's rank correlation, $\rho = 0.89$, $p < 0.0001$, $n = 13$) (Fig. 4.13). In contrast, no significant relationship between those productivities was found during the stationary phase (Spearman's rank correlation, $\rho = -0.60$, $p = 0.35$, $n = 5$). The results in the exponential phase suggested that the TEP were formed from a part of the DOC excreted from *T. nordenskiöldii*.

The averaged cellular DOC productivities during the exponential and stationary phases were $7.8 \pm 2.4 \text{ pg C } [\text{cell}]^{-1} \text{ d}^{-1}$ and $1.3 \pm 0.3 \text{ pg C } [\text{cell}]^{-1} \text{ d}^{-1}$, respectively (Table

4.1). Unexpectedly, the ratio of the TEP/DOC productivity was higher in the exponential phase (2.7 ± 0.01) than in the stationary phase (1.7 ± 0.6), and the ratios were consistently >1 (Table 4.1). This result showed that TEP productivity was higher than the DOC productivity. It was expected that (I) the significantly part of the DOC (including acid polysaccharides) excreted from *T. nordenskiöldii* once attach to the cell surface, and (II) the attachments may accumulate with time. Simultaneously, the attached matters were diffused from the cell surface to the ambient environment, or the fragments formed on the cell surface were released to the ambient environment. Indeed, it was observed that the Alcian blue stainable substances were attached to the cell surface of *T. nordenskiöldii* (Photo 4.2). Such substances should affect to the TEP levels. Therefore, the ratio of >1 might be due to the Alcian blue stainable substances attached on the cell surface. On the other hand, the averaged ratio of TEP/DOC productivity was higher in the exponential phase than in the stationary phase. Perhaps, the differences indicate that the composition of the DOC excreted from *T. nordenskiöldii* changed between the exponential and stationary phases.

4.3.5 POC and PN concentrations

Particulate organic carbon (POC) concentrations in the vessel 1 and 2 changed little between day 0 and day 16 ($277 \pm 87 \mu\text{g C L}^{-1}$ for the vessel 1, $247 \pm 85 \mu\text{g C L}^{-1}$ for the vessel 2), and thereafter the POC concentrations increased (Fig. 4.7). The POC concentrations in the vessels 1 and 2 at days 40 increased to 8,570 and to 11,593 $\mu\text{g C L}^{-1}$, respectively. Assuming that the carbon contents (TEP-C) in the TEP were the 75% as mentioned above, the TEP-C concentrations during this experiment were between 0 and 688 $\mu\text{g C L}^{-1}$ in the vessel 1, and between 12 and 830 $\mu\text{g C L}^{-1}$ in the vessel 2. I estimated the TEP-C/POC ratio from the TEP-C and POC concentrations. Interestingly, the ratio rapidly increased from day 0 (average 2%) to days 12 ($83 \pm 3\%$), and thereafter (i.e., days 32–40) it rapidly decreased to $10 \pm 4\%$ (Fig. 4.8). However, the cause of the high-variably ratios during the exponential phase could not be found.

Particle nitrogen (PN) concentrations were detected between days 18 and 40 (Fig. 4.7). The PN concentrations ranged between 43 $\mu\text{g N L}^{-1}$ and 333 $\mu\text{g N L}^{-1}$ in the vessel 1, and between 46 $\mu\text{g N L}^{-1}$ and 765 $\mu\text{g N L}^{-1}$ in the vessel 2. The ratio of PN/Chl *a* (N/Chl *a*) was not significantly difference between the exponential phase ($9 \pm 3 \mu\text{g N} [\mu\text{g Chl } a]^{-1}$) and the stationary phase ($10 \pm 7 \mu\text{g N} [\mu\text{g Chl } a]^{-1}$) (Table 4.1; Wilcoxon

singled-rank test, $p = 1.0$, $n = 4$). In contrast, a significant difference in the POC/Chl a (C/Chl a) ratio was found between those phases (Wilcoxon singled-rank test, $p < 0.05$, $n = 4$). The averaged ratios of POC/PN in the exponential and stationary phases also showed in the Table 4.1. The ratios were significantly higher in the stationary phase (22.4 ± 4.1) than in the exponential phase (7.9 ± 1.0) (Wilcoxon rank sum test, $p < 0.0001$, $n = 19$). This also indicated that *T. nordenskiöldii* during the stationary phase were in a nitrate deficient condition.

4.3.6 Relationship between the light levels, and DOC and POC productivities

The experiments of the photosynthesis–irradiance (P – E) curve were conducted at day 20 for the vessel 1 and at day 18 for the vessel 2 in the mid-exponential phase, and days 36 for the vessel and days 34 for the vessel in the stationary phase. The P – E curves of the Chl a -normalized POC were showed in Fig. 4.11A. Generally, the productivity was higher in the exponential phase (0.07 – $1.23 \mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1}$) than in the stationary phase (0.17 – $0.01 \mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1}$). The photosynthetic parameters estimated from the P – E curve were summarized in Table 4.2, and the values were also higher in the exponential phase than in the stationary phase. The maximum photosynthetic rate (P_{max}^*) was $1.29 \pm 0.10 \mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1}$ in the exponential phase and $0.15 \pm 0.02 \mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1}$ in the stationary phase. The initial slope (α^*) was $0.012 \pm 0.001 \mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1} [\mu\text{mol photon m}^{-2} \text{ s}^{-1}]^{-1}$ in the exponential phase and $0.002 \pm 0.0001 \mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1} [\mu\text{mol photon m}^{-2} \text{ s}^{-1}]^{-1}$ in the stationary phase. The values of the photoinhibition index (β^*) in the exponential and stationary phases were $0.00299 \pm 0.00025 \mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1} [\mu\text{mol photon m}^{-2} \text{ s}^{-1}]^{-1}$ and $0.00003 \pm 0.00001 \mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1} [\mu\text{mol photon m}^{-2} \text{ s}^{-1}]^{-1}$, respectively. The light saturation index (E_k) was $110 \pm 3 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ in the exponential phase and $76 \pm 7 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ in the stationary phase. The values of Chl a -normalized phytoplankton specific absorption coefficient ($\bar{a}_{\text{ph}}^*$) in the P – E experiments in the exponential and stationary phases were $0.0086 \pm 0.0005 \text{ m}^2 [\mu\text{g Chl } a]^{-1}$ and $0.0092 \pm 0.0004 \text{ m}^2 [\mu\text{g Chl } a]^{-1}$, respectively (Table 4.2). The value of $\Phi_{\text{Chl-a-c-max}}$ in the exponential phase estimated from α^* and $\bar{a}_{\text{ph}}^*$ was $0.031 \pm 0.003 \text{ mol C } [\text{mol photon}]^{-1}$, and the value in the stationary phase was $0.005 \pm 0.0002 \text{ mol C } [\text{mol photon}]^{-1}$. The productivity of DOC was clearly higher in the exponential phase than in the stationary phase as well as the POC productivity (Fig. 4.11B). The values of DOC productivity in the exponential and

stationary phases ranged between 0.020 and 0.133 $\mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1}$, and between 0.0002 and 0.010 $\mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1}$, respectively. In the exponential phase, the DOC productivities of $>1,000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ were significantly higher than the productivity of the $<1,000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Wilcoxon rank sum test, $p < 0.01$, $n = 18$).

Photosynthesis–irradiance (P – E) curves based on the cell-normalized (cellular) POC were showed in Fig. 4.12A. The productivity was higher in the exponential phase (1.0 – $16.7 \text{ pg C } [\text{cell}]^{-1} \text{ h}^{-1}$) than in the stationary phase (0.02 – $0.38 \text{ pg C } [\text{cell}]^{-1} \text{ h}^{-1}$). The photosynthetic parameters estimated from the P – E curve were summarized in Table 4.3, and the values were higher in the exponential phase than in the stationary phase, as well as the parameters of Chl a -normalized P – E curve. The maximum photosynthetic rates ($P_{\text{max}}^{\text{cell}}$) were $16.9 \pm 2.0 \text{ pg C } [\text{cell}]^{-1} \text{ h}^{-1}$ in the exponential phase and $0.33 \pm 0.04 \text{ pg C } [\text{cell}]^{-1} \text{ h}^{-1}$ in the stationary phase. The initial slope (α^{cell}) was $0.154 \pm 0.013 \text{ pg C } [\text{cell}]^{-1} \text{ h}^{-1} [\mu\text{mol photon m}^{-2} \text{ s}^{-1}]^{-1}$ in the exponential phase, whereas that was $0.004 \pm 0.0002 \text{ pg C } [\text{cell}]^{-1} \text{ h}^{-1} [\mu\text{mol photon m}^{-2} \text{ s}^{-1}]^{-1}$ in the stationary phase. The values of the photoinhibition index (β^{cell}) in the exponential and stationary phases were $0.0385 \pm 0.0012 \text{ pg C } [\text{cell}]^{-1} \text{ h}^{-1} [\mu\text{mol photon m}^{-2} \text{ s}^{-1}]^{-1}$ and $0.0001 \pm 0.00003 \text{ pg C } [\text{cell}]^{-1} \text{ h}^{-1} [\mu\text{mol photon m}^{-2} \text{ s}^{-1}]^{-1}$, respectively. The light saturation index (E_k) was $109 \pm 4 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ in the exponential phase, whereas that was $75 \pm 6 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$ in the stationary phase. The values of cellular phytoplankton specific absorption coefficient ($\bar{a}_{ph}^{\text{cell}}$) in the exponential and stationary phases were $113 \pm 5 \times 10^{-12} \text{ m}^2 [\text{cell}]^{-1}$ and $19 \pm 1 \times 10^{-12} \text{ m}^2 [\text{cell}]^{-1}$, respectively (Table 4.3). The value of $\Phi_{\text{cell-c-max}}$ in the exponential phase was $0.032 \pm 0.003 \text{ mol C } [\text{mol photon}]^{-1}$, and the value in the stationary phase was $0.005 \pm 0.0005 \text{ mol C } [\text{mol photon}]^{-1}$. The productivity of DOC was clearly higher in the exponential phase than in the stationary phase (Fig. 4.12B). In the exponential phase, the values of DOC productivity under $>1,000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ were significantly higher than those under $<1,000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Wilcoxon rank sum test, $p < 0.01$, $n = 18$), as well as the Chl a -normalized DOC productivity.

The values of the PER estimated from the DOC and POC productivities ranged between 2 and 57% in the exponential phase and between 0.2 and 5% in the stationary phase (Fig. 4.14). The values in the exponential phase were significantly higher than those in the stationary phase (Wilcoxon singled-rank test, $p < 0.001$, $n = 18$). In the exponential phase, the percentages of the low light intensity ($<100 \mu\text{mol photons m}^{-2}$

s⁻¹) were relatively high compare to those of the mid light intensity (100 – ca. 1,000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) (Wilcoxon rank sum test, $p < 0.001$, $n = 14$). This could relate with relatively high DOC productivity (Figs. 4.11B and 4.12B). It has been reported that the DOC productivity in the low light intensity was higher than that of the high light intensity, and the PER increased (Zlotnik and Dubinsky, 1989; Marañón et al., 2004). Moreover, in the exponential phase, the PRE values in the high PAR ($>1,700 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) were significantly higher than those in the low and middle PAR (ca. $<1,000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) (Wilcoxon rank sum test, $p < 0.05$, $n = 18$). The high percentage contributed to both the photoinhibition of the POC production and the increase in the DOC productivity in high PAR (ca. $>1,000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) (Figs. 4.11 and 4.12). In contrast, the results of the $P-E$ curve experiment in the Ría de Bigo (Galicia, Spain) showed that the photoinhibition of the POC production and the increase in the DOC productivity did not occur even for the high light intensity of ca. $1900 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, and the PER values were low (ca. 10%) (Marañón et al., 2004). In the sea surface of the Oyashio cruise in Chapter 3, the maximum level of PAR in the April was $1,400 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, and that in May reached to $2,200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Based on the $P-E$ curve experiment of *T. nordenskiöldii*, it was suggested that the DOC productivity by phytoplankton in the sea surface was highest within the water column. Since the TEP formations mainly contribute to the DOC excreted by phytoplankton as described above (section 4.3.4) and some papers (e.g., Passow, 2002b; Engel, 2004; Wurl et al., 2011), the TEP productivities might be high in the sea surface. In contrast, the sunlight was reported to decompose the DOC (Stubbins et al., 2012; Helms et al., 2013; Yamashita et al., 2013). Therefore, the TEP formations in the sea surface would be influenced to the intensity of sunlight. Further studies of the relationship between the DOC production by phytoplankton and the DOC decomposition by sunlight are necessary.

Table 4.1 Summary of the results in the exponential and stationary phases. μ : specific growth rate; M : division rate; POC: particulate organic carbon; PN: particulate nitrogen; C: carbon; Chl a : chlorophyll a ; TEP: transparent exopolymer particles; DOC: dissolved organic carbon.

Growth phase	Culture vessel	Term (day)	μ (d ⁻¹)	M (division d ⁻¹)	POC/PN ratio	C/Chl a ratio*	N/Chl a ratio*	Cellular Xanthan gum equiv. [pg Xanthan gum equiv. [cell] ⁻¹ d ⁻¹]	Cellular DOC productivity (pg C [cell] ⁻¹ d ⁻¹)	Ratio of the TEP/DOC productivity
Exponential	1	0 - 28	0.30	0.43	7.4 ± 1.5			25.3	9.5	2.7
Exponential	2	0 - 26	0.33	0.48	8.2 ± 0.9			24.9	9.3	2.7
Exponential	Average of 1 & 2		0.31 ± 0.02	0.45 ± 0.03	7.9 ± 1.0	70 ± 14 (n = 4)	9 ± 3 (n = 4)	25.1 ± 0.2	7.8 ± 1.7	2.7 ± 0.01
Stationary	1	30 - 40	0.04	0.06	21.8 ± 4.2			2.6	1.1	2.3
Stationary	2	28 - 40	0.02	0.03	22.8 ± 4.3			1.5	1.5	1.0
Stationary	Average of 1 & 2		0.03 ± 0.01	0.05 ± 0.02	22.4 ± 4.1	196 ± 92 (n = 4)	10 ± 7 (n = 4)	2.0 ± 0.5	1.3 ± 0.3	1.7 ± 0.6

* Data were calculated from days 20, 28 and 36 of the vessel 1, and from days 18, 20, 28, 34 and 40 of the vessel 2

Table 4.2 Summary of the results obtained in the Chl *a*-normalized photosynthetic-irradiance ($P-E$) curve experiments. $\bar{a}_{ph}^*$: mean chlorophyll (Chl) *a*-specific absorption coefficient of phytoplankton; P_{max}^* : Chl *a*-normalized maximum photosynthetic rate; α^* : the initial slope; β^* : the photo-inhibition index; E_k : light saturation index; $\Phi_{Chl-a-c-max}$: the maximum quantum yield of carbon fixation.

