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Vertical variation of pelagic ciliate communities in the western Arctic Ocean

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ABSTRACT

The vertical distribution and structure of pelagic ciliate communities were investigated at 32 stations (western Arctic Ocean) in a summer sea-ice reduction region from August 1 to September 10, 2012. The distributions of species number, abundance, biomass, dominant species number and abundance, and structural diversity indices showed clear vertical trends associated with vertical changes in the water column. In addition, vertical patterns in community structure accurately reflected those environmental conditions. Multivariate correlation analysis demonstrated that vertical variation in ciliate communities was significantly related to a series of environmental variables. Community structure parameters, especially Shannon diversity (H') and Margalef richness (D), showed strong relationships with vertical changes in chlorophyll *a* and might provide better predictors in future studies. Furthermore, heterotrophic and mixotrophic assemblages both demonstrated clear vertical distribution patterns, and microzooplankton have shown significantly relationship with chlorophyll *a*. These results provide basic data on vertical variation in ciliate communities in the western Arctic Ocean and have considerable potential to understand how pelagic ciliates structured in water column.

1. Introduction

Ciliates are important components of microplankton communities and play an important role in the transfer of energy and material through the pelagic food web (Sherr and Sherr, 2002, 2009). In marine ecosystems, planktonic ciliates are believed to feed on pico- and nanoplankton, which are the dominant size fractions in terms of biomass and primary productivity (Stoecker and McDowell-Cappuzzo, 1990; Sherr et al., 2013), and are expected to be the main grazers as copepods are unable to crop these size classes efficiently (Marshall, 1973). Although the importance of planktonic ciliate ecology is being increasingly recognized and extensive ecological studies in arctic/sub-arctic waters have been investigated in last decade (e.g., Jensen and Hansen, 2000; Lovejoy et al., 2002; Comeau et al., 2011; Lovejoy and Potvin, 2011; Mironova et al., 2013; Franzè and Lavrentyev, 2014), data regarding vertical variation in pelagic ciliate communities and their relationship with water masses are still scant, particularly in the western Arctic Ocean.

Since the late 1990s, catastrophic sea ice reductions during the summer have been observed in the Pacific sector of the Arctic Ocean (western Arctic Ocean) (Shimada et al., 2006; Serreze et al., 2007). As previous studies have shown, the upper several hundred meters of the Arctic Ocean and adjacent seas, such as the Chukchi Sea, are strongly stratified (e.g., Bates et al., 2005). With decreasing sea ice, these regions might experience increased phytoplankton production and diversity compared to ice-covered areas because of the intensification of light in the water column (Lee and Whitledge, 2005) and increased wind-induced mixing, which replenishes sea surface nutrients (e.g., Carmack et al., 2006). Therefore, the distributions of water masses and relationships to nutrient distributions and algal biomass have been examined in detail (e.g., Nishino et al., 2008). High pelagic primary productivity provides the basis for enhanced local secondary production (Lee et al., 2010). However, we still cannot predict whether ongoing vertical changes in water column will affect pelagic ciliates because very little is known about ciliates; species-level community observations are particularly lacking in this region. Thus, to better characterize both the pelagic ciliate community in the western Arctic Ocean and factors that influence its composition and structure, samples of ciliates and other biotic and abiotic parameters were collected from 32 profiles during the late

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summer in a region of melting sea ice. The resulting data were used to provide a more complete picture of the ciliate community in the western Arctic Ocean.

The main objectives of this study were to characterize the vertical distribution of planktonic ciliates, reveal vertical patterns in community structure, determine their potential relationship with water environmental condition, and try to determine factors that influence the vertical distribution of ciliates in the western Arctic Ocean.

2. Material and methods

2.1. Study stations

A multidisciplinary survey was conducted onboard the Korean icebreaker *Araon* in the Chukchi Sea and east Siberian Sea of the western Arctic Ocean, encompassing the area from the Mendeleyev Ridge to the Chukchi Borderland (including the Chukchi Plateau and Northwind Ridge) during late summer from August 1 to September 10, 2012 (Fig. 1). 32 sampling stations were visited (Fig. 1).

2.2. Sampling and sample processing

In total, 227 samples were collected from the 32 stations during the cruise. Vertical profiles of seawater temperature, salinity, density of water, and dissolved oxygen were obtained using a CTD/Rosette system (SeaBird Electronics, SBE 911+) at each sampling station in a depth gradient of 0 m, 10 m, 20 m, 40 m, 60 m, 75 m, 100 m and 150 m.

Water samples for nutrient analysis were collected using the CTD/Rosette sampler holding 24 10-l Niskin bottles. Nutrient samples (100 ml) for measuring nitrate+nitrite (NO_2+NO_3), ammonium (NH_4), phosphate (PO_4), and silicate concentrations (SiO_2) were analyzed onboard the ship using a Bran and Luebbe model Quatro AA (Auto Analyzer), according to the manufacturer's manual.

Water samples (500–1000 ml) for total chlorophyll *a* (Chl *a*) concentration were taken from each depth and immediately filtered through glass fiber filter paper (47 mm; Gelman GF/F). Concentration of Chl *a* was measured onboard using a Turner design trilogy fluorometer after extraction with 90% acetone (Parsons et al., 1984).

To determine the abundance of ciliates, a Niskin Rosette sampler was used to take water samples from each depth. For quantitative studies and the identification of ciliates, 500-ml seawater samples were fixed with Lugol's iodine solution (4% final concentration, volume/volume) (Yang et al., 2009, 2010); these were then stored at 4 °C in darkness until analysis (Pitta et al., 2001; Kchaou et al., 2009; Choi et al., 2012; Yang et al., 2012). Preserved samples were allowed to settle in the mass cylinder for at least 48 h. The upper water was then siphoned off, leaving 20 ml. 1 ml aliquot of each concentrated sample was placed in a Perspex chamber and the ciliates were counted under a light microscope at magnifications from 200× to 400×. Ultimately, 50 ml of seawater was examined to assess abundance. Tintinnids were identified using lorica morphology and the species descriptions of Kofoid and Campbell (1929, 1939); other naked ciliates were identified to the lowest possible taxonomic group or morphologically similar taxa following references such as Maeda (1986), and internet sources (e.g., Strüder-Kypke and Montagnes, 2002). Trophic preference of ciliate was also taken from these sources because the fixative made it impossible to differentiate between heterotrophic or mixotrophic taxa. The taxonomic scheme used was according to Lynn (2008).

