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Composition of algal pigments in surface freshen layer after ice melt in the central Arctic

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Abstract

Seasonal meltwater input creates a thin freshen layer in surface seawater under ice, which largely shifts the algae assemblages. Our recent observation of photosynthetic pigments in the high Arctic showed that ice bottom and 5 m of seawater under ice contained relatively high concentration of fucoxanthin, while chlorophyll *b* and lutein were the major diagnostic pigments in ice-water interface and 0 m of seawater under ice. Additionally, a notable change of dominant phytoplankton occurred in the top 5 m of seawater under ice, from chlorophytes-dominated at surface to diatoms-dominated at 5 m depth, which might attribute to the sharp salinity gradient (salinity from 12.5 to 28.1) in the surface seawater under ice. Our results imply that phytoplankton community in surface layer under ice would become more chlorophytes in the future warming Arctic Ocean.

Key words: the Arctic Ocean, seawater under ice, pigments, nutrients, phytoplankton community

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1 Introduction

Accelerated decline of the Arctic sea ice over the last two decades was one of the most prominent signals of global climate change (Johannessen et al., 1999; Deser and Teng, 2008). Based on the satellite records, the decline rate of Arctic sea ice coverage had already rise from 2.2%–3.0% per decade during the 1979–1996 period to 10.1%–10.7% per decade in present (Comiso et al., 2008), and the ice cover in summer of 2012 declined by at least 40% compared to the average ice cover during 1979–2000 (Parkinson and Comiso, 2013). A freshening trend caused by increased freshwater storage associated with melting of sea ice (Rabe et al., 2011), resulting in a significant change on physical and ecosystem environment in the Arctic Ocean.

How phytoplankton response to sea ice retreat is of central concern due to its ecological importance in marine food web and biological pump. Early studies reported that freshening in the seasonal ice-open basin would favor picophytoplankton thrive but larger cells languish via enhancement of water stability and deepen of nutricline (Li et al., 2009; He et al., 2012). Coupel et al. (2012) also found that distinct distribution of phytoplankton abundance and biomass in marginal ice zone between low ice cover and heavy ice cover in summer. However, information about ecological impact of climate change in the high Arctic is still parse (e.g., Gosselin et al., 1997; Anderson et al., 2003; Rysgaard and Nielsen, 2006; Laney et al., 2013) due to the lack of field work. Therefore it was considered as one of the most required study regions in the Arctic Ocean (Wassmann, 2011).

Thus, it is necessary to have a detailed understanding of phytoplankton response to ice melting and surface freshening in the permanently ice cover water in the high Arctic. In the central Arctic, summer sea ice melting would thin the ice pack, producing a large freshwater input which would lead to dramatic salinity gradient and surface freshening under ice. During the Chinese Arctic cruise in summer 2014, nutrients and phytoplankton observations at a fixed floating ice pack were carried out in order to examine the change of dominant algae at ice bottom and the surface freshening layer under ice. This helps us better understand the ecological and biogeochemical dynamics after ice melting in the high Arctic.

2 Materials and methods

2.1 *Study sites and sampling*

During 19 to 23 August 2014 (CHINARE2014), ice cores and water samples were collected at six sampling sites (IT1–IT6) at a floating ice station in the high Arctic. The GPS track of ice drift and sampling sites were shown in Fig. 1. Ice cores were taken with a Mark ice corer and then seawater of ice-water interface was collected immediately. The bottom 5 cm of ice cores were sectioned and melted in pre-filter saline seawater, to avoid algae cell damage by osmotic pressure change (Garrison and Buck, 1986). Ice cores and water samples of IT1 and IT2 were taken on