Growth phase	Culture vessel	Day	Chl <i>a</i> concentration ($\mu\text{g L}^{-1}$)	Chl <i>a</i> /cell ratio (pg [cell] $^{-1}$)	$\bar{a}_{ph}^*$ ($\text{m}^2 [\mu\text{g Chl } a]^{-1}$)	P_{max}^* ($\mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1}$)	α^* ($\mu\text{g C } [\mu\text{g Chl } a]^{-1} \text{ h}^{-1}$ [$\mu\text{mol photon m}^{-2} \text{ s}^{-1}$] $^{-1}$)	β^* ($\mu\text{mol photon m}^{-2} \text{ s}^{-1}$] $^{-1}$)	E_k ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	$\Phi_{Chl-a-c-max}$ (mol C [mol photon] $^{-1}$)
Exponential	1	20	6.1 ± 0.1	13.6	0.0084 ± 0.0005	1.39	0.012	0.00274	113	0.034 ± 0.002
Exponential	2	18	7.1 ± 0.1	12.4	0.0091 ± 0.0003	1.19	0.011	0.00323	106	0.029 ± 0.001
Exponential	Average of 1 & 2		6.6 ± 0.6	13.0 ± 0.6	0.0086 ± 0.0005	1.29 ± 0.10	0.012 ± 0.001	0.00299 ± 0.00025	110 ± 3	0.031 ± 0.003
Stationary	1	36	42.5 ± 0.3	2.2	0.0095 ± 0.0004	0.13	0.002	0.00002	69	0.005 ± 0.0001
Stationary	2	34	42.6 ± 1.5	1.9	0.0089 ± 0.0003	0.17	0.002	0.00005	82	0.005 ± 0.0001
Stationary	Average of 1 & 2		42.5 ± 1.0	2.1 ± 0.2	0.0092 ± 0.0004	0.15 ± 0.02	0.002 ± 0.0001	0.00003 ± 0.00001	76 ± 7	0.005 ± 0.0002

Table 4.3 Summary of the results obtained in the cell-normalized photosynthetic-irradiance (P - E) curve experiments. $\bar{a}^{*cell}_{ph}$: mean cell-specific absorption coefficient of phytoplankton; P^{cell}_{max} : cell-normalized maximum photosynthetic rate; α^{cell} : the initial slope; β^{cell} : the photo-inhibition index; E_k : light saturation index; $\Phi_{cell-c-max}$: the maximum quantum yield of carbon fixation.

Growth phase	Culture vessel	Day	Cell abundance (cells mL ⁻¹)	$\bar{a}^{cell}_{ph}$ ($\times 10^{12} m^2 [cell]^{-1}$)	P^{cell}_{max} (pg C [cell] ⁻¹ h ⁻¹)	α^{cell} (pg C [cell] ⁻¹ h ⁻¹ [$\mu mol\ photon\ m^{-2}\ s^{-1}$] ⁻¹)	β^{cell} (pg C [cell] ⁻¹ h ⁻¹ [$\mu mol\ photon\ m^{-2}\ s^{-1}$] ⁻¹)	E_k (μmol photons m ⁻² s ⁻¹)	$\Phi_{cell-c\ max}$ (mol C [mol photon] ⁻¹)
Exponential	1	20	448 $\pm$ 14	115 $\pm$ 5	18.9	0.167	0.0373	113	0.034 $\pm$ 0.002
Exponential	2	18	571 $\pm$ 63	111 $\pm$ 4	14.9	0.140	0.0398	106	0.029 $\pm$ 0.001
Exponential	Average of 1 & 2		510 $\pm$ 80	113 $\pm$ 5	16.9 $\pm$ 2.0	0.154 $\pm$ 0.013	0.0385 $\pm$ 0.0012	109 $\pm$ 4	0.032 $\pm$ 0.003
Stationary	1	36	19435 $\pm$ 883	20 $\pm$ 1	0.28	0.004	0.0001	69	0.005 $\pm$ 0.0001
Stationary	2	34	22062 $\pm$ 1553	18 $\pm$ 1	0.37	0.005	0.0001	82	0.006 $\pm$ 0.0001
Stationary	Average of 1 & 2		20748 $\pm$ 1834	19 $\pm$ 1	0.33 $\pm$ 0.04	0.004 $\pm$ 0.0002	0.0001 $\pm$ 0.00003	75 $\pm$ 6	0.005 $\pm$ 0.0005

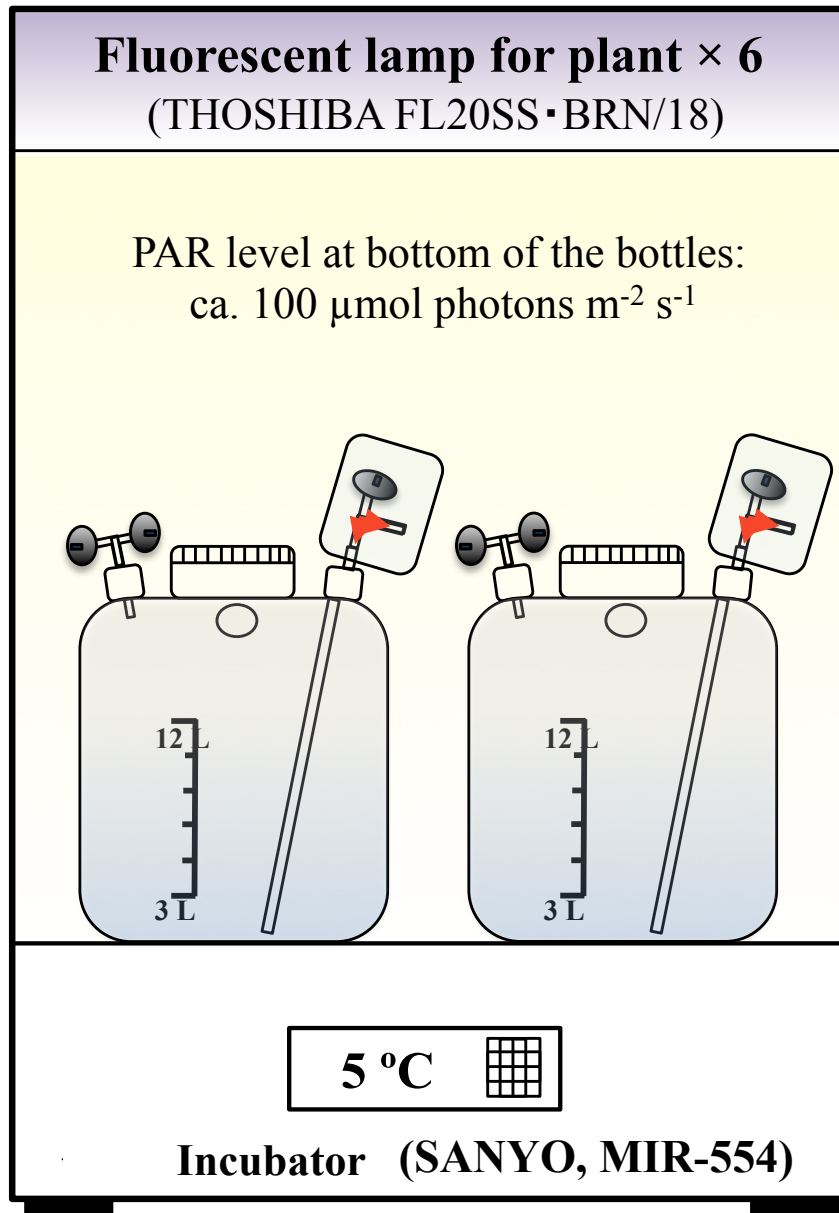


Fig. 4.1 Schematic figure in this experiment. The two 20-L culture vessels were stored in the incubator maintained at 5°C. Six fluorescent lamps were mounted to the upper part in the incubator, and photosynthetic available radiation (PAR) of ca. 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at the base of the bottle was exposed with light dark-cycle of 12 hours vs. 12 hours.

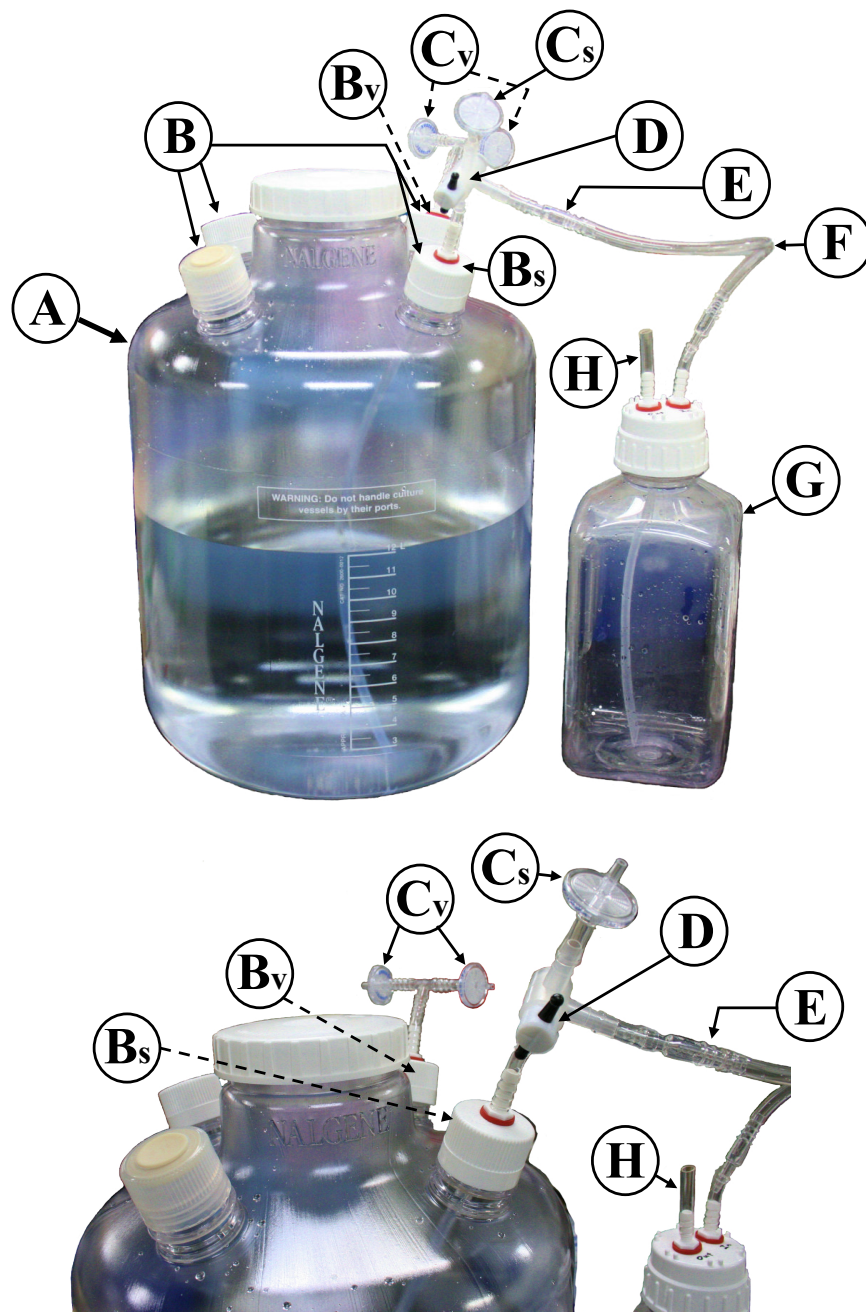


Fig. 4.2 Explanation of the sampling system. The culture experiment was conducted with the 20-L culture vessels (A) with four ports (B). Two ports of the four ports were used for the vent port (B_v) to exchange the air between inside and outside the vessel, and for the sampling port (B_s), respectively. The vent port was mounted the two disposable inline filters (C_v). The sampling port was installed a three-way cock (D) with the inline filter (C_s). When sampling is carried out, the sampling tubing (F) extended from a sampling bottle (G) was connected with the joint (E) extended from the three-way cock (D). Subsequently, the three-way cock was twisted from the atmosphere opening through the inline filter (C_s) to the sampling bottle (G), and an aspirator was connected with the outlet tubing (H) of the sampling bottle (G). The air pressure in the sampling bottle (G) was lowered with the aspirator. Therefore, the water sample was transferred from the 20-L culture vessel to the sampling bottle. After sampling, the three-way cock (D) was re-twisted to the atmosphere opening through the inline filter (C_s), and then the sampling tubing (F) was removed from the joint (E).

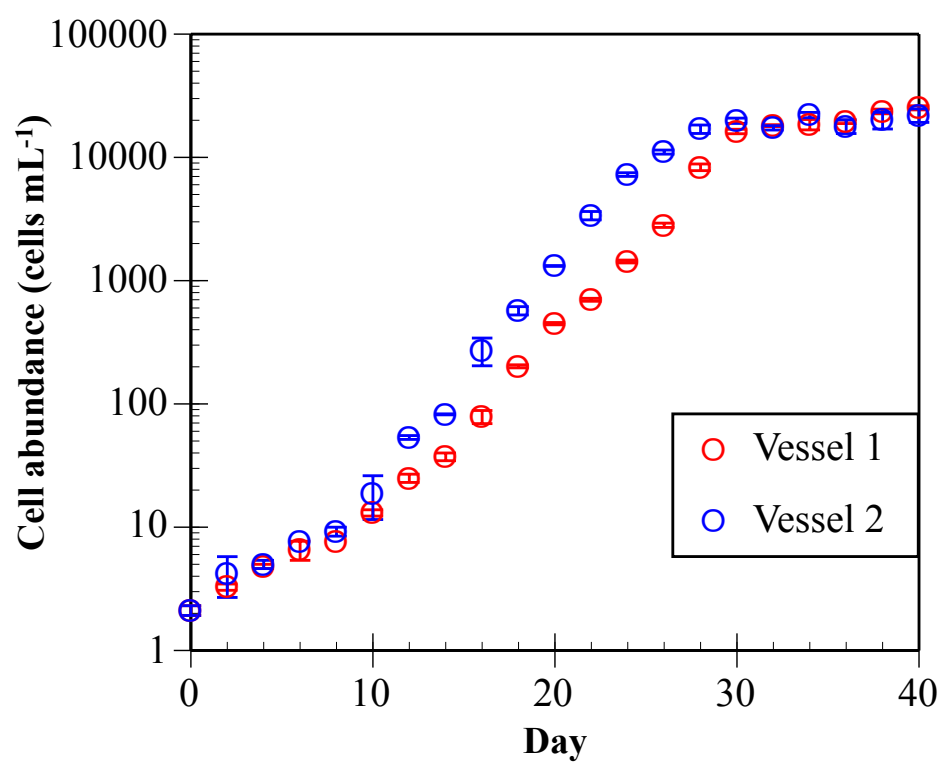


Fig. 4.3 Cell abundances in the culture vessels 1 and 2. The error bar shows the standard deviation ($n = 2$).

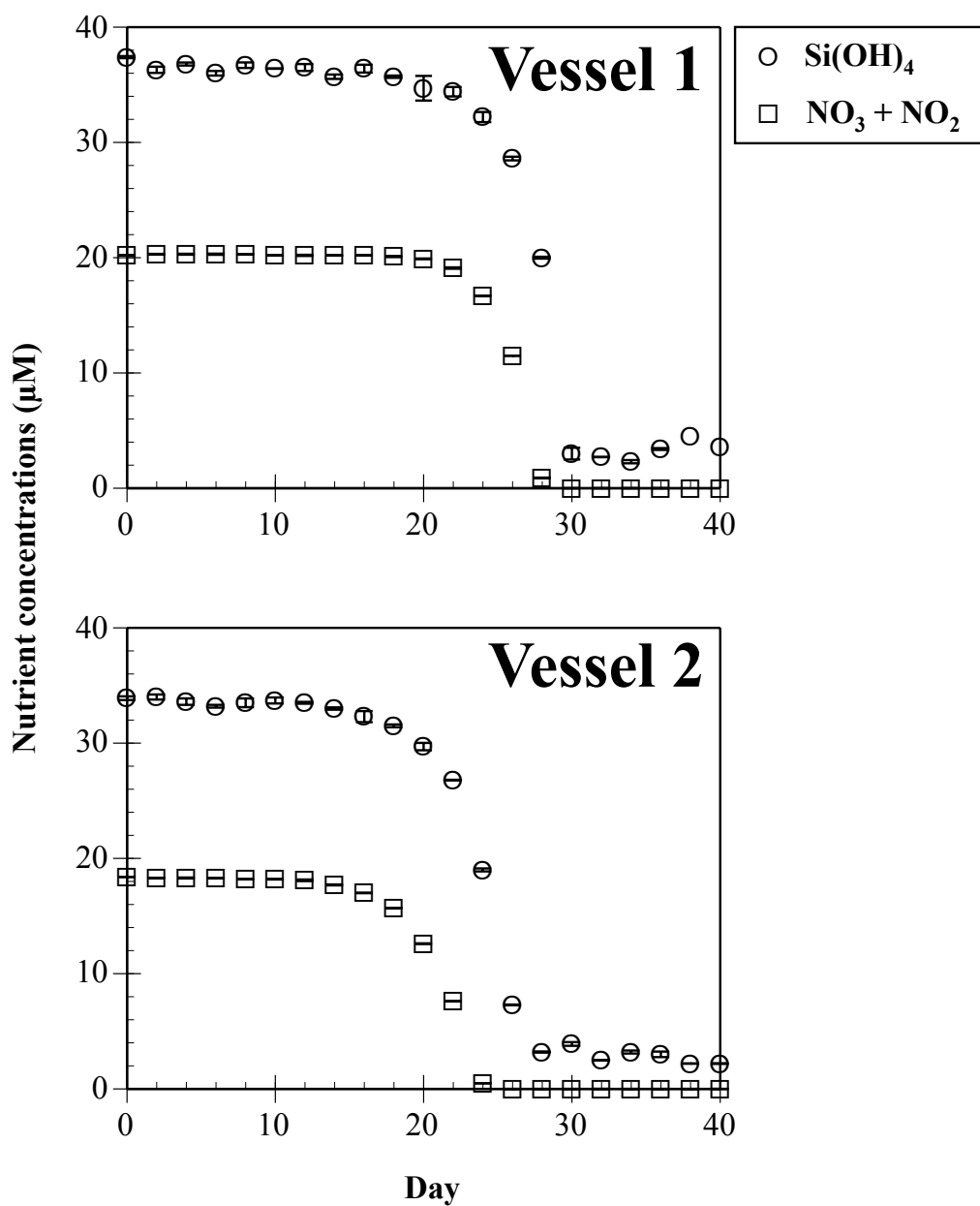


Fig. 4.4 Nitrate (NO_3) plus nitrite (NO_2), and silicate (Si(OH)_4) concentrations in the culture vessels 1 and 2 during this experiment. The error bars show the standard deviation ($n = 2$).