The biovolumes of naked ciliate cell or tintinnid lorica were determined from measurements of their linear dimensions and the volumes were calculated from standard geometric shapes (Hillebrand et al., 1999) and carbon content estimated from relationships described in Menden-Deuer and Lessard (2000). Hereinafter, the term biomass refers to carbon biomass.

2.3. Data analysis of samples

The biodiversity parameters species diversity (Shannon-Wiener H'), species evenness (Pielou's J') and species richness (Margalef D) were computed following the equations:

$$H' = -\sum_{i=1}^{S} P_i(\ln P_i)$$

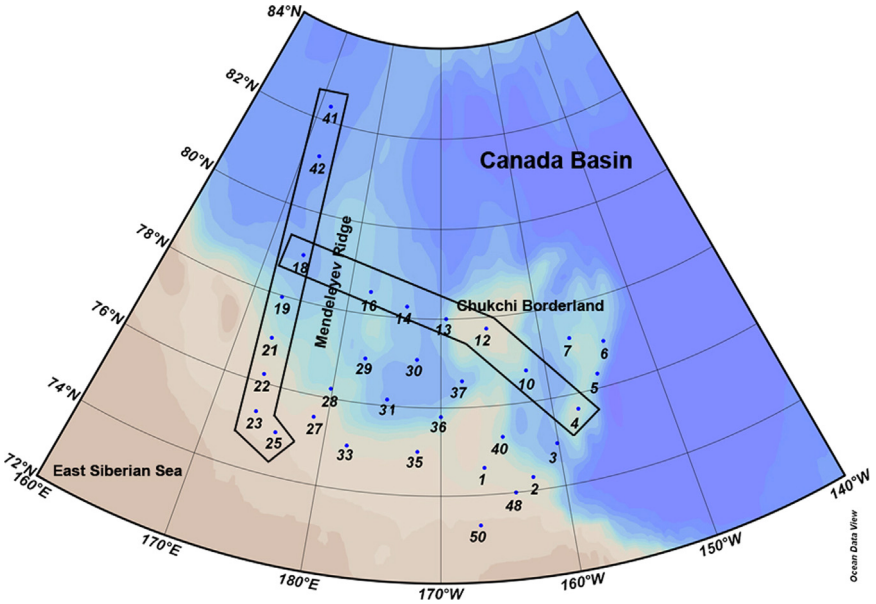

Fig. 1. 32 Sampling stations in western Arctic Ocean from August 1 to September 10, 2012. Samples are coded for stations. Map produced using Ocean Data View (Schlitzer, 2003).

$J' = H'/\ln S$

$D = (S-1)/\ln /N$

where Pi = proportion of the total count arising from the ith species, S = total species, and N = total individuals.

Multivariate analyses of spatial variations in ciliate communities were conducted using the PRIMER v6.1 package and PERMANOVA+ for PRIMER. The contribution of each species to the average Bray–Curtis similarity within each depth and the dissimilarity among groups was examined using the SIMPER (Similarity Percentage Analysis) program (Clarke and Gorley, 2006). Vertical environmental status of the eight sampling depths was summarized using principal components analysis (PCA) based on log-transformed/normalized abiotic data (Clarke and Gorley, 2006; Jiang et al., 2013a). Vertical differences in ciliate communities and relationships with community structure parameters among the eight sampling depths were summarized using the submodule CAP (canonical analysis of principal coordinates) with Bray–Curtis similarities from log-transformed species–abundance data (Anderson et al., 2008; Xu et al., 2013; Jiang et al., 2013b). Differences among groups of samples in PCA and CAP analyses were tested with the analysis of similarities (ANOSIM) (Clarke and Gorley, 2006) and PERMANOVA (Anderson et al., 2008). The significance of biota–environment correlations was tested using the routine RELATE (Mantel test) (Clarke and Gorley, 2006). Submodule biota–environment (BIOENV) was used to explore potential multivariate relationships between biotic parameters and the environmental data (Clarke and Gorley, 2006).

Univariate spearman correlation analyses were carried out using the statistical program SPSS v16.0. Data were log-transformed before analyses.

Geographical map and figures were created using ODV software (R. Schlitzer, Ocean Data View, 2003, http://www.awi-bremerhaven.de/GEO/ODV).

3. Results

3.1. Vertical hydrologic structure of the water column

The vertical sections of physicochemical parameters on a W–E transect including stations 18, 16, 14, 13, 12 10, and 4 are summarized in Fig. 2. The values of water temperature in the east showed higher than the west, and increased first with depth, then decreased gradually (Fig. 2A). However in the west (station 18), the temperature decreased first, and then increased (Fig. 2A). Of particular note, is the deepening of the chlorophyll-*a* (Chl *a*) maxima from west to east (20 m down to 60 m) (Fig. 2F), following the deepening of the pycnocline as shown most clearly in the salinity and water density plots (Fig. 2B and C). Dissolve oxygen (DO), silicate (SiO_2), phosphate (PO_4) and nitrate nitrogen+nitrite nitrogen (NO_2+NO_3) showed the same pattern (Fig. 2D, E, G, and H). In contrast, ammonium nitrogen (NH_4) showed no consistent geographical pattern (Fig. 2I).

With regards to the S–N transect including stations 41, 42, 18, 19–22, 23 and 25 was shown in Fig. 3, the variations of parameters showed a similar vertical pattern as W–E transect. Although differences of measurements between north and south existed, almost all plots showed similar gradually changes except that of NH_4, and water column could be divided basically as two layers by pycnocline, which appears to range from ~20 to 30 m in salinity/density (Fig. 3 B and C). Notably, the higher subsurface chlorophyll-*a* values occurred in the stations (25–21) on the shallow Chukchi shelf compare with those of the stations (18, 42, 41) in the deep sea (Figs. 1 and 3F).

3.2. Vertical variations in taxonomic composition, abundance, biomass and biodiversity parameters

In total, 55 ciliate species were identified during the survey. The information of taxonomic composition and individual dimension was listed in Table S1. Of these, the top-ranked 18 species that provided a cumulative contribution >90% to the ciliate community in each depth were summarized using the SIMPER software and defined as "dominant"; their contributions and ranks are shown in Table 1. Several species provided noticeable contributions to all depths, including *Strombidium acutum*, *Mesodinium rubrum*, *Leegaardiella sol*, *Leegaardiella ovalis,* and *Lohmanniella oviformis*. Other dominant species showed clear vertical distributions. For example, *Tontonia* cf. *gracillima* and *Strombidium* cf. *golbosaneum* were distributed primarily above depth 75 m, while *Strombidium capitatum* was found in surface 0–10 m and *Rimostrombidium* cf. *caudatum*, *Balanion* cf. *comatum*, *Pelagostrobilidium* cf. *neptuni*, *Laboea strobili,* and *Salpingella faurei* were distributed mainly in the middle sampling depths (30–60 m). From the surface to the deep, the highest dominant species number and abundance occurred at 30–60 m, and the lowest dominant species count was found in the deeper (Table 1).