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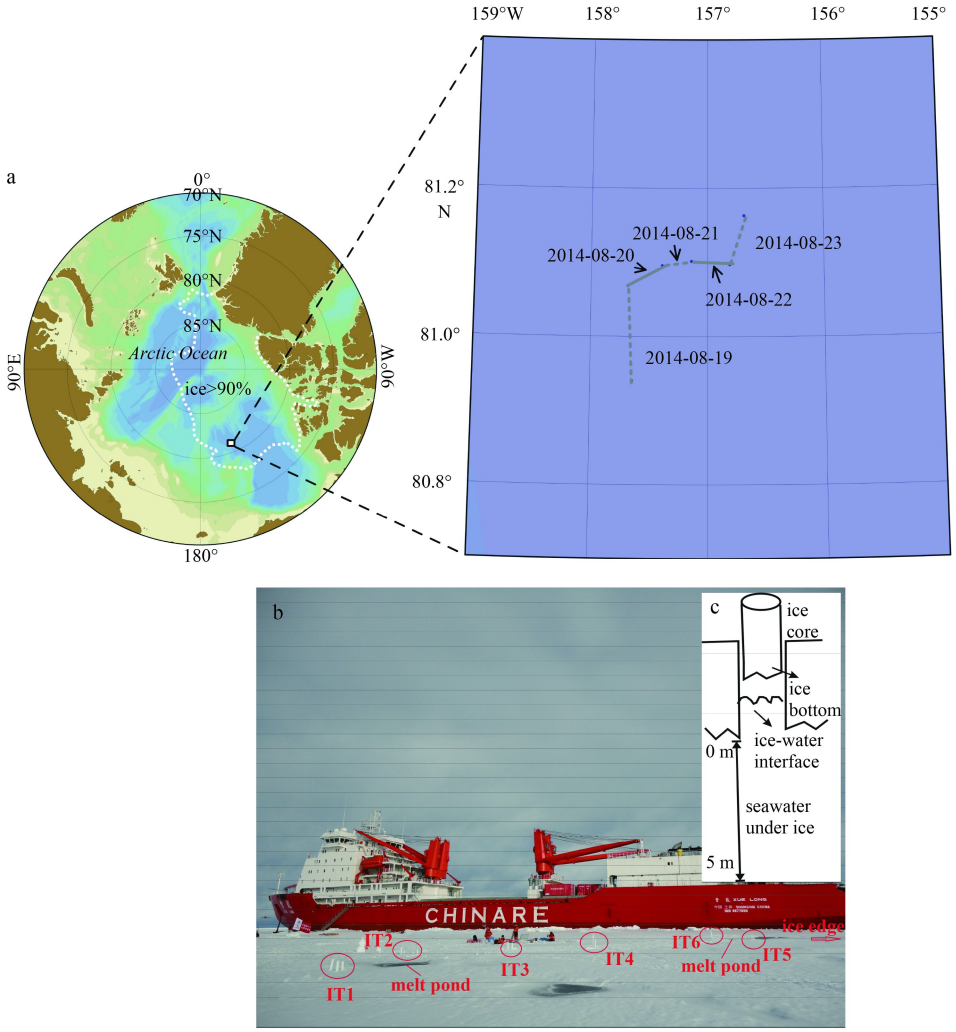

Fig. 1. Study area and drift route of sea-ice (the dashed line represents the ice cover more than 90% in August 2014 (http://nsidc.org/soac)) (a); sampling sites in the ice floating station (b); and schematic diagram of the sampling method of ice bottom, ice-water interface and seawater under ice (c).

19 August, IT3 and IT4 on 20 August, and IT5 and IT6 on 21 August. Seawater under sea-ice was collected at 0 m, 2 m and 5 m water depth on 23 August, of which the salinity was measured with WTW ProfiLine Cond 197i portable salinity sensor (Germany).

2.2 *Nutrients analysis*

Nutrient samples (nitrate plus nitrite, silicate, phosphate) for seawater and ice cores were filtered through pre-washed cellulose acetate membranes (0.45 μm) and measured immediately using a continuous flow analyzer Skarlar San++ (Holland, Breda). Analysis methods for nutrients were referred to the *Specification for Oceanographic Survey* (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (AQSIQ) and Standardization Administration of the People's Republic of China, 2008) and Grasshoff et al. (1999). The detection limits were 0.1 μmol/L for NO_3^-+NO_2^-, 0.1 μmol/L for SiO_3^{2-}, and 0.03 μmol/L for PO_4^{3-}, respectively.

2.3 *Pigments analysis*

For pigment samples, 4–8 L seawater and 1–2 L ice melting water were filtered through GF/F filters under gentle vacuum (<5×10⁴ Pa and dim light condition, and then stored at –80°C until analysis. Pigments were extracted with 3 mL of 100% HPLC-grade methanol at –20°C for 1 h, sonicated (ice-bath) for 30 s and extracted again for 1 h. The extracts were filtered through 0.22 μm microporous membrane. Before 200 μL extract was injected into HPLC system, the extract was premixed with 28 mmol/L tetrabutyl ammonium acetate (TBAA) (1:1 v/v). Pigment was analyzed by HPLC (Waters 600) system equipped with an Eclipse SDB C8 column (150 mm×4.6 mm, 3.5 μm) and with photodiode array detector (Waters 2998), using a solvent system proposed by van Heukelem and Thomas (2001) consisting of Solvent A (methanol) and Solvent B (methanol and TBAA, 70:30, v/v), at a flow rate of 1 mL/min and the column temperature of 45°C. The gradient systems used (min, A%, B%) was as follows: (0, 90, 10), (36, 5, 95), (41, 5, 95), (45, 90, 10), (55, 90, 10). Pigments were qualified and quantified by absorption wavelength at 450 nm for chlorophylls and carotenoids. The peaks were identified by comparing their retention time and spectra with those of pigment standards (DHI water and environment, Demark). Pigments measured were listed in Table 1.