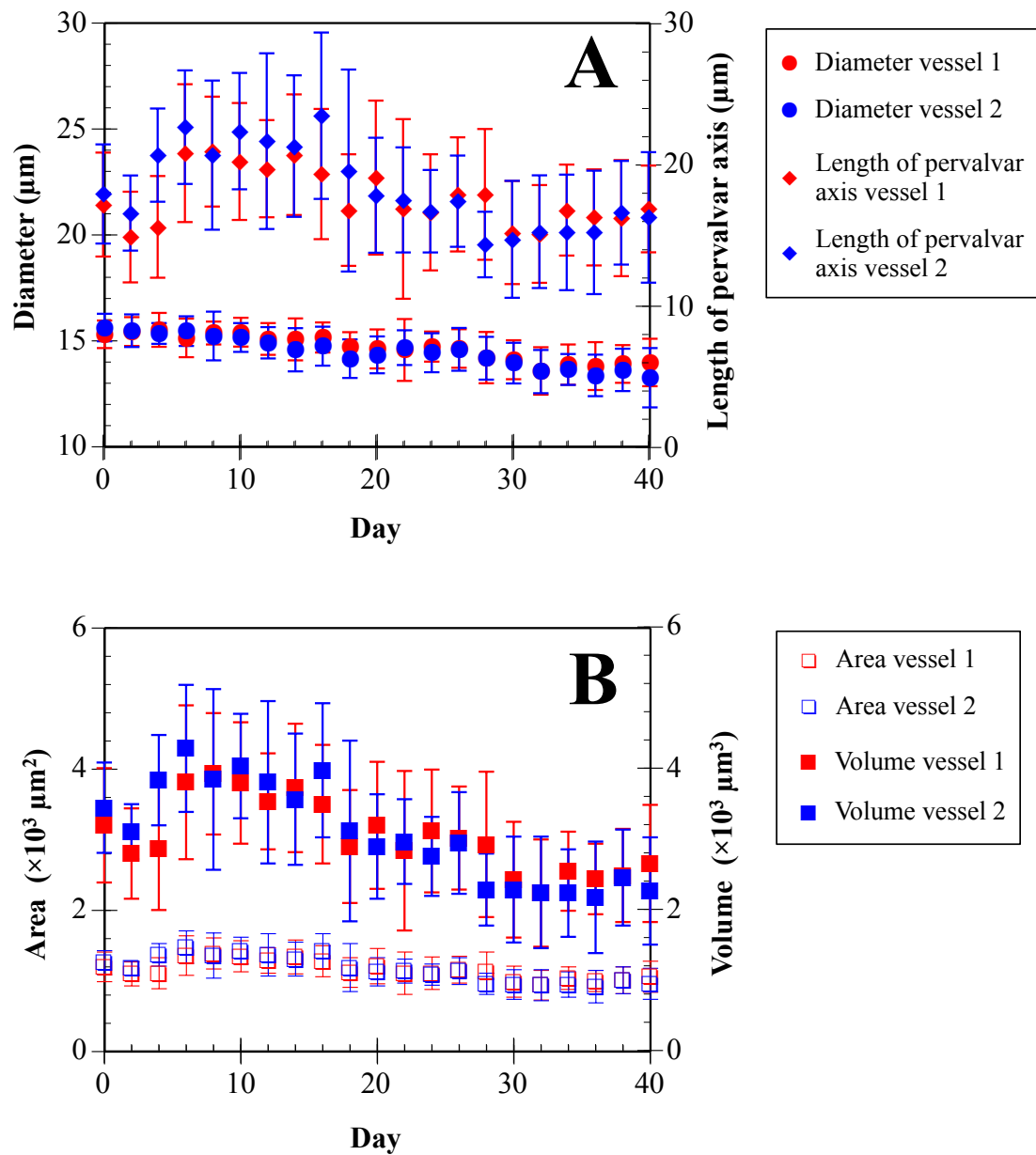


Fig. 4.5 Lengths of the averaged cell diameter and pervalvar axis (A), and the averaged area and volume (B). The error bars show the standard deviation ($n = 11$ for days 0–10; $n = 21$ for days 11–40).

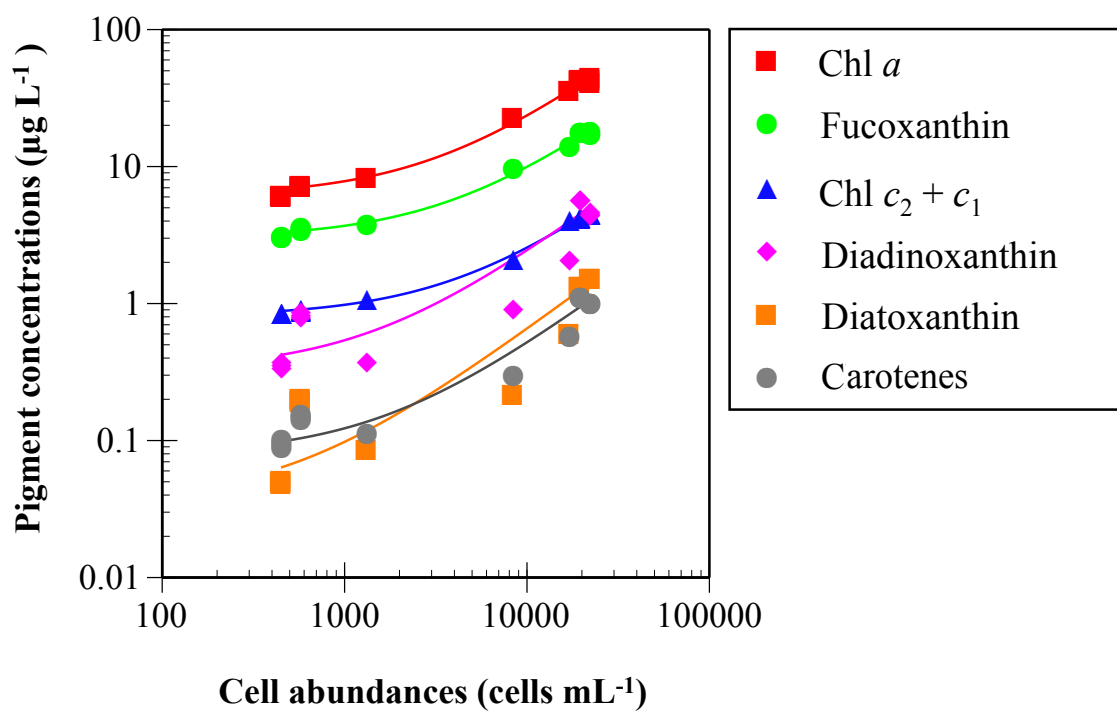


Fig. 4.6 Relationships between the pigment concentrations and the cell abundances. All pigments were carried out the linear fitting.

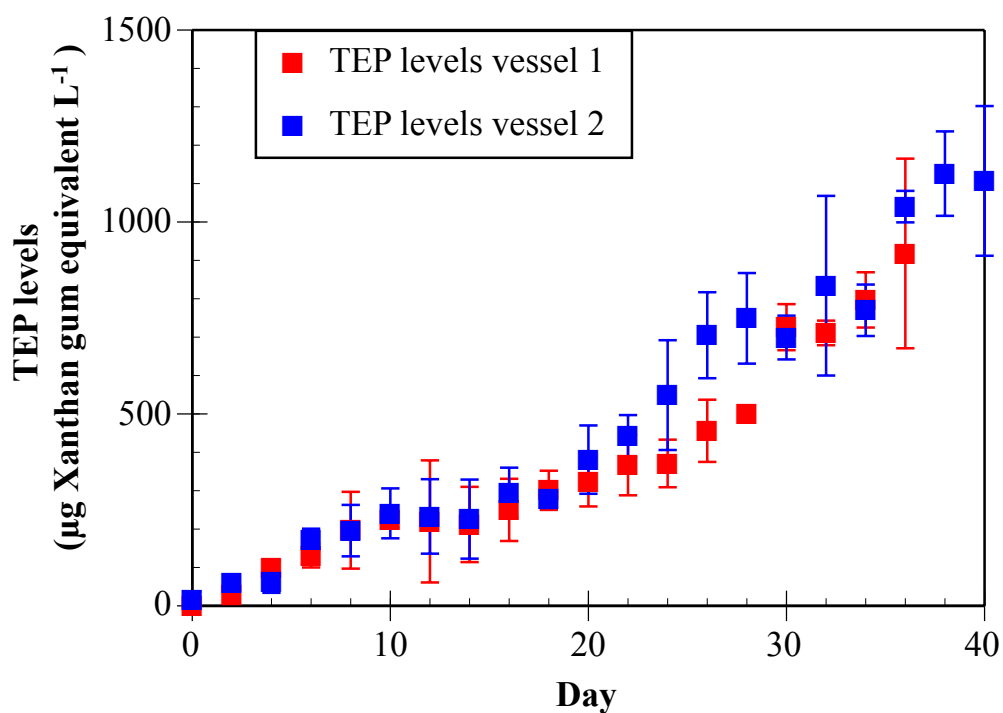


Fig. 4.7 Figure of the TEP levels. The levels increased with days. Unfortunately, the data of days 38 and 40 in the vessel 1 were lost by a mistake during the sampling process. The error bar shows the standard deviation ($n = 3$).

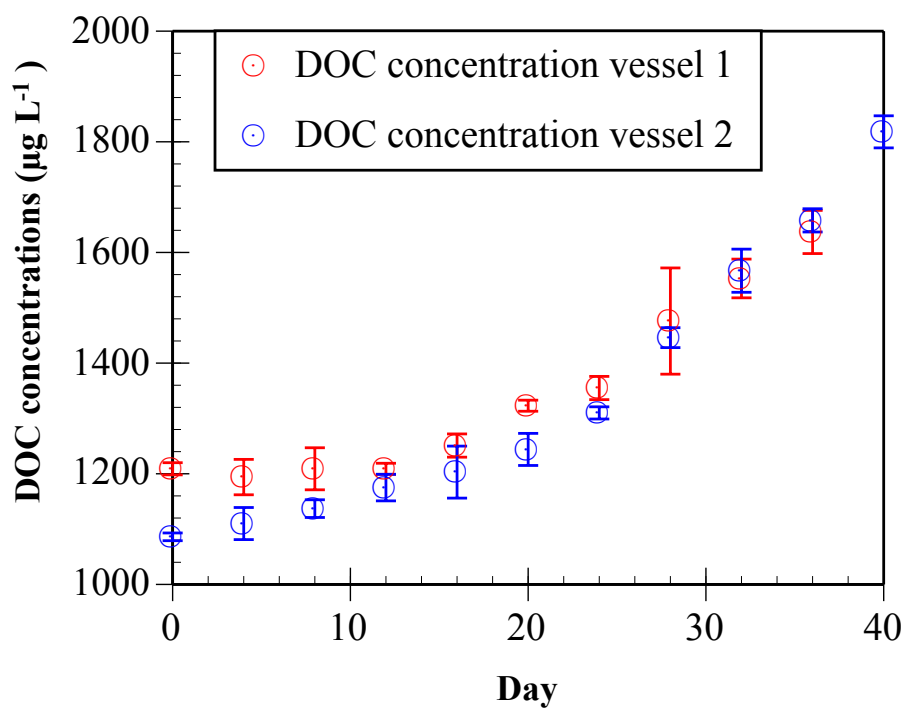


Fig. 4.8 Dissolved organic carbon (DOC) concentrations. Unfortunately, the data of days 40 in the vessel 1 were lost by a mistake during the sampling process. The error bar shows the standard deviation ($n = 5$).

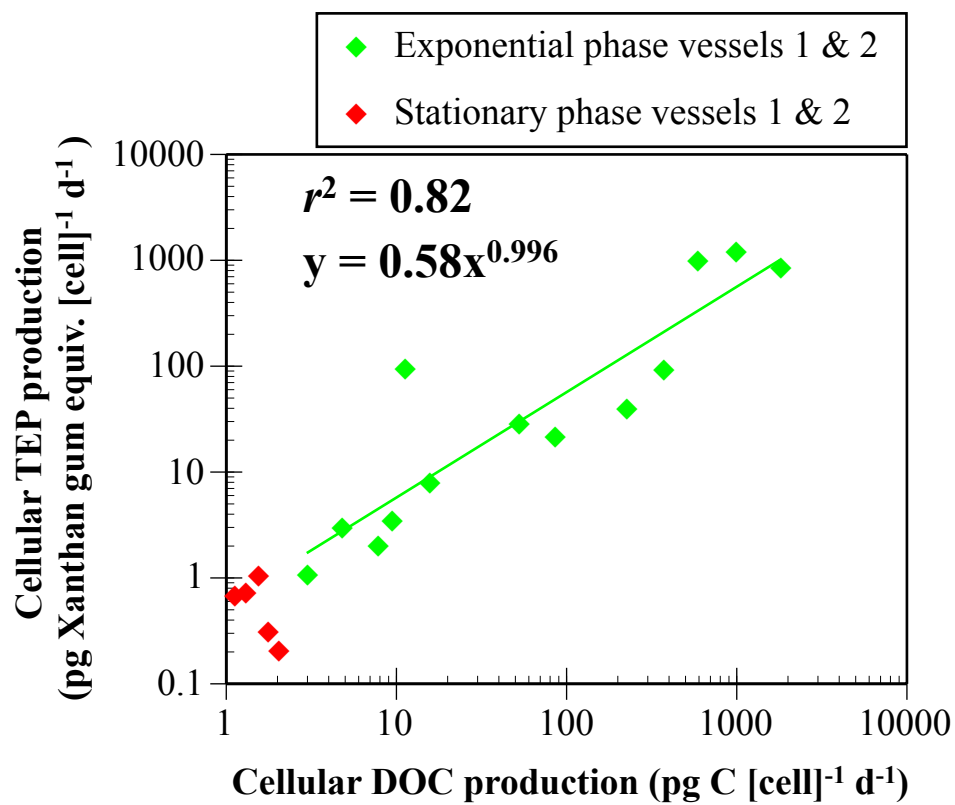


Fig. 4.9 Relationship between the cellular TEP and DOC production.