It is notable that, in upper 0–60 m, the mixotrophic ciliates (e.g., *Mesodinium rubrum*, *S. acutum*, *Tontonia* spp. and *Laboea strobili*) dominated communities (Table 1). However in deeper water, mixotrophic assemblages showed rare distribution and low abundance, but heterotrophic ones (e.g., *Leegaardiella* spp. and *Mesodinium pulex*) were the top contributors to communities (Table 1). In addition, SIMPER analysis revealed that the species composition between samples of 0–60 m and 75–150 m was obviously different at 80.29% dissimilarity level.

The vertical distributions of species number, abundance, biomass, three biodiversity parameters (D, J' and H'), and biomass of mixotrophic and heterotrophic ciliates in a W–E transect and an S–N transect are shown in Figs. 4 and 5 respectively. At two transects, all biotic variables showed obvious decreasing gradients and stratification from the surface to 150 m, and could be basically discriminated into two groups (0–60 m and ~60–150 m), although highest values mainly measured in the upper 0–60 m at western and southern regions (Figs. 4 and 5) and no obvious regular variation was observed in vertical/horizontal distribution of Pielou's J' (Figs. 4 and 5F). Notably, biomass of mixotrophic and heterotrophic assemblages all showed higher values in 0–60 m than deeper, and the distribution pattern of heterotroph biomass is exactly the same with that of Chl *a*.

3.3. Relating vertical community structure patterns to vertical hydrologic structure in the water column

Relationships among the eight depth categories based on all environmental characteristics were summarized by principal components analysis (PCA) (Fig. 6A). The two principal components, which explained 72.0% of the total environmental variability, successfully discriminated the environmental conditions of eight depths (Fig. 6A). The results show that the samples of upper depths (0–60 m, left of the plot) was dramatically different from those of deeper (60–150 m, right of the plot), and could be divided as two groups by axis PC1 which could explained 58.8% of the total environmental variability (Fig. 6A). An ANOSIM test revealed significant differences between samples from two water groups ($R=0.584$, $P=0.001$).

Discrimination among the eight depths was plotted using canonical analysis of principle components (CAP) with Bray–Curtis similarities using log-transformed species-abundance data. Clear vertical patterns were observed in ciliate communities (Fig. 7). The first-squared canonical correlation was large ($\delta^2=0.705$) and the first canonical axis separated communities of upper depths

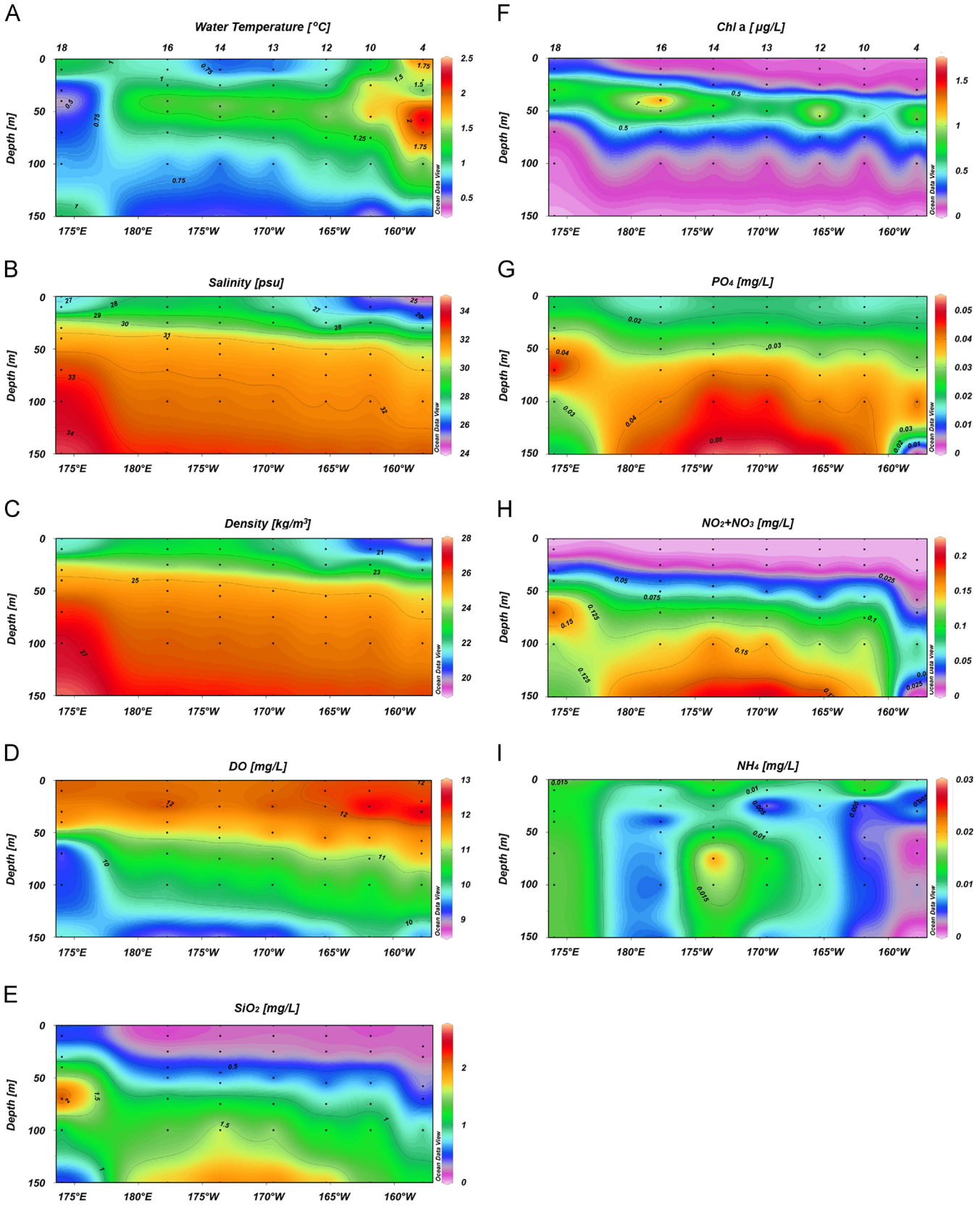

Fig. 2. Vertical section of nine environmental variables along a west–east transect including stations 18, 16, 14, 13, 12 10, and 4 in western Arctic Ocean from August 1 to September 10, 2012. DO, dissolved oxygen; Chl *a*, chlorophyll-*a*; SiO_2, silicate concentrations; PO_4, phosphate; NO_2+NO_3, nitrate+nitrite nitrogen; NH_4, ammonium nitrogen. The label on the top is station number.