2.4 *Relative contributions of different algae assemblagesto chlorophyll a*

Matrix factorization program CHEMTAX proposed by Mackey

Table 1. Major pigment in algal group and abbreviation recommended by Jeffrey et al. (1997)

Pigments	Abbreviation	Algal group and marine processes
a. Chlorophylls		
Chlorophyll *a*	Chl *a*	all photosynthetic microalgae (except prochlorophytes)
Divinyl chlorophyll *a*	DV Chl *a*	prochlorophytes
Chlorophyll *b*	Chl *b*	chlorophytes, prasinophytes
b. Carotenoids		
Alloxanthin	Allo	cryptophytes
19′-Butanoyloxyfucoxanthin	But-fuco	chrysophytes, prymnesiophytes
β, β-carotene	β, β-car	all algae except cryptophytes
Diadinoxanthin	Diadino	diatoms, dinoflagellates, prymnesiophytes, chrysophytes
Neoxanthin	Neo	chlorophytes, prasinophytes
Fucoxanthin	Fuco	diatoms, prymnesiophytes, chrysophytes
19′-Hexanoyloxyfucoxanthin	Hex-fuco	prymnesiophytes
Lutein	Lut	chlorophytes, prasinophytes
Peridinin	Perid	dinoflagellates
Prasinoxanthin	Prasino	prasinophytes
Diatoxanthin	Diato	diatoms, dinoflagellates, prymnesiophytes, chrysophytes
Violaxanthin	Viola	chlorophytes, prasinophytes
Zeaxanthin	Zea	cyanophytes, prochlorophytes
c. Chlorophyll degradation products		
Pheophytin *a*	Phytin *a*	zooplankton faecal pellets, sediments
Pheophorbide *a*	Phide *a*	protozoan faecal pellets

et al. (1998) was the most widely used method to reveal phytoplankton community based on diagnostic pigment ratios. However it is inaccurate for the small dataset of this study, thus a specific pigment ratios method (Marty et al., 2008) was applied to estimate the relative contributions of phytoplankton groups. In this method, the specific ratios of taxonomic pigment to chlorophyll *a* were selected to calculate algae assemblages. Besides, the calculated algae assemblages were classified as three major groups (Not et al., 2005) based on pigment markers: (1) Diatom-group, refers to fucoxanthin-containing group, mainly diatoms, but also including chrysophytes andprymnesiophytes; (2) Green-group, characterized by the presence of Chl *b* and lutein, mainly members of green algae (chlorophytes and prasinophytes share several pigments, but not prasinoxanthin); (3) Cyano-group, including cyanophytes and prochlorophytes. The specific pigment ratios for algae groups' identification were based on Zhuang et al. (2014), which was used on the northern Bering Shelf. The output ratios of diagnostic pigments and chlorophyll *a* were 0.61 for Fuco, 0.26 for Chl *b* and 0.35 for Zea, respectively. The empirical formulas were as follows:

$$C_{\mathrm{TChla}}=3.85C_{\mathrm{Chlb}}+1.64C_{\mathrm{Fuco}}+2.86C_{\mathrm{Zea}},$$

$$F_{\mathrm{diatom}}=1.64C_{\mathrm{Fuco}}/C_{\mathrm{TChla}},$$

$$F_{\mathrm{green}}=3.85C_{\mathrm{Chlb}}/C_{\mathrm{TChla}},$$

$$F_{\mathrm{cyano}}=2.86C_{\mathrm{Zea}}/C_{\mathrm{TChla}},$$

where F_{green}, F_{diatom} and F_{cyano} represented the relative contribution of Green-group, Diatom-group and Cyano-group to total Chl *a* biomass, respectively. C_{Chlb}, C_{Fuco} and C_{Zea} represented the concentration of Chl *b*, Fuco and Zea, respectively.