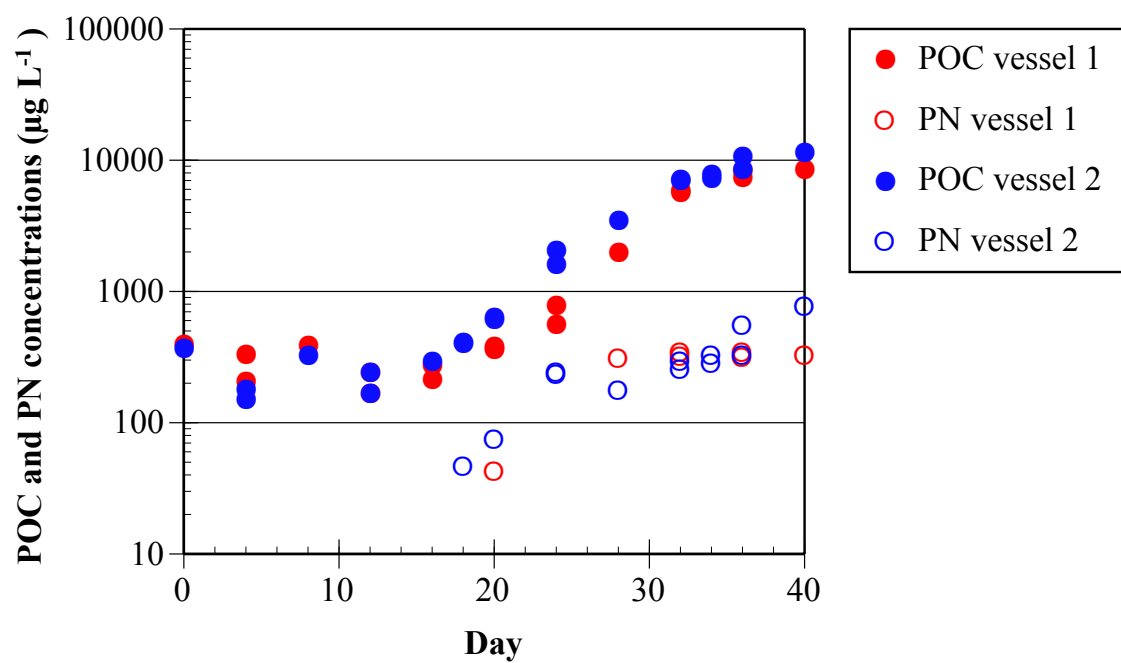


Fig. 4.10 Particulate organic carbon (POC) and particulate nitrogen (PN) concentrations. For the PN, the concentrations during days 0–16 could be not detected due to the detection limit.

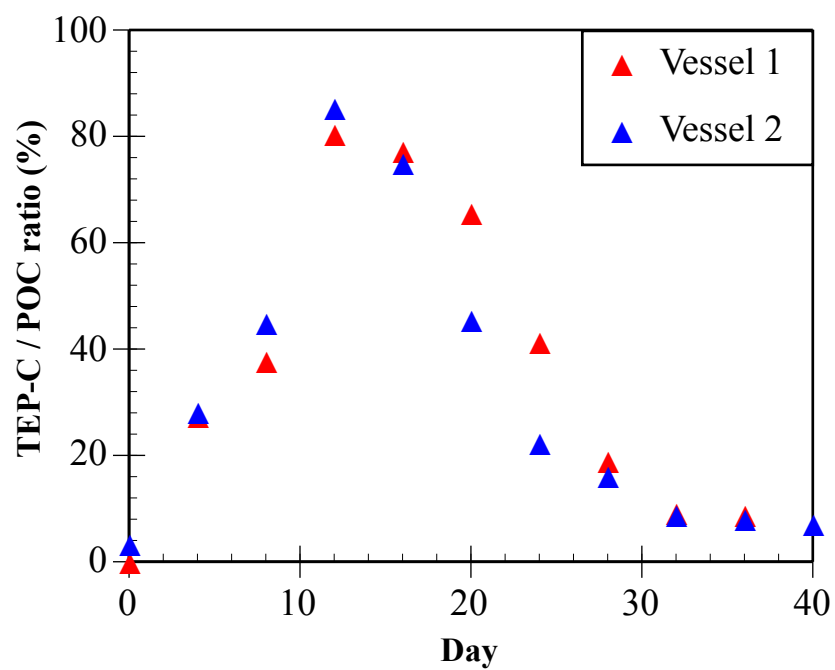


Fig. 4.11 Percentages of the TEP-C/POC concentrations in the vessels 1 and 2.

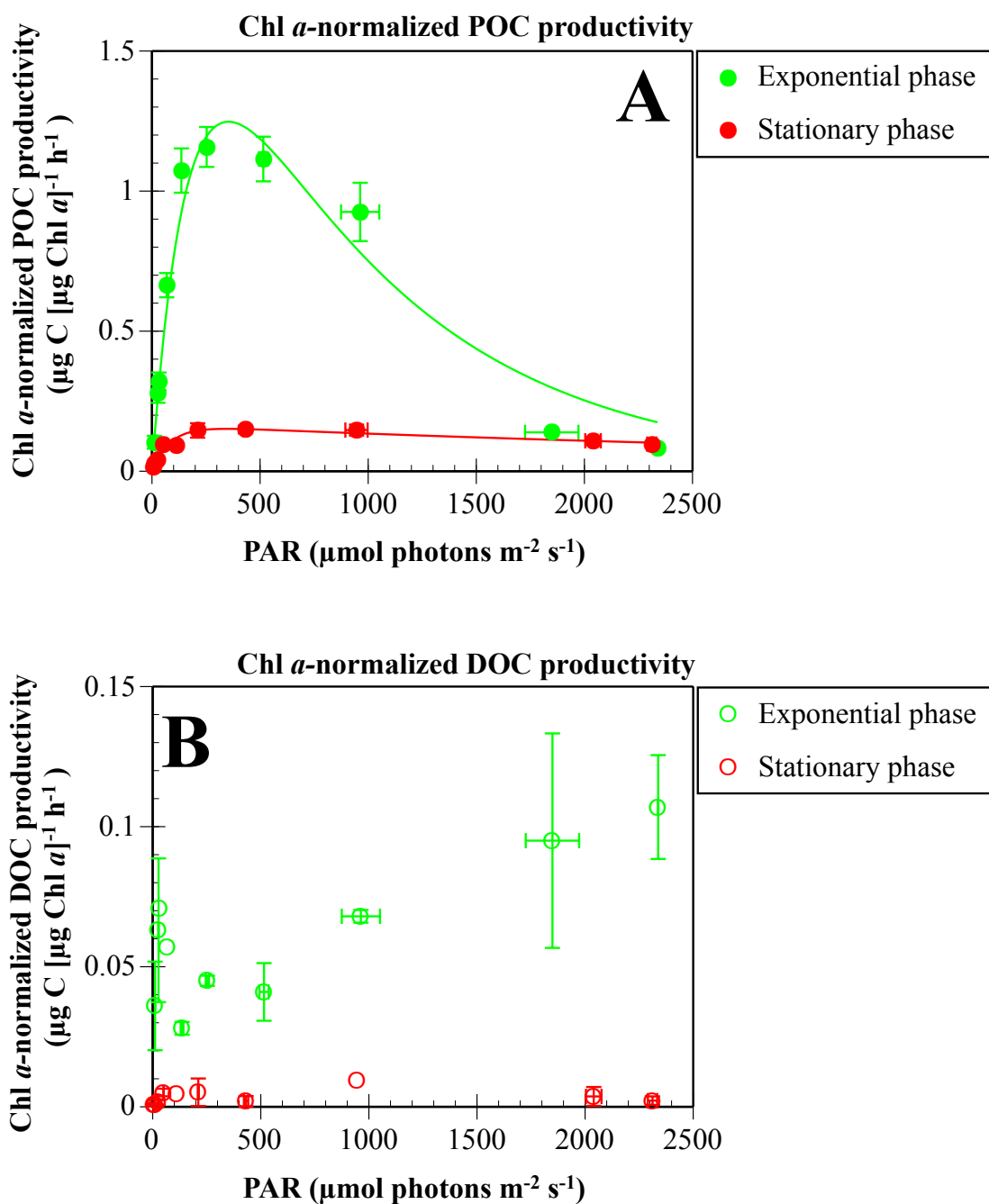


Fig. 4.12 Chl *a*-normalized particulate organic carbon (POC) productivity (A) and dissolved organic carbon (DOC) productivity (B) in the exponential and stationary phases. The error bars show the standard deviation ($n = 2$).

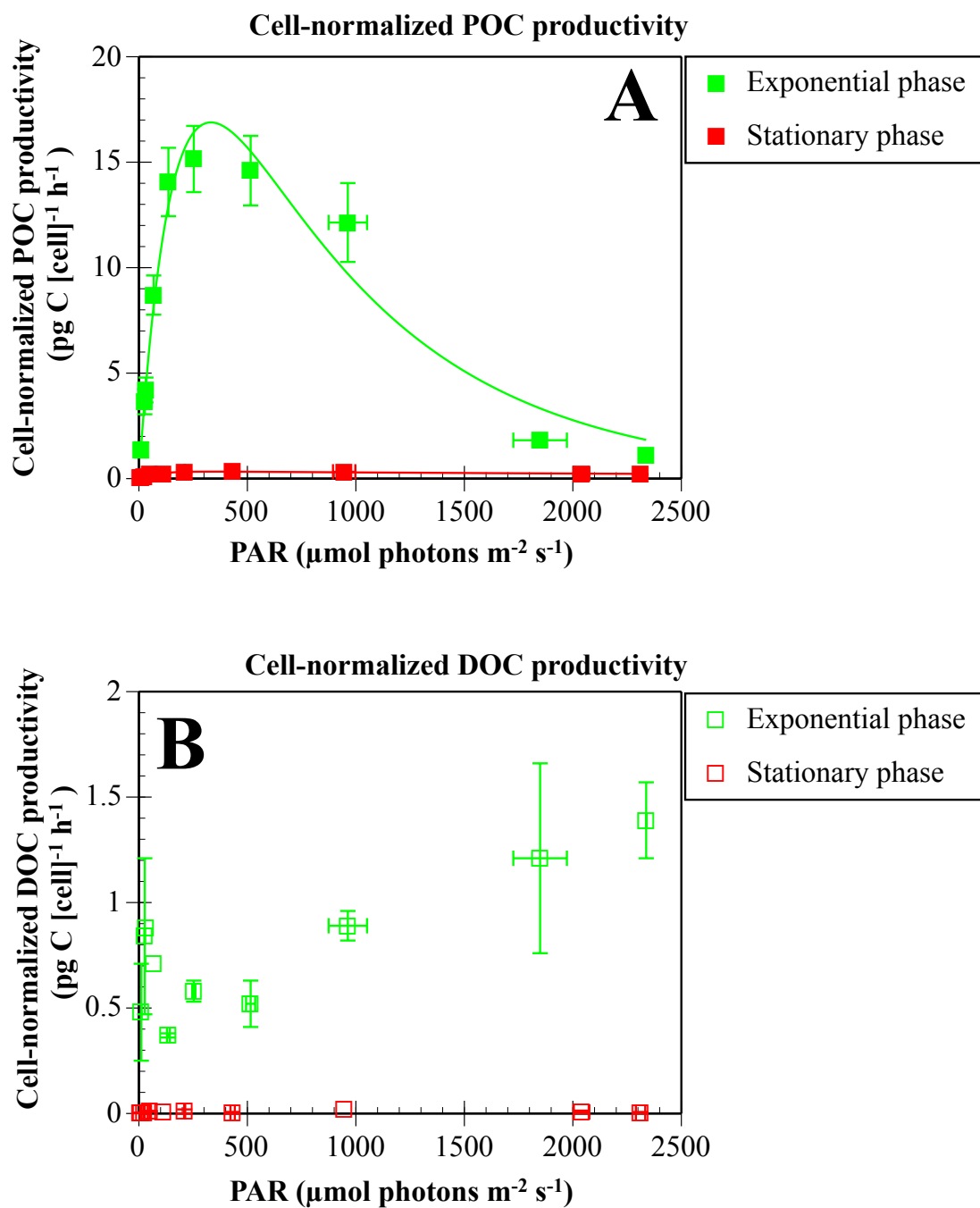


Fig. 4.13 Cell-normalized particulate organic carbon (POC) productivity (A) and dissolved organic carbon (DOC) productivity (B) in the exponential and stationary phases. The error bars show the standard deviation ($n = 2$).

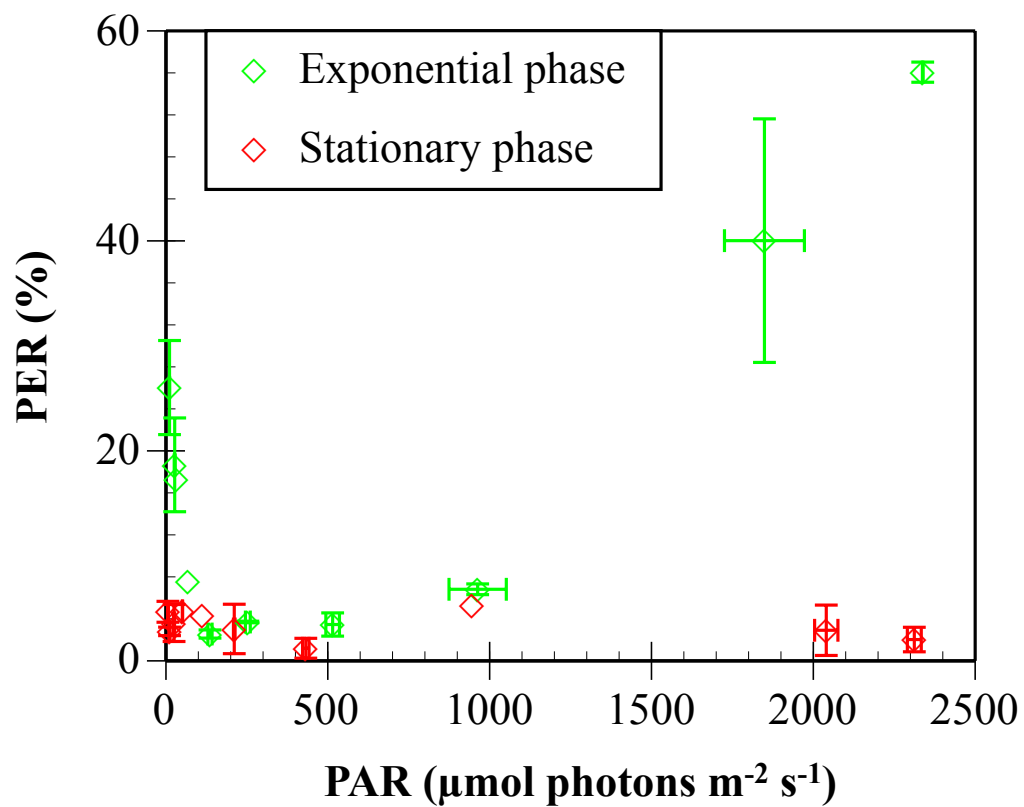


Fig. 4.14 Ratios (PER) of the DOC/Total production. The error bar shows the standard deviation ($n = 2$).