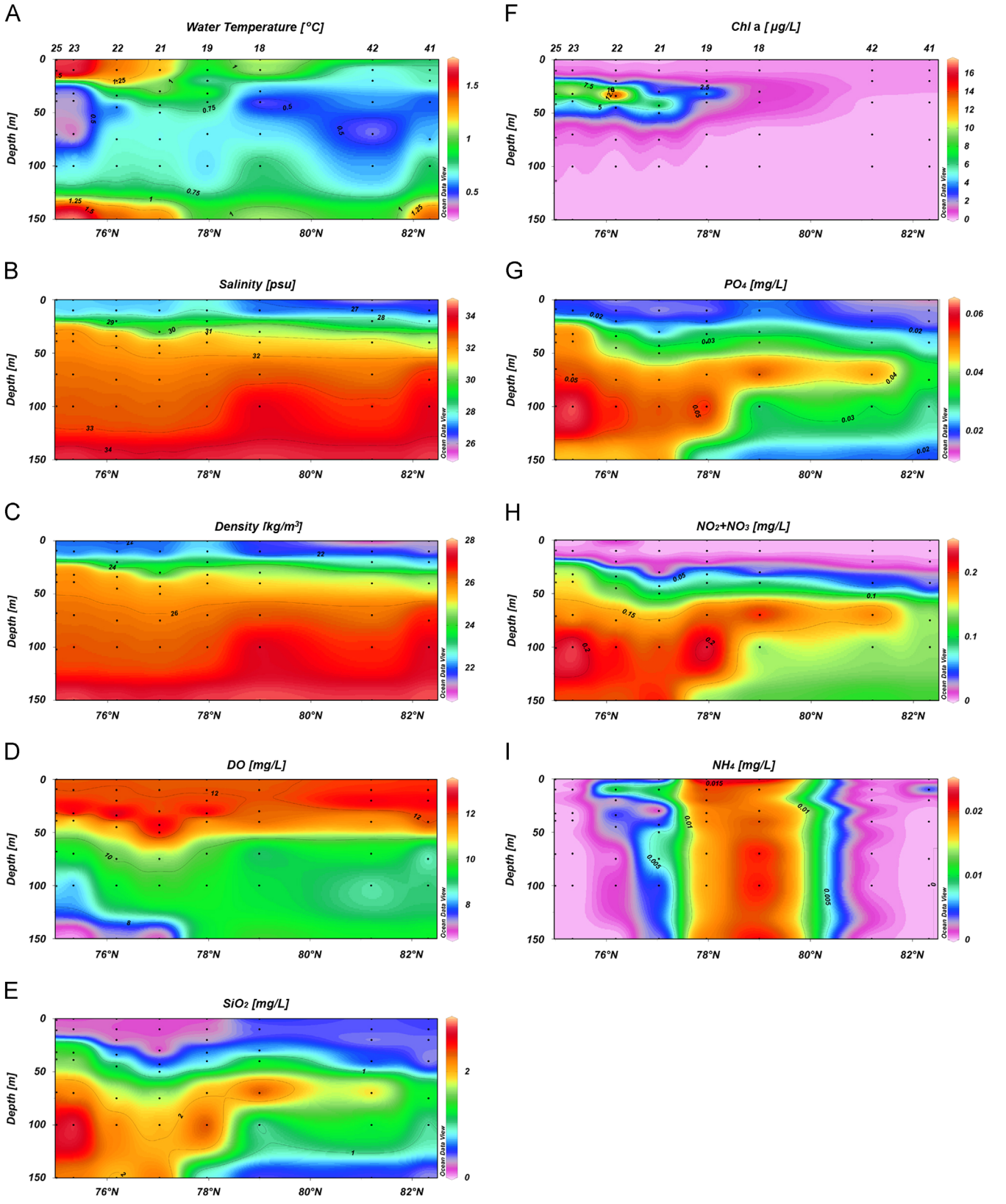

Fig. 3. Vertical distribution of nine environmental variables along a south-north transect including stations 41, 42, 18, 19, 21–23 and 25 in western Arctic Ocean from August 1 to September 10, 2012. DO, dissolved oxygen; Chl *a*, chlorophyll-*a*; SiO_2, silicate concentrations; PO_4, phosphate; NO_2+NO_3, nitrate+nitrite nitrogen; NH_4, ammonium nitrogen.

(0–60 m, on the right of the plot) from those of deeper ones (60–150 m, on the left of plot). The second canonical axis had a much smaller eigenvalue ($\delta^2=0.117$) and there was actually no clear separation (Fig. 7). A PERMANOVA test demonstrated that there was a significant effect of the two water groupings (pseudo-$F=27.204$, $P=0.001$).

Table 1
Average species number, abundance (ind. l^{-1}) and biomass ($\mu g\ C\ l^{-1}$) among each sampling depth, and dominant species list with average abundance (Av. *N*, ind. l^{-1}) and cumulative contribution (%) to communities. Hetero, heterotrophy; Mixo, mixotrophy.