3 Results

3.1 *Nutrients distribution*

The concentrations of nutrients in the bottom section of the ice core were similar at six sampling sites (Fig. A1) and characterized by severe oligotrophic condition, with concentration in average (0.1±0.1) μmol/L (NO_3+NO_2), (0.04±0.01) μmol/L (PO_4) and (1.3±0.0) μmol/L (SiO_2), respectively (Table 2). In contrast, phosphate and silicate concentration in ice-water interface were slightly higher than those in ice bottom, with mean value of (0.20±0.11) μmol/L and (1.6±0.2) μmol/L, while inorganic nitrogen was under detected (0.1 μmol/L), suggesting that nitrogen was the absolute limiting nutrient.

As shown in Table 2, seawater under ice (0–5 m) also suffered the nitrogen limitation with inorganic nitrogen ranged from 0 to 0.5 μmol/L, which was lower than phytoplankton growth threshold (defined by 1 μmol/L). Phosphate and silicate in seawater under ice (0–5 m) were relatively abundant with a range from 0.16 to 0.71 μmol/L and 1.8 to 2.4 μmol/L, respectively. Compared to ice bottom and ice-water interface, seawater under ice had relatively high nutrients stocks, with a mean concentration of (0.52±0.19) μmol/L (PO_4) and (2.1±0.3) μmol/L (SiO_2) respectively.

Table 2. Average concentration of nutrients (μmol/L) and pigments (ng/dm^3) in ice-related habitats (ice bottom, ice-water interface and seawater under ice) at the sampling sites (IT1–IT6) in ice floes in high Arctic

Ice-related habitats	Nutrients/μmol·L^{-1}			Pigments/ng·dm^{-3}				
	NO_3+NO_2	PO_4	SiO_2	Chl *a*	Fuco	Chl *b*	Lut	Zea
Ice bottom	0.1±0.1	0.04±0.01	1.3±0.0	82±53	35±30	0±0	2±4	0±0
Ice-water interface	0.0±0.0	0.20±0.11	1.6±0.2	112±52	17±4	26±23	29±17	5±6
Seawater under ice (0 m)	0.1±0.2	0.28±0.13	1.7±0.2	132±78	16±18	60±34	46±41	6±9
Seawater under ice (2 m)	0.1±0.1	0.58±0.09	2.2±0.1	55±8	13±7	11±13	8±7	0±0
Seawater under ice (5 m)	0.1±0.0	0.69±0.02	2.3±0.0	38±7	18±3	2±5	1±2	0±0

3.2 *Algal pigments*

Pigments analysis revealed that Chl *a*, Fuco, Diadino and Diato were major pigments detected in the bottom segment of the ice cores, with average values of (82±53) ng/dm^3, (35±30) ng/dm^3, (23±17) ng/dm^3 and (5±6) ng/dm^3 respectively (Fig. A2), indicating a dominance of diatoms in the algae assemblages of the ice bottom.

Chlorophyll *a* concentration in ice-water interface ranged from 59 to 198 ng/dm^3, with a mean concentration of (112±52) ng/dm^3 (Table 2). The highest amount was encountered at IT6 (Fig. A2). Lut was the most abundant carotenoids with an average value of (29±17) ng/dm^3, while the averaged concentrations of Chl *b* and Viola were up to (26±23) ng/dm^3 and (16±15) ng/dm^3 respectively. Since prasinoxanthin was not detected (Prasinoxanthin was the diagnostic pigment of prasinophyts), the high abundance of Chl *b* and Viola indicated that chlorophytes was the dominant algae in ice-water interface. Besides, Fuco had a mean concentration of (17±4) ng/dm^3 and Zea, a diagnostic pigment of cyano-group, had a mean value of (5±6) ng/dm^3.

In the seawater under ice, both the amount and composition of photosynthetic pigments had a significant change along the water depth. Pigments at 0 m depth reached the highest concentration, with Chl *a* ranged from 62 ng/dm^3 to 285 ng/dm^3. Chl *b* and Lut was the major diagnostic pigments, with averaged concentration of (60±34) ng/dm^3 and (46±41) ng/dm^3 respectively, indicating the distribution of chlorophytes. Algal pigments at 5 m depth were relatively low compared to those at 0 m depth. However pigments at different water depths vary widely. Chl *a* and Fuco were major components of pigments with average concentration of (38±7) ng/dm^3 and (18±3) ng/dm^3, whereas others diagnostic pigments were quite low or undetected, suggesting a diatoms-dominated at 5 m depth. While at 2 m depth, two opposite distributed pattern of pigments composition were found in seawater. Fuco was the main diagnostic pigments at Stas IT1 and IT3, while Chl *b* and Lut were relatively high at the others station. The average concentration of pigments at 0 m depth were (55±8) ng/dm^3 (Chl *a*), (13±7) ng/dm^3 (Fuco), (11±13) ng/dm^3 (Chl *b*) and (8±7) ng/dm^3 (Lut).