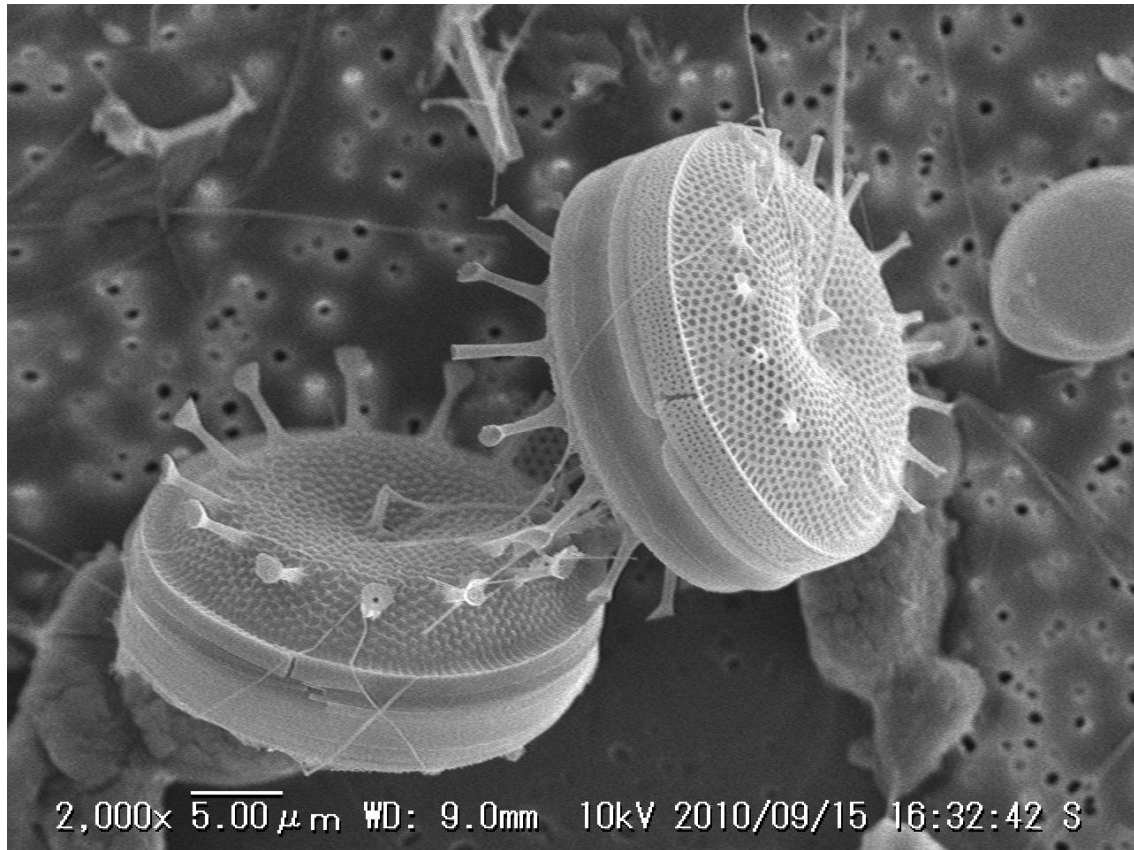


Photo 4.1 *Thalassiosira nordenskiöldii* photographed with Scanning electronic microscope (SEM).

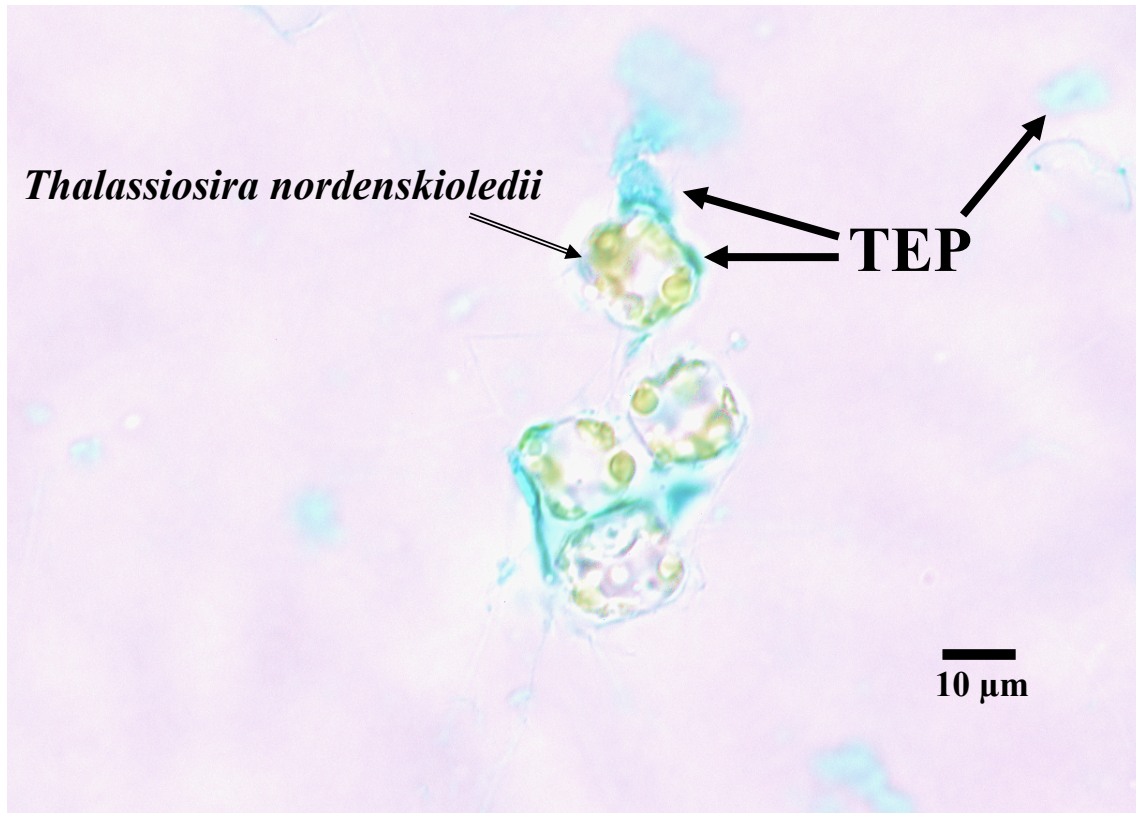


Photo 4.2 *Thalassiosira nordenskiöldii* and TEP in this experiment were photographed with a optical microscope. The TEP were attaching to the surface of *T. nordenskiöldii*. The four cells of the center in the photo were *T. nordenskiöldii*. The Blue substances were TEP stained by the Alcian blue.

Chapter 5 – General conclusions and perspectives

5.1 General conclusions

Main primary producer in the ocean is phytoplankton. The massive organic matter produced by phytoplankton support the almost all heterotrophic organisms in the ocean. Simultaneously, the carbon fixation from inorganic carbon to organic carbon by phytoplankton has affect to the carbon cycles not only in the ocean but also in the atmosphere. Atmospheric CO₂ has increased from the industrial revolution. This originates in the burning of fossil fuels. The level rose 140% from 280 ppm at pre-industrial revolution (1750) to 390 ppm at present (2011). The CO₂ dissolved in the ocean surface can use for photosynthesis by phytoplankton. A part of the CO₂ fixed into organic carbon by the phytoplankton may be transported from the surface to deeper layer (called biological carbon pump), and the organic carbon is decomposed by the respiration of heterotrophic organisms such as bacteria in the deep ocean. In the deep layer, it is considered that the seawater is transported by deep circulation (i.e., the great ocean conveyor belt). Therefore, the carbon transported to the deep ocean is fixed during up to ca. 2,200 years in the ocean, and the upwelling occur in the some regions of the world's ocean. Since the upwelling current transports the various substances such as nutrients from the deep layer to the surface, the regions are the higher primary production than other oceans.

Western subarctic Pacific is located in the upwelling region. Massive phytoplankton blooms in the spring occurs by both of the stratification within the water column and the relief of light limitation. During the spring phytoplankton bloom, the primary productivity was reported to reach 3,200 mg C m⁻² d⁻¹, and the high values of the water-column light utilization efficiency (Ψ) of phytoplankton photosynthesis were estimated. In contrast, the western subarctic gyre (WSG) during summer have been recognized as high-nitrate, low-chlorophyll (HNLC) waters, and the HNLC phenomenon in the WSG mainly attributable to low iron availability of phytoplankton and high zooplankton grazing. The observations of the Ψ in the WSG during summer showed that the variability was very high. Therefore, in Chapter 2, I investigated the Ψ in the WSG of the North Pacific during summer 2008. The Ψ values (0.64–1.86 g C [g Chl *a*]⁻¹ [mol photon]⁻¹ m²) obtained significantly increased with decreasing daily PAR

(photosynthetic available radiation) and were generally higher than those of previous studies, not only from the subarctic Pacific but also from the world's oceans. To examine the effect of iron availability on Ψ in the WSG, Ψ values were estimated from the data of two in situ iron fertilization experiments: the Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study I (SEEDS-I) and II (SEEDS-II). The results found that iron availability did not affect the Ψ values. Overall, in Chapter 2 revealed that Ψ values changed remarkably in the WSG during the summer and that higher values were found at the stations where moderate PAR levels (ca. 10–30 mol photons $\text{m}^{-2} \text{d}^{-1}$) were observed and autotrophic flagellates predominated in the phytoplankton assemblages. The high values of the water-column light utilization efficiency (Ψ) of phytoplankton photosynthesis can contribute to the high efficiency of biological carbon pumping observed in the western subarctic Pacific.

For increase the biological carbon pump efficiency, the particle size was one of the important factors. Transparent exopolymer particles (TEP) are very sticky particles that exhibit the characteristics of gels, and particularly important for the aggregate of particles. The TEP concentrations in the seawater relate with the phytoplankton bloom, and in particular, the production of the TEP or TEP precursors of the diatoms are known to be relatively high, compared with the other phytoplankton groups. The Oyashio region in the western subarctic Pacific is reported that the high productivity and biological pump efficiency are high during spring diatom bloom. However, the TEP in the Oyashio region have not been investigated to date. Then, in Chapter 3, I studied the dynamics of the TEP in the Oyashio region during spring diatom blooms of 2010 and 2011. The TEP levels were highest in April (171 μg Xanthan gum equivalent L^{-1}), and generally decreased from April to June. From the summary of the TEP levels in the world's oceans, it comprehended that the variability of the TEP levels was very high (0–14,800 μg Xanthan gum equiv. L^{-1}), and that generally, the high levels were found in the semi-closed regions such as estuary, bay and sea ice. Chlorophyll (Chl) *a* concentrations in the mixed layer during Oyashio spring phytoplankton bloom were significantly correlated with the TEP levels. Therefore, it was suggested that the TEP were formed from the TEP precursors excreted by phytoplankton. However, the Chl *a*-normalized TEP levels increased from April to June. This might show that the cellular TEP productivities of the diatoms in the Oyashio spring bloom were relatively low compare with the other phytoplankton groups appeared in June. On the other hand, the

dissolved organic carbon (DOC) productivity was investigated as the first study in the Oyashio region. The DOC productivity changed little during the spring bloom ($1.3\text{--}4.2\ \mu\text{g C m}^{-3}\ \text{d}^{-1}$). Since it is reported that the TEP were mainly formed from the dissolved acid polysaccharides excreted by phytoplankton, the relationship between the TEP levels and the DOC productivity in the sea surface was examined. However, no significant relationship was found between those. It was suggested that the TEP or TEP precursors produced by phytoplankton in the Oyashio region during the spring bloom were affected to decomposition by bacteria, and to predation by zooplankton. Hence, to evaluate the net TEP production by diatom, and to recognize the mechanisms of the formation from DOC (TEP precursors) excreted by diatom to TEP, I carried out the laboratory experiment using *Thalassiosira nordenskiöldii* dominated in the Oyashio region of spring diatom bloom.

The laboratory experiment was carried out during 40 days in the incubator. The temperature, salinity and macronutrient concentrations in the culture vessels were adjusted with those at before of the Oyashio spring bloom. The PAR level and the light:dark cycle were $100\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$ and 12-hours:12-hours, respectively. The medium was autoclaved twice. The axenic *T. nordenskiöldii* were inoculated into the medium, and this experiment was started. The DOC and TEP concentrations increased with the incubate time. Moreover, the cellular DOC productivity in the exponential growth phase was significantly correlated with the cellular TEP productivities. This result suggested that the TEP were formed from the DOC excreted by *T. nordenskiöldii*. Again, the TEP were formed from the dissolved acid polysaccharides among DOC. Nevertheless, the TEP/DOC ratio was more than 1. It was considered that the DOC excreted by *T. nordenskiöldii* once attach on the cell surface. The results of photosynthetic–irradiance ($P\text{--}E$) curve experiment were showed that the POC and DOC productivities were higher in the exponential phase than in the stationary phase. The intensity of PAR also affected to the POC and DOC productivities. In particular, the strong intensity ($>1,000\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$) of PAR increased the DOC productivity. In addition, the ratio (PER) of the DOC productivity and Total productivity (DOC + POC productivities) were relatively high in the PAR intensity of $>1,700\ \mu\text{mol photons m}^{-2}\ \text{s}^{-1}$. Those results might show that the DOC concentration increase, when ocean surface exposed to the strong sunlight. Therefore, the TEP levels in the sea surface possibly increase.

5.2 Perspectives

Overall, this doctoral dissertation is divided to the Ψ study in the WSG and the TEP study in the Oyashio region. To link the stories between Chapters 2 and 3, I connected the Ψ study with the TEP study by focus on the biological carbon pump in the western subarctic Pacific. However, those parameters are fundamentally different. In addition, although those study regions were broadly same in the western subarctic Pacific, it is also different for the space-time interval. To examine the relation between the Ψ and the TEP, simultaneous observations of those parameters are necessary.

For the Ψ study, I found that the higher Ψ values in the WSG were obtained in region where autotrophic flagellates predominated in the phytoplankton assemblages. However, hitherto, it has been reported that the higher Ψ value related with diatoms. I am considering that the high Ψ value during the spring bloom in the western subarctic Pacific contribute with diatoms, whereas, that the higher Ψ values in the WSG during the summer attribute to autotrophic flagellates. To clarify of the contribution of autotrophic flagellate, the study focused on the relationship between the Ψ and the flagellates should conduct in the WSG during summer.

For the TEP study in *in-situ*, bacteria had to contribute with the decomposition of the TEP, and a part of the TEP production might cause by bacteria. I could not do those assessments. Hence, the one of future work is to evaluate the bacterial impact to the TEP in the Oyashio region during spring bloom. In addition, the key of the TEP study would be to measure the relationship between the setting POC and the TEP. Therefore, observation of the TEP corrected in the sediment trap is also needed for the assessment of the relationship. On the other hand, based on the results of photosynthesis–irradiance ($P-E$) curve experiments in the laboratory experiments, I considered that since the strong sunlight increased the DOC productivity, the TEP formations during the Oyashio spring diatom blooms would rise in ocean surface. In contrast, the sunlight was reported to decompose the DOC. Therefore, the study of micro layer in the surface will be interesting. Further studies of the relationships between the DOC and TEP production by phytoplankton, and between the DOC and TEP decompositions by the sunlight are necessary.

Acknowledgements

I am deeply grateful to Associate Prof. Koji Suzuki (Hokkaido University) for giving me the opportunity to study, his stimulations and helpful discussions throughout my study.

I would like to thanks to Associate Prof. Yohei Yamashita (Hokkaido University) for his helpful technical assistance and helpful discussions in Chapters 3 and 4 in this thesis.

I would like to express my heartfelt thanks Prof. Hisayuki Yoshikawa-Inoue, Associate Prof. Yukata W. Watanabe, Associate Prof. Jun Nishioka, Associate Prof. Takafumi Hirata, Assistant Prof. Sohiko Kameyama and Assistant Prof. Tomonori Isada (Hokkaido University) for their helpful support and valuable comments in this study.

I would like to express my heartfelt thanks Prof. Hiroshi Hattori (Tokai University) for his helpful technical assistance and kind support accomplish this study.

I thank to Prof. Isao Kudo (Hokkaido University), Dr. Hiroaki Saito (Tohoku National Fisheries Research Institute) and Prof. Atsushi Tsuda (The University of Tokyo) for their helpful support and valuable comments in this study. Thanks are extended to Associate Prof. Kazutaka Takahashi (The University of Tokyo) for his useful comments and sampling assistances. I am grateful to Prof. Atsuko Sugimoto and Ms. Yumi Hoshino for technical support in the POC, PN and the ^{13}C measurements. Thanks are also extended to Prof. Shinichiro Noriki (Fuji Women's University) for his helpful scientific suggestions.