		0 m		10 m		20 m		40 m		60 m		75 m		100 m		150 m	
Species number		36		37		43		47		44		29		21		24	
Abundance		875		1379		1363		2466		1113		196		145		107	
Biomass		3.64		7.03		5.00		9.12		3.43		0.44		0.25		0.55	
		Av. *N*	%	Av. *N*	%	Av. *N*	%	Av. *N*	%	Av. *N*	%	Av. *N*	%	Av. *N*	%	Av. *N*	%
Balanion cf. *comatum*	Hetero							32	1.7	23	3.4						
Laboea strobila	Mixo							37	1.5								
Leegaardiella ovalis	Hetero	36	4.4	45	4.8	86	14.9	48	4.1	35	4.2	13	5.0	12	10.0	11	16.8
Leegaardiella sol	Hetero	42	5.4	42	10.1	58	10.3	69	10.7	58	9.5	18	23.3	13	9.3	12	13.7
Lohmanniella oviformis	Hetero	57	3.1	97	4.6	108	2.1	225	10.4	102	9.6	10	6.5	9	7.5	6	6.8
Mesodinium pulex	Hetero			67	4.3	253	8.3	496	7.7	77	6.9	49	40.9	51	62.7	24	37.0
Mesodinium rubrum	Mixo	111	11.5	263	26.2	156	16.3	229	15.7	58	12.3	17	7.2			8	12.1
Pelagostrobilidium cf. *neptuni*	Hetero							32	1.5	19	2.2						
Pelagostrobilidium cf. *spirale*	Hetero					40	3.1	50	2.6	37	3.6						
Rimostrombidium cf. *caudatum*	Hetero							43	2.3								
Salpingella faurei	Hetero									89	3.9						
Spirotontonia cf. *grandis*	Mixo	22	2.5	50	2.2			83	2.1	25	2.5						
Strombidium acutum	Mixo	221	37.2	274	18.1	121	21.5	122	12.0	94	11.9	13	4.3	10	3.8	7	4.9
Strombidium capitatum	Mixo	27	3.6	66	2.7												
Strombidium cf. *globosaneum*	Hetero	78	8.2	83	6.9	65	2.4	198	6.3	66	7.4	10	3.0				
Strombidium cf. *pollostomum*	Hetero	34	3.3					67	3.4	72	5.6						
Tontonia cf. *gracillima*	Mixo	40	8.5	53	7.0	55	12.2	68	6.0	54	5.0						
Tontonia sp.	Mixo	32	3.1	43	3.6			61	2.2	30	2.5						

A vector overlay of the Spearman's correlations for the community structure parameters among the eight depths with the CAP axes is shown in the inset of Fig. 7. Species number, abundance, biomass, richness (*D*), Shannon diversity (*H′*), and biomass of mixo/heterotrophs had the same orientation, toward the upper right of the plot which is sample cloud of upper depths. The species evenness (*J′*) index pointed toward the bottom left of the plot, in contrast to the other indices (Fig. 7). From the lengths of the vectors, it is not surprising that *J′* played a relatively minor role once the others were taken into account (Fig. 7).

To improve the consistency in vertical variability for the abiotic and biotic data from the above CAP and PCA analyses, a RELATE analysis (Mantel test) was conducted to identify potential relationships. A large ($\rho=0.765$) and significant ($P=0.001$) correlation was found between vertical variations in planktonic ciliate abundances and environmental variables.

Correlations between ciliate abundances and environmental variables were established using multivariate biota–environment (BIOENV) analysis (Table 2). The best match with ciliate abundances occurred with dissolved oxygen ($\rho=0.894$, $P=0.01$). In combination with dissolved oxygen, Chl *a*, water temperature, SiO_2, NO_2+NO_3, and NH_4 were the most common variables and were included in all correlations with statistical significance ($P<0.05$) (Table 2).

A PCA plot that included vectors for both community structure parameters (species count, abundance, biomass, diversity, richness, evenness and biomass of mixo/heterotrophs) of ciliate communities and physicochemical variables is shown in Fig. 6B to examine relationships among these variables. Most of ciliate variables, except Pielou's evenness *J′*, were correlated with the first principal component and showed correlations with physicochemical variable vectors (e.g., water temperature, salinity, DO, NO_2+NO_3, and PO_4) toward the lower left the plot where deeper depth samples located (Fig. 6B). Notably, comparing to mixotrophs, biomass of heterotrophs showed higher correlation with the second principal component and vector of Chl *a* (Fig. 6B). Spearman's correlation analysis (Table 3) found similar correlations, with the being positively correlated with Chl *a* and DO, but negatively correlated with salinity, water density, SiO_2, and nutrients. For example, species number (*S*), richness (*D*), diversity (*H′*) and biomass of heterotrophs were significantly positively correlated ($P<0.01$) with Chl *a* but negatively correlated with NH_4, while abundance, biomass and mixotrophs were positively correlated ($P<0.01$) with DO but negatively correlated with nutrients ($P<0.05$). The only exception was species evenness (*J′*), which showed the opposite pattern, being positively correlated ($P<0.05$) with salinity, water density, and SiO_2 (Table 3).

4. Discussion

In the last decades, the sea ice extent in the Arctic has been shrinking dramatically during summers (Arctic Climate Impact Assessment, 2004). Vertical structure in water column over the Chukchi Plateau is recognized as a typical stratification structure in the western Arctic Ocean (Nishino et al., 2008). Upper water masses over the Chukchi Sea shelves and the Arctic Ocean normally include the Polar Mixed Layer (PML; upper 0–50 m, salinity typically <31) and the Upper Halocline Layer (UHL; ~50–150 m deep; core layer has a salinity of 33.1) (Shimada et al., 2001; Bates et al., 2005). To examine the vertical distribution of water masses and environmental variation in present study, we studied vertical profiles of environmental variables. The salinity and density contours represented isohaline surfaces and nearly correspond to isopycnal surfaces because density at low temperatures is strongly dependent on salinity (Nishino et al., 2008). In terms of salinity/density, the pycnocline appears to range from ~20 m at 175°E to ~50 m at 155°W and divided the water column into two sections. In the eastern part, those two sections were in consistent with Polar Mixed Layer (PML) and Upper Halocline Layer (UHL) basically, which have been found in previous studies in the same sea ice reduction region in the western Arctic Ocean (e.g., Shimada et al., 2001; Bates et al., 2005). As previously reported, Pacific-origin Summer Water (PSW) reaches the Chukchi Sea and then changes its direction toward the northwest along the northern slope of the Chukchi Sea and is delivered to the Chukchi Borderland region (Shimada et al., 2006). The horizontal heat transportation and heat release from PSW in that region are the main reasons for the rapid and extensive sea-ice retreat and for changes in the water column structure (Shimada et al., 2006). In eastern