3.3 *Algae assemblages*

The relative contributions of algae assemblages calculated based on the specific pigment ratios were shown in Table 3. As mentioned above, diatoms were major contributor of diatoms-group and chlorophytes were major contributor of green-group. A high percentage of diatoms (up to 90%) were found in ice bottom and at 5 m depth of seawater under ice. Meanwhile, in ice-water interface and at 0 m depth of seawater under ice, chlorophytes contributed 77% and 87%, respectively, to thetotal chlorophyll *a* biomass. At 2 m depth of seawater under ice, diatoms and chlorophytes co-dominated the phytoplankton community. Cyanobacteria had a significant distribution in ice-water interface and at 0 m depth of seawater under ice, with a relative contribution of 6% and 3%.

4 Discussion

4.1 *Dramatic change of dominant algae in ice ecosystem*

As shown in Fig. 2, pigment-induced phytoplankton community shown that most of algae biomass in ice bottom was contributed by diatoms, with a relative contribution higher than 90%, which was consistent with previous observation in the central Arctic. Gradinger (1999) also found that pennate diatoms dominated in the bottom layer of ice cores, while phototrophic flagellates ruled the upper parts. Besides, pigments biomass of ice bottom in the central Arctic was two or three orders of magnitude lower than that in Chukchi shelf (Gradinger, 2009), which probably ascribed to nutrients availability in the bottom layer.

Phytoplankton community in ice-water interface was dominated by chlorophytes, which was different from ice bottom (Fig. 2), suggesting a distinct mechanism of algal physiology between ice and water. The low salinity in ice-water interface might benefit chlorophytes and others flagellates. Also a green algae bloom was recorded in under-ice ponds with salinity of ice-water interface as low as 9.1 (Gradinger, 1996). It has been reported that the coverage of under-ice ponds accounted for 5% of the Arctic sea ice cover (Eicken, 1994). However, nutrients stock indicated ice-water interface might hardly sustain an algae bloom in the study area.

Chlorophyll *a* biomass in seawater under ice decreased gradually with the increase of depth, which might be related to light intensity (Fig. 2). With the increase of depth and salinity, the chlorophytes biomass decreased whereas the diatoms biomass increased. Thus, a significant shift of dominant phytoplankton was observed at the top 5 m of seawater under ice, with the algae assemblages shifted from dominance of chlorophytes at 0 m depth to co-dominate by diatoms and chlorophytes at 2 m depth, and then to diatoms-dominated at 5 m depth (Fig. 2). The rapid change of phytoplankton composition in seawater under ice was unique in the global ocean.

Generally, nutrients availability played an important role in phytoplankton selection. For instance large diatoms usually dominated in the nutrients-rich region and picophytoplankton dominated in the oligotrophic water. In here, spearman correlation coefficient analysis showed that change of algae assemblages significantly correlated with environmental variables, such as depth, salinity, phosphate and silicate, but not related with inorganic nitrogen (Table 4). Extremely low concentration of inorganic nitrogen absolutely limited the phytoplankton growth. Thus nutrients were not the major cause of the rapid change of dominant phytoplankton. The salinity change, however, might be the key factor for the phytoplankton shift in such thin layer of seawater under ice. As mentioned, a strong salinity gradient formed in the top 5 m of seawater under ice, with salinity increase from in average 12.5 at 0 m to 28.1 at 5 m (Fig. 2). Appar-

Table 3. Average concentration of Chl *a* (ng/dm^3) and average contribution of different algae assemblages in ice-related habitats (ice bottom, ice-water interface and seawater under ice)

Ice-related habitats	Chl *a*/ng·dm^{-3}	Percentage/%		
		Diatoms-group	Green-group	Cyano-group
Ice bottom	82	92	8	0
Ice-water interface	112	17	77	6
Seawater under ice (0 m)	132	9	87	3
Seawater under ice (2 m)	55	47	53	0
Seawater under ice (5 m)	38	93	7	0