I wish to the captains, crews and scientists during the cruises of the *R/V Hakuho-Maru* (KH08-2), *Wakataka-Maru* (WK1004 and WK1006) and *Tansei-Maru* (KT11-07). This study would not have been possible without the secure cruises by their efforts.

Special acknowledgements are due to Drs. Hisashi Endo, Shintaro Takao, Yuki Takabe-Saito, Ai Saito-Hattori, Takafumi Kataoka, Yuya Tada, Koji Sugie, Tomomi R. Takamura, Daiki Nomura, Masahito Shigemitu, Chunmao Zhu, Shunsuke Tei, and colleagues at laboratory.

Finally, I would like to express my deepest gratitude to my parents Shinichi and Tamae, and my sister Yuri with limitless love for their continuous encouragements and supports.

– Blank page –

References

- Allredge, A. L., U. Passow and B. E. Logan (1993): The abundance and significance of a class of large, transparent organic particles in the ocean. *Deep-Sea Res. I*, **40**, 1131–1140.
- Alonso-Sáez, L., E. Vázquez-Domínguez, C. Cardelús, J. Pinhassi, M. Sala, I. Lekunberri, V. Balagué, M. Vila-Costa, F. Unrein, R. Maassana, R. Simó and J. Gasol (2008): Factors controlling the year-round variability in carbon flux through bacteria in a coastal marine system. *Ecosystems*, **11**, 397–409.
- Arakaki, A. K., E. A. Ceccarelli and N. Carrillo (1997): Plant-type ferredoxin-NADP⁺ reductases: A basal structural framework and multiplicity of functions. *FASEB J.*, **11**, 133–140.
- Ayers, J. M. and M. S. Lozier (2012): Unraveling dynamical controls on the North Pacific carbon sink. *J. Geophys. Res.*, **117**, C01017, Doi: 10.1029/2011JC007368.
- Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil and F. Thingstad (1983): The ecological role of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.*, **10**, 257–263.
- Azam, F. and D. C. Smith (1991): Bacterial influence on the variability in the ocean's biogeochemical state: a mechanistic view. In S. Demers (Ed.), *Particle Analysis in Oceanography* (pp. 213–233). Berlin: Springer Verlag NATO ASI Series G27.
- Azetsu-Scott, K. and U. Passow (2004): Ascending marine particles: Significance of transparent exopolymer particles (TEP) in the upper ocean. *Limnol. Oceanogr.*, **49**, 741–748.
- Baines, B. and M. L. Pace (1991): The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. *Limnol. Oceanogr.*, **36**, 1078–1090.
- Barber, J. (2008): Photosynthetic generation of oxygen. *Phil. Trans. R. Soc. B*, **363**, 2665–2674.
- Barsanti, L. and P. Gualtieri (2006): *Algae – Anatomy, Biochemistry, and Biotechnology*. CRC Press. 3, 42.

- Barrera-Alba, J. J., G. A. O. Moser, S. M. F. Gíanesella and F. M. P. Saldanha-Corrêa (2012): Role of transparent exopolymer particles on phytoplankton dynamics in a subtropical estuary, Cananéia-Iguape (SP, Brazil). *Open Journal of Scientific Research*, **2**, 25–32.
- Bar-Zeev, E., I. Berman-Frank, N. Stambler, E. V. Domínguez, T. Zohary, E. Capuzzo, E. Meeder, D. J. Sggett, D. Iluz, G. Dishon and T. Berman (2009): Transparent exopolymer particles (TEP) link phytoplankton and bacterial production in the Gulf of Aqaba. *Aquat. Microb. Ecol.*, Doi: 10.3354/ame01322.
- Bar-Zeev, E., T. Berman, E. Rahav, G. Dishon, B. Herut, N. Kress and I. Berman-Frank (2011): Transparent exopolymer particle (TEP) dynamics in the eastern Mediterranean Sea. *Mar. Ecol. Prog. Ser.*, **431**, 107–118.
- Beauvais, S., M. L. Predrotti, E. Villa and R. Lemée (2003): Transparent exopolymer particles (TEP) dynamics in relation to trophic and hydrological conditions in the NW Mediterranean Sea. *Mar. Ecol. Prog. Ser.*, **262**, 97–109.
- Berman, T. and Y. Viner-Mozzini (2001): Abundance and characteristics of polysaccharide and proteinaceous particles in Lake Kinneret. *Aquat. Microb. Ecol.*, **24**, 255 – 264.
- Bhaskar, P. V. and N. B. Bhosle (2006): Dynamics of transparent exopolymer particles (TEP) and particle-associated carbohydrates in the Dona Paula bay, west coast of India. *J. Earth Syst. Sci.*, **115**, 403–413.
- Bjørnsen, P. K. (1988): Phytoplankton exudation of organic matter: Why do healthy do it? *Limnol. Oceanogr.*, **33**, 151–154.
- Blankenship, R. E. and R. C. Prince (1985): Excited-state redox potentials and the Z scheme of photosynthesis. *Trans Biochem. Sci.*, **10**, 382–383.
- Blankenship, R. E. (2002): *Molecular Mechanisms of Photosynthesis*. Iowa State University Press. 113.
- Broecker, W. S. (1991): The great ocean conveyor. *Oceanography*, **4**, 79–89.
- Calvin, M. (1989): Forty years of photosynthesis and related activities. *Photosynth. Res.*, **21**, 3–16.
- Charette, M. A. and W. H. F. Smith (2010): The volume of Earth's ocean. *Oceanography*, **23**, 112–114.
- Chiba, S., T. Ono, K. Tadokoro, T. Midorikawa and T. Saino (2004): Increased

- stratification and decreased lower trophic level productivity in the Oyashio region of the north Pacific: A 30-year retrospective study. *J. Oceanogr.*, **60**, 149–162.
- Claquin, P., I. Probert, S. Lefebvre and B. Veron (2008): Effects of temperature on photosynthetic parameters and TEP production in eight species of marine microalgae. *Aquat. Microb. Ecol.*, **51**, 1–11.
- Cleveland, J. S. and Weidemann, A. D. (1993) Quantifying absorption by aquatic particles: A multiple scattering correction for glass-fiber filters. *Limnol. Oceanogr.*, **38**, 1321–1327.
- Cota, G. F., W. O. Smith Jr. and B. Greg Mitchell (1994): Photosynthesis of *Phaeocystis* in the Greenland Sea. *Limnol. Oceanogr.*, **39**, 948–953.
- Corzo, A., S. Rodríguez-Gálvez, L. Lubian, P. Sangrá, A. Martínez and A. Morillo (2005): Spatial distribution of transparent exopolymer particles in the Bransfield Strait, Antarctica. *J. Plankton Res.*, **27**, 635–646.
- Cramer, M. A., G. M. Soriano, M. Ponomarev, D. Huang, H. Zhang, S. E. Martinez and J. L. Smith (1996): Some new structural aspects and old controversies concerning the cytochrome b6f complex of oxygenic photosynthesis. *Annu. Rev. Plant Physiol. Plank Mol. Biol.*, **47**, 477–508.
- Chisholm, S. W., R. J. Olson, E. R. Zettler, R. Goericke, J. B. Waterbury and N. A. Welschmeyer (1988): A novel free-living prochlorophyte abundant in the oceanic euphotic zone. *Nature*, **334**, 340–343.
- Chisholm, S. W. (2000): Stirring times in the Southern Ocean. *Nature*, **407**, 685–687.
- Demers, S., S. Roy, R. Gagnon and C. Vignault (1991): Rapid light-induced changes in cell fluorescence and in xanthophyll-cycle pigments of *Alexandrium excavatum* (Dinophyceae) and *Thalassiosira pseudonana* (Bacillariophyceae): a photo-protection mechanism. *Mar. Ecol. Prog. Ser.*, **76**, 185–193.
- Dumont, I., V. Schoemann, D. Lannuzel, L. Chou, J. -L. Tison and S. Becquevort (2009): Distribution and characterization of dissolved and particulate organic matter in Antarctic pack ice. *Polar. Biol.*, **32**, 733–750.
- Dunne, J. A., J. Harte and K. Taylor (2003): Subalpine meadow flowering phenology responses to climate change: integrating experimental and gradient methods. *Ecological Monographs.*, **73**, 69–86.

- Emerson, S. R. and J. I. Hedges (2008): *Chemical Oceanography and the Marine Carbon Cycle*. Cambridge University Press. 26, 121, 162.
- Endo, H., T. Yoshimura, T. Kataoka and K. Suzuki (2013): Effects of CO₂ and iron availability on phytoplankton and eubacterial community compositions in the northwest subarctic Pacific. *J. Exp. Mar. Biol. Ecol.*, **439**, 160–175.
- Engel, A., W. Koeve and B. Zeitzschel (1997): Verteilung transparenter exopolymerer partikel (TEP) im nordostatlantik-untersuchungen entlang eines 20° W schnittes (Meteor 36/2). *Berichte Fachbereich Geowissenschaften Universität Bremen*, **109**, 23.
- Engel, A. (1998): *Bildung, Zusammensetzung und Sinkgeschwindigkeiten mariner Aggregate*. Doctoral Dissertation in Garman, pp. 145.
- Engel, A. and U. Passow (2001): Carbon and nitrogen content of transparent exopolymer particles (TEP) in relation to their Alcian Blue adsorption. *Mar. Ecol. Prog. Ser.*, **219**, 1–10.
- Engel, A., M. Meyerhöfer and K. von Brökel (2002): Chemical and biological composition of suspended particles and aggregates in the Baltic Sea in summer (1999). *Estuarine, Coastal and Shelf Science*, **55**, 729–741.
- Engel, A. (2004): Distribution of transparent exopolymer particles (TEP) in the northeast Atlantic Ocean and their potential significance for aggregation processes. *Deep-Sea Res. I*, **51**, 86–92.
- Engel, A., S. Thoms, U. Riebesell, E. Rochelle-Newall and I. Zondervan (2004a): Polysaccharide aggregation as a potential sink of marine dissolved organic carbon. *Nature*, **428**, 929–932.
- Engel, A., B. Delille, S. Jacquet, U. Riebesell, E. Eochelle-Newall, A. Terbrüggen and I. Zondervan (2004b): Transparent exopolymer particles and dissolved organic carbon production by *Emiliania huxleyi* exposed to different CO₂ concentrations: a mesocosm experiment. *Aquat. Microb. Ecol.*, **34**, 93–104.
- Engel, A., J. Harlay, J. Piontek and L. Chou (2012): Contribution of combined carbohydrates to dissolved and particulate organic carbon after the spring bloom in the northern Bay of Biscay (North-Eastern Atlantic Ocean). *Continental Shelf Research*, **45**, 42–53.
- Fabricius, K. E., C. Wild, E. Wolanski and D. Abele (2003): Effects of transparent exopolymer particles and muddy terrigenous sediments on the survival of

- hard coral recruits. *Estuarine, Coastal and Shelf Science*, **57**, 613–621.
- Falkowski, P. G. (1981): light-shade adaptation and assimilation numbers. *J. Plankton Res.*, **3**, 203–216.
- Falkowski, P. G. (2002): The ocean's invisible forest. *Sci. Am.*, **287**, 54–61.
- Falkowski, P. G. and F. A. Raven (2007): *Aquatic Photosynthesis second edition*. Princeton University Press. pp. 127, 341–342, 343, 351–353.
- Favorite, F., A. J. Dodimead and K. Hirano (1976): Oceanography of the Subarctic Pacific region, 1960–71. *Bull. Int. North Pacific Comm.*, **13**, p. 195.
- Fernández, M., M. Bianchi and F. V. Wambeke (1994): Bacterial biomass, heterotrophic production and utilization of dissolved organic matter photosynthetically produced in the Almeria-Oran front. *J. Marine Syst.*, **5**, 313–325.
- Fogg, G. E. (1983): The ecological significance of extracellular products of phytoplankton photosynthesis. *Botanica Marina*, **XXVI**, 3–14.
- Frankignoulle, M., C. Canon and J.P. Gattuso (1994): Marine calcification as a source of carbon dioxide: positive feedback of increasing atmospheric CO₂. *Limnol. Oceanogr.*, **39**, 458–462.
- Frankignoulle, M., M. Pichon and J.P. Gattuso (1995): Aquatic calcification as a source of carbon dioxide. *NATO ASI Series*, **33**, 265–271.
- Fujiki, T., T. Toda, T. Kikuchi and S. Taguchi (2003): Photoprotective response of xanthophyll pigments during phytoplankton blooms in Sagami Bay, Japan. *J. Plankton Res.*, **25**, 317–322.
- Fukao, T., S. Kitahara, N. Karino, T. Yamatogi, K. Kimoto and Y. Kotani (2011): Dynamics of transparent exopolymer particles in spring and autumn in Isahaya Bay, Japan. *Nippon Suisan Gakkaishi*, **77**, 1027–1033 (in Japanese with English abstract).
- Fukao, T., K. Kimoto and Y. Kotani (2012): Effect of temperature on cell growth and production of transparent exopolymer particles by the diatom *Coscinodiscus granii* isolated from marine mucilage. *J. App. Phycol.*, **24**, 181–186.
- Fukuyo, Y., H. Takano, M. Chihara and K. Matsuoka (eds.) (1990): *Red Tide Organisms in Japan—An Illustrated Taxonomic Guide—*. Uchida Rokakuho, Tokyo, Japan, pp. 162–331.
- Furnas, M. J. (1982): Growth rates of summer nanophytoplankton (<10 µm)

- populations in lower Narragansett Bay, Rhode Island, USA. *Mar. Biol.*, **70**, 105–115.
- Goes, I., Joaquim, K. Sasaoka, H. R. Gomes, S. Saitoh and T. Saino (2004): A comparison of the seasonality and interannual variability of phytoplankton biomass and production in the western and eastern gyres of the subarctic Pacific using multi-sensor satellite data. *J. Oceanogr.*, **60**, 75–91.
- García, C. M., L. Prieto, M. Vargas, F. Echevarría, J. García-Lafuente, J. Ruiz and J. P. Rubín (2002): Hydrodynamics and the spatial distribution of plankton and TEP in the Gulf of Cádiz (SW Iberian Peninsula). *J. Plankton Res.*, **24**, 817–833.
- Grossart, H. -P. and M. Simon (1997): Formation of macroscopic organic aggregates (lake snow) in a large lake: The significance of transparent exopolymer particles, phytoplankton, and zooplankton. *Limnol. Oceanogr.*, **42**, 1651–1659.
- Guillard, R. R. L. and J. H. Ryther (1962): Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Can. J. Microbiol.*, **8**, 229–239.
- Guillard, R. R. L. (1973): Division rates. In: Stein, J. R. (Ed.) *Handbook of Phycological Methods: Culture Methods and Growth Measurements*. Cambridge University Press, Cambridge, pp. 289 – 312.
- Guillard, R. R. L. (1975): Culture of phytoplankton for feeding marine invertebrates. In Smith W. L. and Chanley M. H. (Eds.) *Culture of Marine Invertebrate Animals*. Plenum Press, New York, USA, pp. 26–60.
- Hama, T., T. Miyazaki, Y. Ogawa, T. Iwakuma, M. Takahashi, A. Otsuki and S. Ichimura (1983): Measurement of photosynthetic production of a marine phytoplankton population using a stable ^{13}C isotope. *Marine Biology*, **73**, 31–36.
- Hama, T. and K. Yanagi (2001): Production and neutral dldose composition of dissolved carbohydrates excreted by natural marine phytoplankton populations. *Limol. Oceanogr.*, **46**, 1945–1955.
- Harlay, J., A.V. Borges, C. Van Der Zee, B. Delille, R. H. M. Godoi, L. -S.