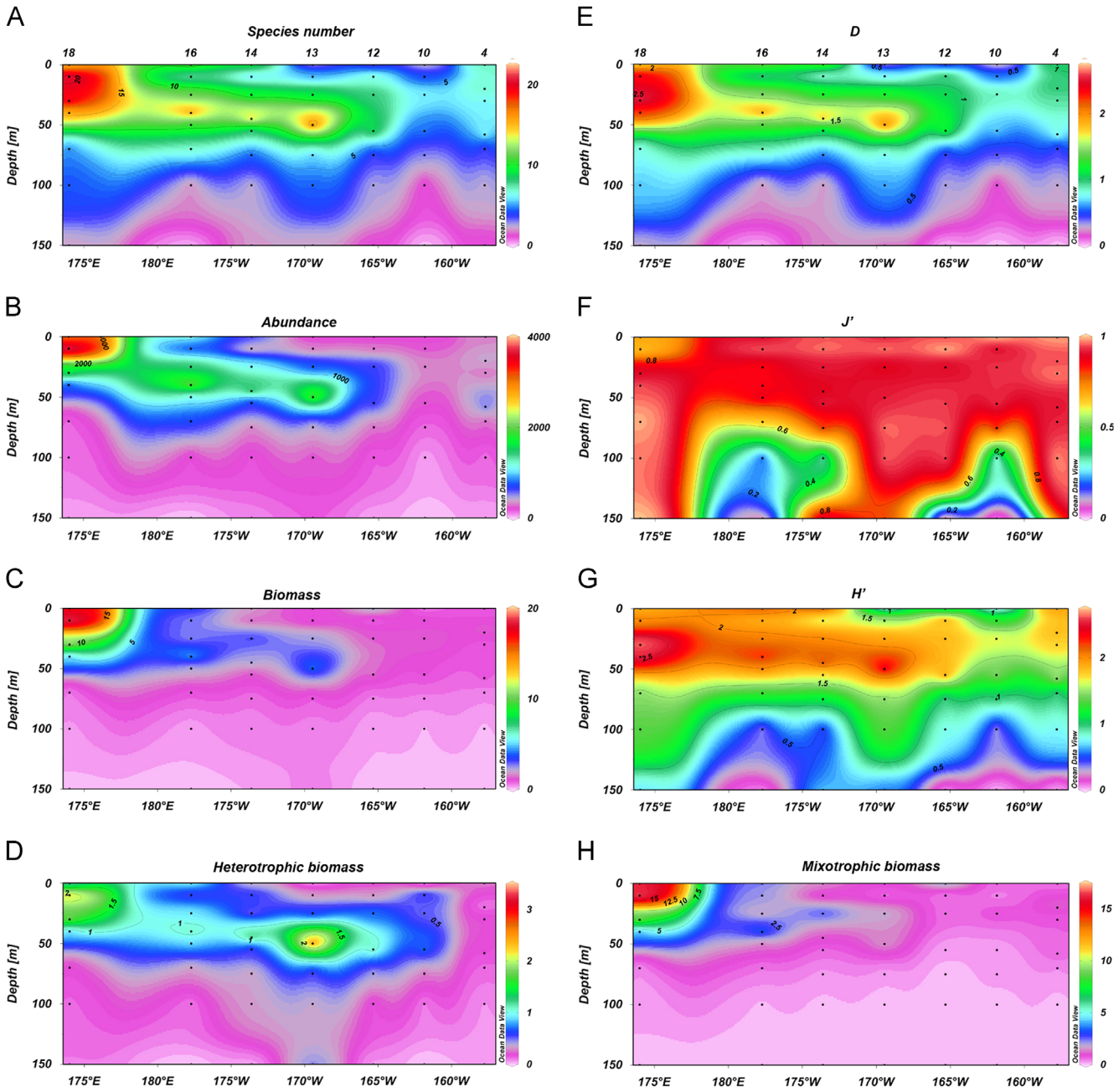

Fig. 4. Vertical variations in species number (A), abundance (ind. l^{-1}) (B), biomass (μg C l^{-1}) (C), heterotrophic ciliate biomass (μg C l^{-1}) (D), Margalef *D* (E), Pielou's *J′* (F), Shannon *H′* (G), and mixotrophy biomass (μg C l^{-1}) (H) of planktonic ciliates from transect shown in Fig. 2.

Chukchi Borderland region, water temperature increased first with depth, and then decreased, which was consistent with the hydrographic records of the heat release of PSW (e.g., Shimada et al., 2006). However, the western part (Mendeleyev Ridge) was experienced less influence by PSW, and temperature decreased first then increased. This kind of east–west difference has been revealed by previous investigations (e.g., Nishino et al., 2008; Jiang et al., 2013c). For Chl *a*, the high values occurred at depth ~40 m because light penetration without sea ice cover can produce a prominent Chl *a* layer (Nishino et al., 2008). Probably because of the shallower nutrient supply from sea ice melt water in the southwest of Chukchi shelf, the maximum values occurred. To understand the vertical environmental variations in this area, a multivariate approach using all environmental variables from each depth and station is proved that the vertical environmental structure of the water column in present study region included two parts: 0–60 m and 60–150 m.

The pelagic ciliate community in the study region was diverse and 55 ciliate species were identified during the surveys (Jiang et al., 2013c). Taxonomic composition of present study is similar to previous reports of planktonic ciliates in arctic/sub-arctic waters (Paranjape, 1987; Sherr et al., 1997; Johansson et al., 2004; Mironova et al., 2009; Niemi et al., 2011), particularly concerning global distribution of ciliates (Agatha, 2011). Comparing the vertical variations to previous Arctic studies is difficult in that most previous works with detailed species-level resolution from the Arctic Ocean have concentrated on temporal/horizontal distribution although the present abundance and biomass is closely comparable with other reports (e.g., Andersen, 1988; Sherr et al., 1997; Niemi et al., 2011). In summary, the ciliates found in the