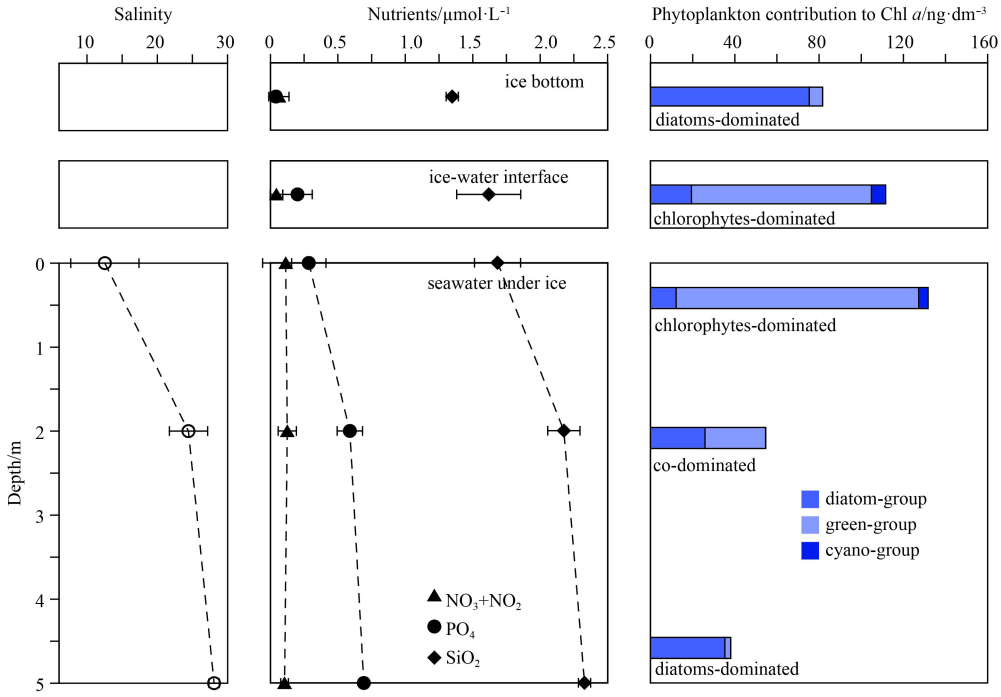

Fig. 2. Distribution of averaged salinity, nutrients and algae assemblage based on the observation in the ice-system (ice bottom, ice-water interface and seawater under ice) in the central Arctic.

Table 4. Spearman correlation coefficient analysis between environmental variables and algae assemblages

		Depth	Salinity	P	N+N	Si
Diatom-group	correlation coefficient	0.605**	0.683**	0.597**	0.288	0.663**
	significance (bilateral)	0.008	0.002	0.009	0.246	0.003
Green-group	correlation coefficient	–0.878**	–0.871**	–0.789**	–0.343	–0.860**
	significance (bilateral)	0.000	0.000	0.000	0.164	0.000
Cyano-group	correlation coefficient	–0.545*	–0.469*	–0.470*	–0.307	–0.517*
	significance (bilateral)	0.019	0.050	0.049	0.215	0.028

Note: ** When confidence level (bilateral) is 0.01, the correlation is significant; * when confidence level (bilateral) is 0.05, the correlation is significant.

ently, phytoplankton responses promptly to the surface freshening layer in the sea-ice system. Therefore ice melt in summer was an important factor influencing the algae assemblage in the central Arctic.

4.2 *Comparison of dominant algae in ice-system with others oligotrophic waters*

As in the other oceans, nitrogen limitation constrains sustainability of primary production in the Arctic Ocean (Falkowski, 1997). The surface seawater under ice was considered as the most nitrogen limitation part in the Arctic Ocean (Cota et al., 1990). In tropical and temperate oceans, prochlorophytes and cyanobacteria were usually the dominant algae in oligotrophic surface waters (Table 5) owing to their low nutrients demand. However, distribution of prochlorophytes and cyanobacteria were limited in the Arctic Ocean, due to the low temperature and light availability. Previous study (Coupel et al., 2012) suggested that pico-flagellates (mainly prasinophytes) dominated in phytoplankton community at the ice edge of the Arctic Ocean (Table 5). While in perennially ice-covered central Arctic, algae assemblages of surface water under ice was dominate by chlorophytes. Chlorophytes were widely distributed in the freshwater, thus the extremely low salinity in surface water under ice might beneficial to chlorophytes. In other words, environment factors such as salinity significantly influence the phytoplankton community in different oligotrophic waters, although primary production was subject to nutrients deficiency.