- Schiettecatte, N. Roevros, K. Aerts, P. -E. Lapernat, L. Rebreanu, S. Groom, M. -H. Daro, R. Van Grieken and L. Chou (2010): Biogeochemical study of a coccolithophore bloom in the northern Bay of Biscay (NE Atlantic Ocean) in June 2004. *Prog. Oceanogr.*, **86**, 317–336.
- Harrison, W. G. and T. Platt (1980): Variations in assimilation number of coastal marine phytoplankton: effects of environmental covariates. *J. Plankton Res.*, **2**, 249–260.
- Hashimoto, S. and A. Shiomoto (2002): Light utilization efficiency of size-fractionated phytoplankton in the subarctic Pacific, spring and summer 1999: high efficiency of large-sized diatom. *J. Plankton Res.*, **24**, 83–87.
- Hattori, H., M. Koike, K. Tachikawa, H. Saito and K. Nagasawa (2004): Spatial variability of living coccolithophore distribution in the western subarctic Pacific and western Bering Sea. *J. Oceanogr.*, **60**, 505–515.
- Hellebust, J. A. (1965): Excretion of some organic compounds by marine phytoplankton. *Limnol. Oceanogr.*, **10**, 192–206.
- Helms, J. R., A. Stubbins, E. M. Perdue, N. W. Green, H. Chen and K. Mopper (2013): Photochemical bleaching of oceanic dissolved organic matter and its effect on absorption spectral slope and fluorescence. *Mar. Chem.*, **155**, 81–91.
- Hoganson, C. W. and G. T. Babcock (1997): A metalloradical mechanism for the generation of oxygen from water in photosynthesis. *Science*, **277**, 1953–1956.
- Honda, M. C. (2003): Biological pump in the northwestern north Pacific. *J. Oceanogr.*, **59**, 671–684.
- Honda, C. M., K. Sasaoka, H. Kawakami, K. Matsumoto, S. Watanabe and T. Dickey (2009): Application of underwater optical data to estimation of primary productivity. *Deep-Sea Res. I*, **56**, 2281–2292.
- Hong, Y., W. O. Smith, Jr. and A.-M. White (1997): Studies on transparent exopolymer particles (TEP) production in the Ross Sea (Antarctica) and by *Phaeocystis antarctica* (prymnesiophyceae). *J. Phycol.*, **33**, 368–376.
- Ichinomiya, M., Y. Gomi, M. Nakamachi, T. Ota and T. Kobari (2010): Temporal patterns in silica deposition among siliceous plankton during the spring bloom in the Oyashio region. *Deep-Sea Res. II*, **57**, 1665–1670.
- Imai, K., Y. Nojiri, N. Tsurushima and T. Sanino (2002): Time series of seasonal variation of primary productivity at station KNOT (44°C, 155°E) in the

- sub-arctic western North Pacific. *Deep-Sea Res. II*, **49**, 5395–5408.
- Isada, T., A. Kuwata, H. Saito, T. Ono, M. Ishii, H. Yoshikawa-Inoue and K. Suzuki (2009): Photosynthetic features and primary productivity of phytoplankton in the Oyashio and Kuroshio-Oyashio transition regions of the northwest Pacific. *J. Plankton Res.*, **31**, 1009–1025.
- Isada, T., A. Hattori-Saito, H. Saito, T. Ikeda and K. Suzuki (2010): Primary productivity and its bio-optical modeling in the Oyashio region, NW Pacific during the spring bloom 2007. *Deep-Sea Res. II*, **57**, 1653–1664.
- Iversen, M. H. and H. Ploug (2010): Ballast minerals and the sinking carbon flux in the ocean: carbon-specific respiration rates and sinking velocity of marine snow aggregates. *Biogeosciences*, **7**, 2613–2624.
- Junge, W., H. Lill and S. Engelbrecht (1997): ATP synthase: an electrochemical transducer with rotatory mechanics. *Trends Biochem. Sci.*, **22**, 420–423.
- Junge, W. (1999): ATP synthase and other motor proteins. *Proc. Natl. Acad. Sci. USA*, **96**, 4735–4737.
- Junge, W. (2004): Protons, proteins and ATP. *Photosynth. Res.*, **80**, 197–221.
- Kaiser, M. J., M. J. Attrill, S. Jennings, D. N. Thomas, D. A. Barnes, A. S. Brierley, J. G. Hiddink, H. Kaartokallio, N. V. C. Polunin and D. G. Raffaelli (2011): *Marine Ecology – Processes, Systems, and Impacts second edition*. Oxford University Press. pp. 36, 45, 96.
- Karp-Boss, L., E. Boss and P. A. Jumars (1996): Nutrient fluxes to planktonic osmotrophs in the presence of fluid motion. *Oceanography and Marine Biology: an Annual Review*, **34**, 71–107.
- Kasai, H., H. Saito, A. Yoshimori and S. Taniguchi (1997): Variability in timing and magnitude of spring bloom in the Oyashio region, the western subarctic Pacific off Hokkaido, Japan. *Fish. Oceanogr.*, **6**, 118–129.
- Kasai, H., H. Saito and A. Tsuda (1998): Estimation of standing stock of chlorophyll a and primary production from remote-sensed ocean color in the Oyashio region, the western subarctic Pacific, during the spring bloom in 1997. *J. Oceanogr.*, **54**, 527–537.
- Kawakami, H., Y.-L. Yang, M. C. Honda and M. Kusakabe (2004): Particulate organic carbon fluxes estimated from ^{234}Th deficiency in winters and spring in the northwestern North Pacific. *Geochemical Journal*, **38**, 581–592.

- Key, R. M., A. Kozyr, C. L. Sabine, K. Lee, R. Wanninkhof, J. L. Bullister, R. A. Feely, F. J. Millero, C. Mordy and T. -H. Peng (2004): A global ocean carbon climatology: Results from Global Data Analysis Project (GLODAP). *Global Biogeochem. Cycles*, **18**, GB4031, doi:10.1029/2004GB002247.
- Kjørboe, T. (1993): Turbulence, phytoplankton cell size, and the structure of pelagic food webs. *Adv. Mar. Biol.*, **29**, ISBN 0-12-026129-4.
- Kjørboe, T. and J. Hansen (1993): Phytoplankton aggregate formation: observations of patterns and mechanisms of cell sticking and the significance of exopolymeric material. *J. Plankton Res.*, **15**, 993–1018.
- Kjørboe, T., J. L. S. Hansen, A. L. Alldredge, G. A. Jackson, U. Passow, H. G. Dam, D. T. Drapeau, A. Waite and C. M. Garcia (1996): Sedimentation of phytoplankton during a diatom bloom: Rates and mechanisms. *J. Mar. Res.*, **54**, 1123–1148.
- Kirk, J. T. O. (2011): *Light and Photosynthesis – in Aquatic Ecosystems third edition*. Cambridge: Cambridge University Press. 36–44, 308–329.
- Kishino, M., M. Takahashi, N. Okami and S. Ichimura (1985): Estimation of the spectral absorption coefficients of phytoplankton in the sea. *Bull. Mar. Sci.*, **37**, 634–642.
- Klaas, C. and D. E. Archer (2002): Association of sinking organic matter with various types of mineral ballast in the deep sea; implications for the rain ratio. *Global Biogeochem. Cycles*, **16**, 1–13, 1116, doi: 10.1029/2001GB001765.
- Klain, C., P. Claquin, A. Pannard, C. Napoléon, B. L. Roy and B. Véron (2011): Dynamics of soluble extracellular polymeric substances and transparent exopolymer particle pools in coastal ecosystems. *Mar. Ecol. Prog. Ser.*, **427**, 13–27.
- Komuro, C., H. Narita, K. Imai, Y. Nojiri and R. W. Jordan (2005): Microplankton assemblages at Station KNOT in the subarctic western Pacific, 1999–2000. *Deep-Sea Res. II*, **52**, 2206–2217.
- Kono, T. and H. Saito (2010): A mixing analysis of surface water in the Oyashio region: Its implications and application to variations of the spring bloom. *Deep-Sea Res. II*, **57**, 1595–1607.
- Krembs, C. and A. Angel (2001): Abundance and variability of microorganisms and transparent exopolymer particles across the ice-water interface of melting

- first-year sea ice in the Laptev Sea (Arctic). *Marine Biology*, **138**, 173–183.
- Kudo, I., Y. Noiri, K. Imai, Y. Nojiri, J. Nishioka and A. Tsuda (2005): Primary productivity and nitrogenous nutrient assimilation dynamics during the Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study. *Prog. Oceanogr.*, **64**, 207–221.
- Kudo, I., Y. Noiri, W. P. Cochlan, K. Suzuki, T. Aramaki, T. Ono and Y. Nojiri (2009): Primary productivity, bacterial productivity and nitrogen uptake in response to iron enrichment during the SEEDS II. *Deep-Sea Res. II*, **56**, 2755–2766.
- Kumar, M. D., V. V. S. S. Sarma, N. Ramaiah, M. Graus and S. N. de Sousa (1998): Biogeochemical significance of transport exopolymer particles in the Indian Ocean. *Geophys. Res. Lett.*, **25**, 81–84.
- Lalli, C. M. and T. R. Parsons (1997): *Biological Oceanography an Introduction Second edition*. Elsevier, pp. 41, 113–117.
- Latasa, M. (2007): Improving estimations of phytoplankton class abundances using CHEMTAX. *Mar. Ecol. Prog. Ser.*, **329**, 13–21.
- Lam, P. J. and J. K. Bishop (2008): The continental margin is a key source of iron to the HNLC North Pacific Ocean. *Geophys. Res. Lett.*, **35**, L07608, Doi: 10.1029/2008GL033294.
- Ling, S. C. and A. L. Alldredge (2003): Does the marine copepod *Calanus pacificus* consume transparent exopolymer particles (TEP)? *J. Plankton Res.*, **25**, 507–515.
- Lopez-Sandval, D. C., A. Fernández and E. Marañón (2011): Dissolved and particulate primary production along a longitudinal gradient in the Mediterranean Sea. *Biogeosciences*, **8**, 815–825.
- Mackey, M., D. J. Mackey, H. W. Higgins and S. W. Wright (1996): CHEMTAX— a program for estimating class abundance from chemical markers: application to HPLC measurements of phytoplankton. *Mar. Ecol. Prog. Ser.*, **144**, 265–283.
- Mahowald, N. M., A. R. Baker, G. Bergametti, N. Brooks, R. A. Duce, T. D. Jickells, N. Kubilay, J. M. Prospero and I. Tegen (2005): Atmospheric global dust cycle and iron input to the ocean. *Global Biogeochem. Cycles*, **19**, GB4025, Doi: 10.1029/2004GB002402.
- Malpezzi, M. A., L. P. Sanford and B. C. Crump (2013): Abundance and distribution of

- transparent exopolymer particles in the estuarine turbidity maximum of Chesapeake Bay. *Mar. Ecol. Prog. Ser.*, **486**, 23–35.
- Marañón, E., P. Cermeño and E. Fernández (2004): Significance and mechanisms of photosynthetic production of dissolved organic carbon in a coastal eutrophic ecosystem. *Limnol. Oceanogr.*, **49**, 1652–1666.
- Mari, X. and T. Kiørboe (1996): Abundance, size distribution and bacterial colonization of transparent exopolymer particles (TEP) during spring in the Kattegat. *J. Plankton Res.*, **18**, 969–986.
- Matsumoto, Katsumi (2007): Radiocarbon-based circulation age of the world oceans. *J. Geophys. Res.*, **112**, C09004, Doi:10.1029/2007JC004095.
- Mayklestad, S. (1974): Production of carbohydrates by marine planktonic diatoms. I. Comparison of nine different species in culture. *J. Exp. Mar. Biol. Ecol.*, **15**, 261–274.
- Moisan, T. A., M. Olaizola and B. G. Mitchell (1998): Xanthophyll cycling in *Phaeocystis antarctica*: changes in cellular fluorescence. *Mar. Ecol. Prog. Ser.*, **169**, 113–121.
- Myklestad, S.M., H.-M. Vårum and B. Volcani (1989): Rate of release of extracellular amino acids and carbohydrates from the marine diatom *Chaetoceros affinis*. *J. Plankton Res.*, **11**, 763–773.
- Myklestad, S. M. (1995): Release of extracellular products by phytoplankton with special emphasis on polysaccharides. *The Science of the Total Environment*, **165**, 155–165.
- Midorikawa, T., S. Iwano, S. Kazuhiro, H. Takano, H. Kamiya, M. Ishii and H. Inoue (2003): Seasonal changes in oceanic pCO₂ in the Oyashio region from winter to spring. *J. Oceanogr.*, **59**, 871–882.
- Mochizuki, M., N. Shiga, M. Saito, K. Imai, Y. Nojiri (2002): Seasonal changes in nutrients, chlorophyll a and the phytoplankton assemblage of the western subarctic gyre in the Pacific Ocean. *Deep-Sea. Res. II*, **49**, 5421–5439.
- Nelson, D. M., P. Tréguer, M. A. Brzezinski, A. Leynaert and B. Quéguiner (1995): Production and dissolution of biogenic silica in the ocean: Revised global estimates, comparison with regional data and relationship to biogenic sedimentation. *Global Biochem. Cycles*, **9**, 359–372.
- Najdek, M, M. Blazina, D. Fuks, I. Ivancic, T. Silovic (2011): Intrusion of high-salinity

- water causes accumulation of transparent exopolymer particles (TEP) in the northern Adriatic Sea. *Aquat. Microb. Ecol.*, **63**, 69–74.
- Nishioka, J., T. Ono, H. Saito, T. Nakatsuka, S. Takeda, T. Yoshimura, K. Suzuki, K. Kuma, S. Nakabayashi, D. Tsumune, H. Mitsudera, W. K. Johnson and A. Tsuda (2007): Iron supply to the western subarctic Pacific: Importance of iron export from the Sea of Okhotsk. *J. Geophys. Res.*, **112**, C10012, Doi: 10.1029/2006JC004055.
- Nishioka, J., T. Ono, H. Saito, K. Sasaoka and T. Yoshimura (2011): Oceanic iron supply mechanisms which support the spring diatom bloom in the Oyashio region, western subarctic Pacific. *J. Geophys. Res.*, **116**, C02021, Doi: 10.1029/2010JC006321.
- Obernosterer, I. and G. J. Herndl (1995): Phytoplankton extracellular release and bacterial growth: dependence on the inorganic N:P ratio. *Mar. Ecol. Prog. Ser.*, **116**, 247–257.
- Oostende, N. V., T. C. W. Moerdijk-Poortvliet, H. T. S. Boschker, W. Vyverman and K. Sabbe (2013): Release of dissolved carbohydrates by *Emiliania huxleyi* and formation of transparent exopolymer particles depend on algal life cycle and bacterial activity. *Environ. Microbiol.*, **15**, 1514–1531.
- Ortega-Retuerta, E., I. Reche, E. Pulido-Vilena, S. Agustí and C. M. Duarte (2009): Uncoupled distributions of transparent exopolymer particles (TEP) and dissolved carbohydrates in the Southern Ocean. *Mar. Chem.*, **115**, 59–65.
- Ortega-Retuerta, E., C. M. Duarte and I. Reche (2010): Significance of bacterial activity for the distribution and dynamics of transparent exopolymer particles in the Mediterranean Sea. *Microb. Ecol.*, **59**, 808–818.
- Passow, U. and A. L. Alldredge (1994): Distribution, size and bacterial colonization of transparent exopolymer particles (TEP) in the ocean. *Mar. Ecol. Prog. Ser.*, **113**, 185–198.
- Passow, U., W. Kozlowski and M. Vernet (1995): Palmer LTER: Temporal variability of transparent exopolymer particles in Arthur Harbor during the 1994–1995 growth season. *Antarctic Journal*, **Review 1995**, 265–266.
- Passow, U. and A. L. Alldredge (1995a): Aggregation of a diatom bloom in a mesocosm: The role of transparent exopolymer particles (TEP). *Deep-Sea Res. II*, **42**, 99–109. *Continental Shelf Research*, **21**, 327–346.