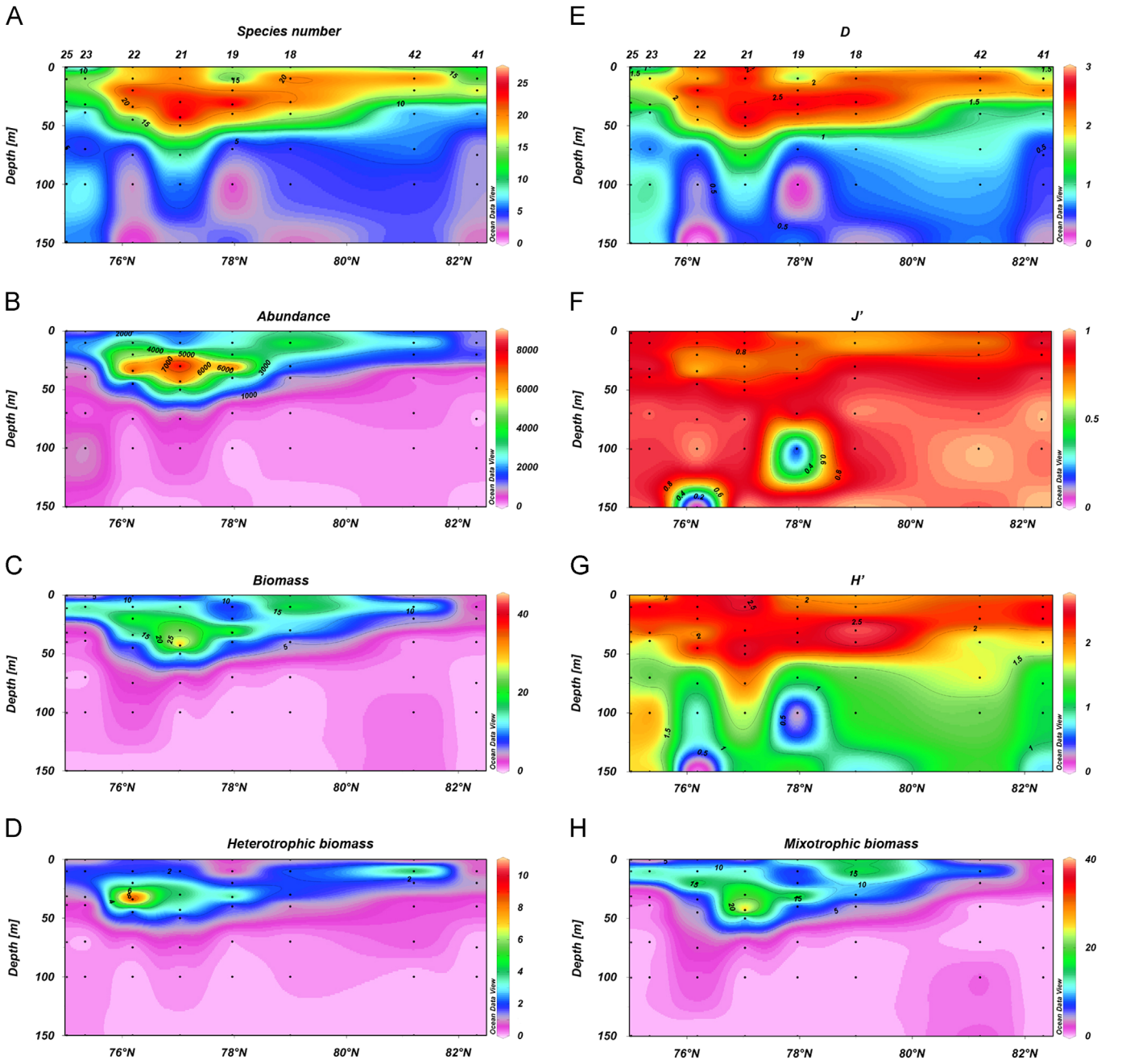

Fig. 5. Vertical variations in species number (A), abundance (ind. l^{-1}) (B), biomass (μg C l^{-1}) (C), heterotrophic ciliate biomass (μg C l^{-1}) (D), Margalef *D* (E), Pielou's *J′* (F), Shannon *H′* (G), and mixotrophy biomass (μg C l^{-1}) (H) of planktonic ciliates from stations shown in Fig. 3.

current study are roughly comparable to those reported from previous research. Although, their diversity was based on morphotypes distinguished using an inverted microscope with relatively low magnification and, actual species diversity might be higher. As is shown by the dominant species at each depth in our results, there was an overwhelming dominance of aloricate oligotrichs, both in terms of abundance and species number, which is consistent with many other studies of broad-scale habitats in marine ecosystems (Agatha, 2011). From the surface to 150-m depth, average species number, abundance, biomass, and dominant species number increased at first and then decreased, with the maximum values at about 40 m, and could be divided into two layers which are basically following two layers above and below the pycnocline. In terms of vertical variation in the community structure parameters (species number, abundance, biomass, Shannon diversity, Pielou's evenness, and Margalef richness) demonstrated similar trends. Furthermore, the depths and locations where maximum values were observed are consistent with those of the high chlorophyll *a* concentrations. According to analogous variations in environmental variables and community structure parameters, we suspect that vertical diversity in ciliate communities was correlated with environmental variables.

In addition, heterotrophic and mixotrophic assembleges mainly distributed in upper surface and their biomass also showed same vertical trends. It is demonstrated that mixotrophic ciliates were the most numerous trophic assemblage in surface waters, while heterotrophs dominant the deeper layer communities and contribution of mixotrophs is much fewer because the low light condition. A significant correlation was found between vertical distribution of heterotrophs and that of Chl *a*. However, there is no such significant