4.3 *Impact of freshening on the algal community in the high Arctic*

The consequences of the freshening on phytoplankton dynamics in the Arctic Ocean have been well studied in recent years. As shown in Fig. 3, the surface freshening by sea ice reduction and/or higher river runoffs (Yamamoto-Kawai et al., 2009) as well as accelerated nutrient consumption in surface layer, resulted in a more oligotrophic Arctic Ocean with a deepened nutricline (Coupel et al., 2014). Consequently phytoplankton became smaller (Li et al., 2009) and subsurface chlorophyll maximum (SCM) deeper (McLaughlin and Carmack, 2010). In perennially ice-covered area, a strong salinity gradient (from salinity about 5 to 28) formed in the surface layer under sea ice and then a second

Table 5. Comparison of the algae assemblages in the oligotrophic ecosystem

Area	Characteristic	Depth	Dominant algae assemblage	Reference
Equatoiral Pacific	warm pool	upper ocean (<150 m)	Prochlorococcus, Synechococcus and haptophytes	Mackey et al. (2002)
Western EquatoiralPacific	warm pool	upper ocean (<200 m)	prochlorophytes, cyanobacteria and prymnesiophytes	Zhuang et al. (2012)
Offshore of East China Sea	Kuroshio water	upper ocean (<150 m)	prochlorophytes, chrysophytes and prymnesiophytes	Furuya et al. (2003)
Northern South China Sea	warm eddy formed by the Kuroshio intrusion	euphotic zone (<100 m)	prochlorophytes	Huang et al. (2010)
Northern Bering Sea	nutrients-depleted surface water	surface seawater	diatom and chrysophytes co-dominated	Zhuang et al. (2014)
Canada Basin	marginal ice zone	surface seawater	Prasinophytes-dominated	Coupel et al. (2012)
High Arctic	ice-system	sea-ice interface	chlorophyte-dominated	this paper

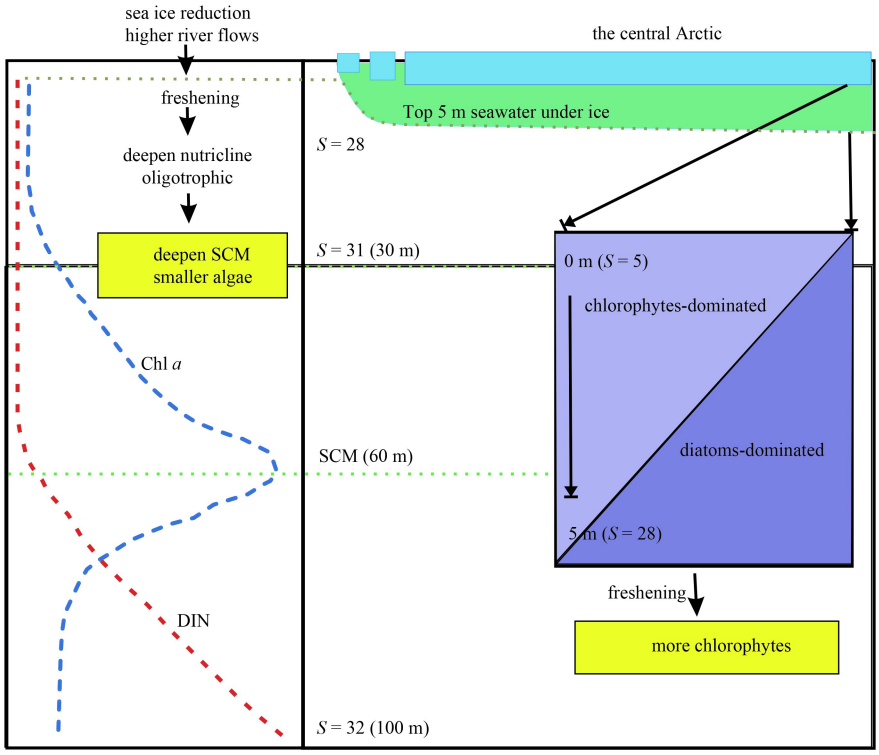

Fig. 3. The sketch of the freshening impact on the phytoplankton dynamics in the central Arctic.

salinity gradient in halocline layer. Ashift of dominant algae associated with salinity change occurred, from a chlorophytes-dominated community to a diatoms-dominated one. Therefore the increased freshening in the surface layer under sea ice caused by the global warming might shift the phytoplankton community to more chlorophytes in the central Arctic in the future.

5 Conclusions

In our observation, a dramatic salinity gradient together with an alternation of dominant algae occurred at the top 5 m of the seawater under ice in the high Arctic Ocean. Chlorophytes dominated at 0 m in the seawater with low salinity. The phytoplankton rapidly shifted to diatom-dominated at 5 m with relatively high salinity. Our results imply that phytoplankton community in the surface layer under ice would become more chlorophytes in the future freshening Arctic Ocean. This shift might alter the food web in the perennially ice-covered Arctic Ocean since the community size and structure are strong determinants of the ecosystem carbon flux. A chlorophytes-based system tended not to support large exports of biogenic carbon. Our observation in top 5 m of the seawater under ice was a complement of study on phytoplankton dynamics response to the freshening in the high Arctic Ocean.