- Passow, U. and A. L. Alldredge (1995b): A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). *Limnol. Oceanogr.*, **40**, 1326–1335.
- Passow, U. (2000): Formation of transparent exopolymer particles, TEP, from dissolved precursor material. *Mar. Ecol. Prog. Ser.*, **192**, 1–11.
- Passow, U., R. F. Shipe, A. Murray, D. K. Pak, M. A. Brzezinski and A. L. Alldredge (2001): The origin of transparent exopolymer particles (TEP) and their role in the sedimentation of particulate matter. *Continental Shelf Research*, **21**, 327–346.
- Passow, U. (2002a): Production of transparent exopolymer particles (TEP) by phyto- and bacterioplankton. *Mar. Ecol. Prog. Ser.*, **236**, 1–12.
- Passow, U. (2002b): Transparent exopolymer particles (TEP) in aquatic environments. *Prog. Oceanogr.*, **55**, 287–333.
- Pearlstein, R. M. (1996): Coupling of excitation motion in the core antenna and primary charge separation in the reaction center. *Photosynth. Res.*, **48**, 75–82.
- Pedrotti, M. L., F. Peters, S. Beauvais, M. Vidal, J. Egge, A. Jacobsen and C. Marrase (2010): Effects of nutrients and turbulence on the production of transparent exopolymer particles: a mesocosm study. *Mar. Ecol. Prog. Ser.*, **419**, 57–69.
- Platt, T., C. L. Gallegos and C. L. Harrison (1980): Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *J. Mar. Res.*, **38**, 687–701.
- Platt, T. (1986): Primary production of the ocean water column as a function of surface light intensity: algorithms for remote sensing. *Deep-Sea Res.*, **33**, 149–163.
- Platt, T. and S. Sathyendranath (1988): Oceanic primary production: estimation by remote sensing at local and regional scales. *Science*, **241**, 1613–1620.
- Prieto, L., G. Navarro, A. Cózar, F. Echevarría and C. M. García (2006): Distribution of TEP in the euphotic and upper mesopelagic zones of the southern Iberian coasts. *Deep-Sea Res. II*, **53**, 1314–1328.
- Prieto, L. and J. P. Cowen (2007): Transparent ecopolymer particles in a deep-sea hydrothermal system: Guaymas Basin, Gulf of California. *Mar. Biol.*, **150**, Doi: 10.1007/s00227-006-0430-1.
- Prince, R. C. (1985): Redox-driven proton gradients. *BioScience*, **35**, 22–26.
- Radić, T., R. Kraus, D. Fuks, J. Radić and O. Pečar (2005): Transparent exopolymer particles' distribution in the northern Adriatic and their relation to

- microphytoplankton biomass composition. *Sci. Total Environ.*, **353**, 151–161.
- Ramaiah, M., T. Yoshikawa, K. Furuya (2001): Temporal variations in transparent exopolymer particles (TEP) associated with a diatom spring bloom in a subarctic ria in Japan. *Mar. Ecol. Prog. Ser.*, **212**, 79–88.
- Ramaiah, M. and K. Furuya (2002): Seasonal variations in phytoplankton composition and transparent exopolymer particles in a eutrophic coastal environment. *Aquat. Microb. Ecol.*, **30**, 69–82.
- Ramaiah, N., S. Takeda, K. Furuya, T. Yoshimura, J. Nishioka, T. Aono, Y. Nojiri, K. Imai, I. Kudo, H. Saito and A. Tsuda (2005): Effect of iron enrichment on the dynamics of transparent particles in the western subarctic Pacific. *Prog. Oceanogr.*, **64**, 253–261.
- Raven, J. R. (2006) Sensing inorganic carbon: CO₂ and HCO₃⁻. *Biochem. J.*, **396**, e5–e7.
- Raven, J. R., M. Giordano, J. Beardall and S. C. Maberly (2011): Algal and aquatic plant carbon concentrating mechanisms in relation to environmental change. *Photosynth. Res.*, Doi: 10.1007/s11120-011-9632-6.
- Reigstad, M. and P. Wassmann (2007): Does *Phaeocystis* spp. contribute significantly to vertical export of organic carbon? *Biogeochemistry*, **83**, 217–234.
- Riebesell, U., M. Reigstad, P. Wassmann, T. Noji and U. Passow (1995): On the trophic fate of *Phaeocystis pouchetii* (Hariot): VI. Significance of *Phaeocystis*-derived mucus for vertical flux. *Netherlands Journal of Sea Research*, **33**, 193–203.
- Round, F. E., R. M. Crawford and D. G. Mann (2007): *the diatoms BIOLOGY & MORPHOLOGY OF THE GENERA*. Cambridge University Press, America.
- Saito, H., A. Tsuda and H. Kasai (2002): Nutrient and plankton dynamics in the Oyashio region of the western subarctic Pacific Ocean. *Deep-Sea Res. II*, **49**, 5463–5486.
- Sarthou, G., K. R. Timmermans, S. Blain and P. Tréguer (2005): Growth physiology and fate of diatoms in the ocean: a review. *J. Sea Res.*, **53**, 25–42.
- Scott, F. J. and H. J. Marchant (2005): *Antarctic Marine Protists*. Goanna Print, Canberra, Australia, pp. 13–201.
- Shackelford, R. and J. P. Cowen (2006): Transparent exopolymer particles (TEP) as a component of hydrothermal plume particle dynamics. *Deep-Sea Res. II*, **53**,

1677–1694.

- Shin, M. and D. I. Arnon (1965): Enzymic mechanisms of pyridine nucleotide reduction in chloroplasts. *J. Biol. Chem.*, **240**, 1405–1411.
- Shimeta, J. (1993): Diffusional encounter of submicrometer particles and small cells by suspension feeders. *Limnol. Oceanogr.*, **38**, 456–465.
- Shiomoto, A. (2000): Efficiency of water-column light utilization in the subarctic northwestern Pacific. *Limnol. Oceanogr.*, **45**, 982–987.
- Siedler, G., J. Church and J. Gould (2001): *Ocean Circulation and Climate – Observing and Modelling the Global Ocean*. Academic Press. p. 16.
- Smith, D. C., G. F. Steward, R. A. Long and F. Azam (1995): Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. *Deep-Sea Res. II*, **42**, 75–97.
- Staats, N., L. J. Stal and L. R. Mur (2000): Exopolysaccharide production by the epipelagic diatom *Cylindrotheca closterium*: effects of nutrient conditions. *J. Exp. Mar. Biol. Ecol.*, **249**, 13–27.
- Stokes, M. A. (1845): On the theories of the internal friction of fluids in motion, and of the equilibrium and motion of elastic solids. *Trans. Camb. Phil. Soc.*, **8**, 287–319.
- Stubbins, A., J. Niggemann and T. Dittmar (2012): Photo-lability of deep ocean dissolved black carbon. *Biogeosciences*, **9**, 1661–1670.
- Sugimoto, K., H. Fukuda, M. A. Baki and I. Koike (2007): Bacterial contribution to formation of transparent exopolymer particles (TEP) and seasonal trends in coastal waters of Sagami Bay, Japan. *Aquat. Microb. Ecol.*, **46**, 31–41.
- Sun, C. -C., Y. -S. Wang, Q. P. Li, W. -Z. Yue, Y. -T. Wang, F. -L. Sun and Y. -L. Peng (2012): Distribution characteristics of transparent exopolymer particles in the Pearl River estuary, China. *J. Geophys. Res.*, **117**, G00N17, Doi: 10.1029/2012JG001951.
- Sunda, W., G. and S. A. Huntsman (1997): Interrelated influence of iron, light and cell size on marine phytoplankton growth. *Nature*, **390**, 389–392, doi: 10.1038/37093.
- Suzuki, K., A. Tsuda, H. Kiyosawa, S. Takeda, J. Nishioka, T. Saino, M. Takahashi and C. S. Wong (2002): Grazing impact of microzooplankton on diatom bloom in a mesocosm as estimated by pigment-specific dilution technique. *J. Exp. Mar.*

Biol. Ecol., **271**, 99–120.

- Suzuki, K., A. Hinuma, H. Saito, H. Kiyosawa, H. Liu, T. Saino and A. Tsuda (2005): Responses of phytoplankton and heterotrophic bacteria in the northwest subarctic Pacific to in situ iron fertilization as estimated by HPLC pigment analysis and flow cytometry. *Prog. Oceanogr.*, **64**, 167–187.
- Suzuki, K., H. Saito, T. Isada, A. Hattori-Saito, H. Kiyosawa, J. Nishioka, R. Michael, L. McKey, A. Kuwata and A. Tsuda (2009): Community structure and photosynthetic physiology of phytoplankton in the northwest subarctic Pacific during an in situ iron fertilization experiment (SEEDS-II). *Deep-Sea Res. II*, **56**, 2733–2744.
- Suzuki, K., A. Kuwata, N. Yoshie, A. Shibata, K. Kawanobe and H. Saito (2011): Population dynamics of phytoplankton, heterotrophic bacteria, and viruses during the spring bloom in the western subarctic Pacific. *Deep-Sea Res. I*, **58**, 575–589.
- Tada, Y., A. Taniguchi, I. Nagao, T. Miki, M. Uematsu, A. Tsuda and K. Hamasaki (2011): Differing growth responses of major phylogenetic groups of marine bacteria to natural phytoplankton blooms in the western north Pacific Ocean. *Appl. Environ. Microbiol.*, **77**, 4055, Doi: 10.1128/AEM.02952-10.
- Taiz, L. and E. Zeiger (2002): *Plant Physiology Third edition*. Academic Press. p. 46.
- Takahashi, T., S. C. Sutherland, C. Sweeney, A. Poisson, N. Metzl, B. Tilbrook, N. Bates, R. Wanninkhof, R. A. Feely, C. Sabine, J. Olafsson and Y. Nojiri (2002): Global sea-air CO₂ flux based on climatological surface ocean pCO₂, and seasonal biological and temperature effects. *Deep-Sea Res. II.*, **49**, 1601–1622.
- Tamaru, Y., Y. Takani, T. Yoshida and T. Sakamoto (2005): Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium *Nostoc commune*. *Appl. Environ. Microbiol.*, **71**, 7327–7333.
- Thingstad, T. F. (1998): A theoretical approach to structuring mechanisms in the pelagic food web. *Hydrobiologia*, **363**, 59–72.
- Thomas, J. P. (1971): Release of dissolved organic matter from natural populations of marine phytoplankton, *Mar. Biol.*, **11**, 311–323.
- Tomas, C. R. (ed.) (1997): *Identifying Marine Phytoplankton*. Academic Press, San

- Diego, USA, pp. 5–385.
- Thornton, D. C. O., E. M. Fejes, S. F. DiMarco and K. M. Clancy (2007): Measurement of acid polysaccharides in marine and freshwater samples using Alcian blue. *Limnol. Oceanogr.: Methods*, **5**, 73–87.
- Tréguer, P. and P. Pondaven (2000): Silica control of carbon dioxide. *Nature*, **406**, 358–359.
- Tsuda, A., S. Takeda, H. Saito, J. Nishioka, Y. Nojiri, I. Kudo, H. Kiyosawa, A. Shiimoto, D. Tsumune, T. Yoshimura, T. Aono, A. Hinuma, M. Kinugasa, K. Suzuki, Y. Sohrin, Y. Noiri, H. Tani, Y. Deguchi, N. Tsurushima, H. Ogawa, K. Fukami, K. Kuma and T. Saino (2003): A mesoscale iron enrichment in the Western Subarctic Pacific induces a large centric diatom bloom. *Science*, **300**, 958–961, Doi: 10.1126/science.1082000.
- Tsuda, A., S. Takeda, H. Saito, J. Nishioka, I. Kudo, Y. Nojiri, K. Suzuki, M. Uematsu, M. L. Wells, D. Tsumune, T. Yoshimura, T. Aono, T. Aramaki, W. P. Cochlan, M. Hayakawa, K. Imai, T. Isada, Y. Iwamoto, W. K. Johnson, S. Kameyama, S. Kato, H. Kiyosawa, Y. Kondo, M. Levasseur, R. J. Machida, I. Nagao, F. Nakagawa, T. Nakanishi, S. Nakatsuka, A. Narita, Y. Noiri, H. Obata, H. Ogawa, K. Oguma, T. Ono, T. Sakuragi, M. Sasakawa, M. Sato, A. Shimamoto, H. Takata, C. G. Trick, Y. W. Watanabe, C. S. Wong and N. Yoshie (2007): Evidence for the grazing hypothesis: Grazing reduces phytoplankton responses of the HNLC ecosystem to iron enrichment in the western subarctic Pacific (SEEDS II). *J. Oceanogr.*, **63**, 983–994.
- Umena, Y., K. Kawakami, J. – R. Shen and N. Kamiya (2011): Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature*, **473**, 55–60.
- Underwood, G. J. C., S. Fietz, S. Papadimitriou, D. N. Thomas and G. S. Diekmann (2010): Distribution and composition of dissolved extracellular polymeric substances (EPS) in Antarctic sea ice. *Mar. Ecol. Prog. Ser.*, **404**, 1–19.
- Van Heukelem, L. and C. S. Thomas (2001): Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments. *J. Chromatogr. A*, **910**, 31–49.
- Webb, W. L., Newton, M. and Starr, D. (1974) Carbon dioxide exchange of *Alnus rubra*: a mathematical model. *Oecologia*, **17**, 281–291.
- Welschmeyer, N. A. (1994): Fluorometric analysis of chlorophyll a in the presence of

- chlorophyll *b* and pheopigments. *Limnol. Oceanogr.*, **39**, 1985–1992.
- Wetz, M. S., M. C. Robbins and H. W. Paerl (2009): Transparent exopolymer particles (TEP) in a river-dominated estuary: spatial-temporal distributions and an assessment of controls upon TEP formation. *Estuaries and Coast*, **32**, 447–455.
- Whitney, F. A. (2011): Nutrient variability in the mixed layer of the subarctic Pacific ocean, 1987–2010. *J. Oceanogr.*, **67**, 481–492.
- WMO, World Meteorological Organization (2012): *WMO Greenhouse gas bulletin*. **8**.
- Wurl, O., L. Miller and S. Vagle (2011): Production and fate of transparent exopolymer particles in the ocean. *J. Geophys. Res.*, **116**, C00H13, Doi; 10.1029/2011JC007342.
- Yamada, Y., H. Fukuda, K. Inoue, K. Kogure and T. Nagata (2013): Effects of attached bacteria on organic aggregate setting velocity in seawater. *Aquat. Microb. Ecol.*, **70**, 261–272.
- Yamashita, Y., Y. Nosaka, K. Suzuki, H. Ogawa, K. Takahashi and H. Saito (2013): Photobleaching as a factor controlling spectral characteristics of chromophoric dissolved organic matter in open ocean. *Biogeoscience*, **10**, 7207–7217.
- Yasuda, I. (2003): Hydrographic structure and variability in the Kuroshio-Oyashio transition area. *J. Oceanogr.*, **59**, 389–402.
- Young, A. J., D. Phillip, A. V. Ruban, P. Horton and H. A. Frank (1997): The xanthophyll cycle and carotenoid-mediated dissipation of excess excitation energy in photosynthesis. *Pure & Appl. Chem.*, **69**, 2125–2130.
- Young, J. R., M. Geisen, L. Cros, A. Kleijne, C. Sprengel, I. Probert and J. B. Østergaard (2003): *A Guide to Extant Coccolithophore Taxonomy*. International Nannoplankton Assosiation.
- Zhang, J., T. Nishijima and K. Fukami (1996): Production of vitamin B12 binder by *Heterosigma akashiwo* and some of its properties. *Nippon Suisan Gakkaishi*, **62**, 647–653. (in Japanese with English abstract)
- Zlotnik, I. and Z. Dubinsky (1989): The effect of light and temperature of DOC excretion by phytoplankton. *Limnol. Oceanogr.*, **34**, 831–839.