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Appendix:

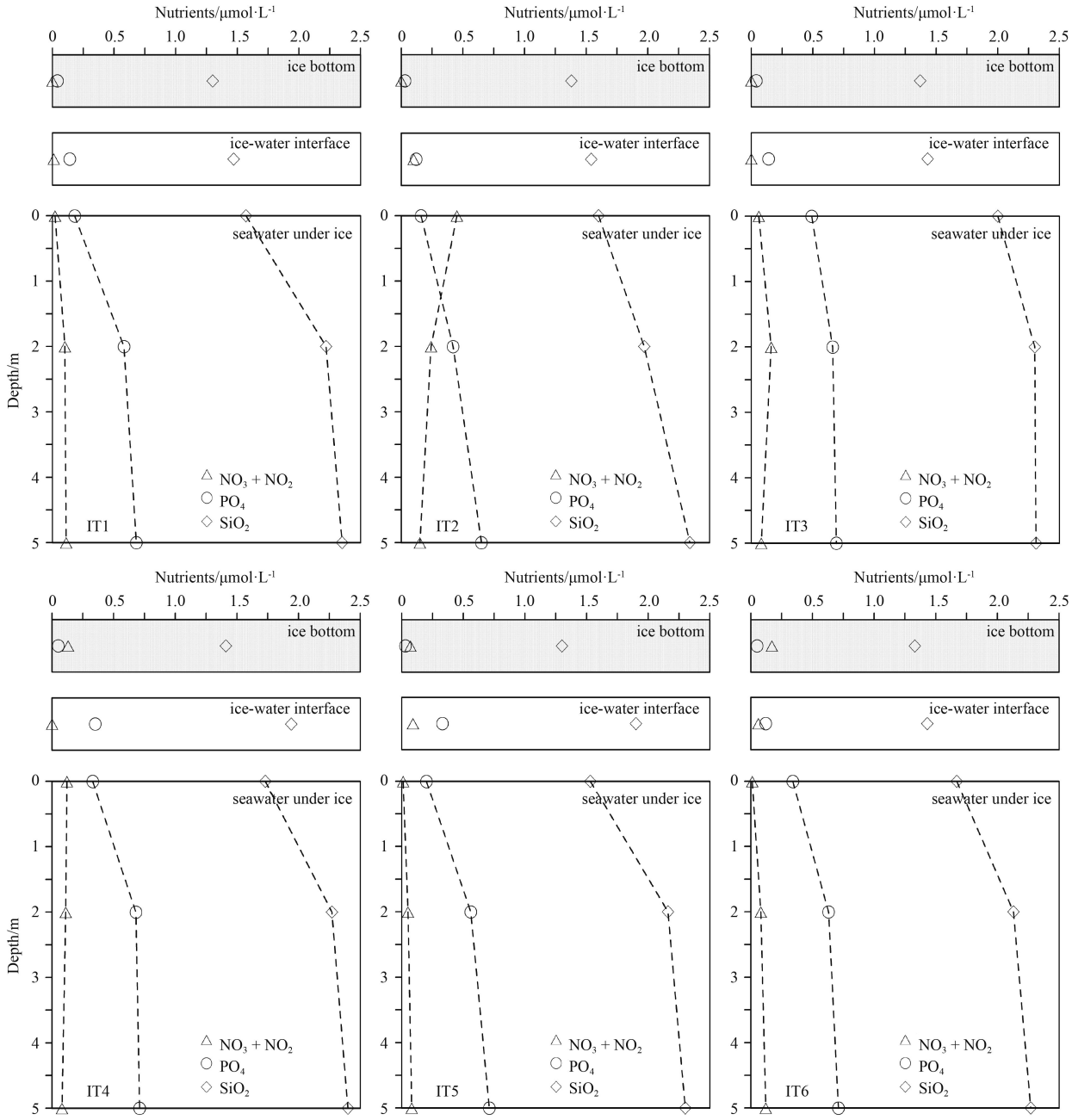

Fig. A1. Nutrients distribution (μmol/L) of ice bottom, ice-water interface and seawater under ice at the sampling sites (IT1-IT6) in ice floes in high Arctic.

Fig. A2. Distribution of Chl *a* and four major diagnostic pigments (ng/dm³) in ice bottom, ice-water interface and seawater under ice at the sampling sites (IT1-IT6) in ice floes in the high Arctic, different color represented Chl *a*, Fuco, Chl *b*, Lut and Zea, respectively.