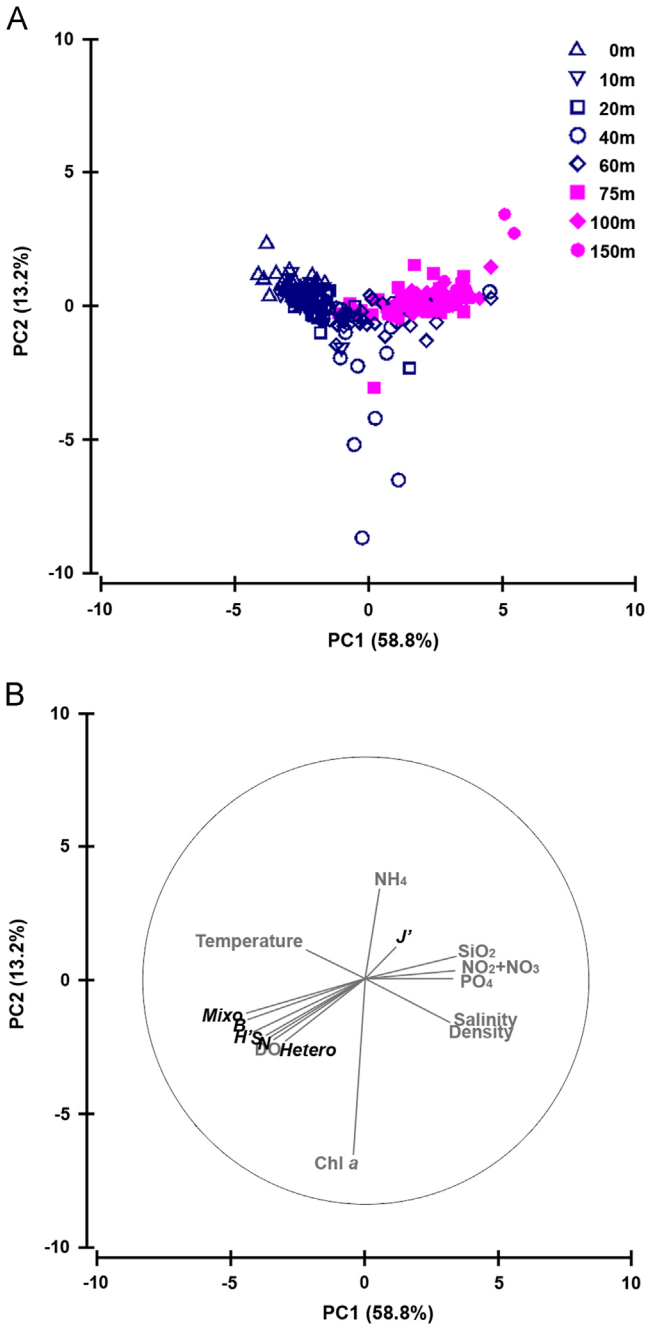

Fig. 6. Principal component analysis (PCA) plot based on log-transformed abiotic data of samples from eight depths in western Arctic Ocean during the period from August 1 to September 10, 2012. Axes 1 and 2 respectively accounted for 58.8% and 13.2% of the total variation present.

result between mixotrophs and Chl *a*. It is still unclear that mixotrophs is whether and under what conditions do ciliates become mostly heterotrophic or mostly autotrophic. When doing correlation analysis with whole spatial data including all depths and stations, the relationships between mixotrophs and Chl *a* in specific station or depth might be buried. But, as the S–N transect showing, the distribution of mixotrophs in water column correlated to that of Chl *a* obviously.

Multivariate analyses are more sensitive than univariate analyses in terms of detecting changes in complex biotic and abiotic data. They are also extremely useful for analyzing differences between communities at various spatial scales and for illustrating how these communities vary along gradients of environmental conditions (Jiang et al., 2011a, 2011b, 2012a, 2012b, 2012c; Xu et al., 2011a, 2011b, 2011c). In the present study, PCA revealed clear vertical pattern in environmental conditions. The eight sampling depths were distinguished as two statistically significant groups. CAP ordinations also demonstrated that ciliate communities exhibited vertical pattern with two groups that were similar to those in environmental data. Especially, result showed that species compositions between two groups were significantly different. Furthermore, a Mantel test identified significant linkage between vertical variation in ciliate community structure and certain environmental variables, and that supported our hypotheses the community distributed according to the vertical environmental changes. Multivariate correlation analysis demonstrated that vertical variation in ciliate communities was significantly related to several environmental variables, especially dissolved oxygen, either alone or in combination with water temperature, SiO_2, Chl *a*, and nutrients. Thus, we suggest that the vertical distribution of ciliate communities in this study was significantly related to vertical variation of water masses. Although an important factor was not considered, the predation by mesozooplankton is potential to structure ciliate communities. Because many copepods are selective predators on ciliates, choosing ciliates over phytoplankton prey (Jonsson and Tiselius, 1990, Atkinson et al., 2002).

Species diversity (Shannon H'), evenness (Pielou's J'), and richness (Margalef D) indices are commonly used in community-level investigations and are suitable for simple statistical analyses (Magurran, 1991). In the present study, diversity and richness indices had higher values at depths of ~40–50 m, which was consistent with the vector result of the CAP analysis. Furthermore, PCA analysis showed that community structure indices were significantly correlated with environmental variables. Spearman's

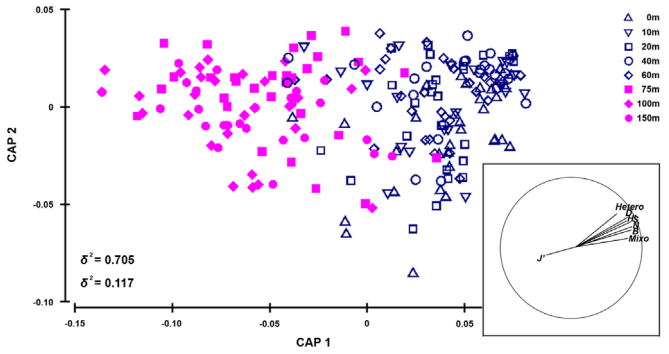

Fig. 7. Canonical analysis of principal coordinates (CAP) on Bray–Curtis similarities from log-transformed species-abundance data of eight depths in western Arctic Ocean during the period from August 1 to September 10, 2012, and correlations of community structure parameters with the two CAP axes.

Table 2
Summary of results from biota–environment (BIOENV) analysis showing the best matches of combinations of environmental variables with ciliate communities.

Rank	Abundance-environment	
	ρ	Variables
1	0.894	DO
2	0.883	Chl *a*, DO, SiO_2, NO_2+NO_3, NH_4
3	0.880	Tem, DO, NO_2+NO_3, NH_4
4	0.874	Tem, DO, SiO_2, NO_2+NO_3, NH_4
5	0.873	Chl *a*, Tem, DO, PO_4, NH_4
6	0.872	DO, SiO_2
7	0.872	Chl *a*, Tem, DO, NO_2+NO_3, NH_4
8	0.871	Tem, DO, SiO_2, NH_4
9	0.867	Tem, DO, NO_2+NO_3
10	0.866	DO, NO_2+NO_3, NH_4

ρ=Spearman correlation coefficient; Tem, water temperature; see Fig. 2 for other abbreviations.

Table 3
Spearman correlations of ciliate species number (S), abundance (N), biomass (B), Margalef richness (D), Pielou's evenness (J'), Shannon diversity (H'), heterotrophic ciliate biomass and mixotrophic biomass with the environmental parameters measured. For each parameter, the Spearman correlation coefficient (ρ) and the probability (P) are shown.

	S		N		B		D		J'		H'		*Hetero*		*Mixo*	
	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P	ρ	P
Chl *a*	0.905**	0.002	0.786*	0.021	0.571	0.139	0.929**	0.001	0.190	0.651	0.929**	0.001	0.905**	0.002	0.500	0.207
Salinity	−0.524	0.183	−0.690	0.058	−0.714*	0.047	−0.310	0.456	0.762*	0.028	−0.381	0.352	−0.595	0.120	−0.857**	0.007
Tem	0.667	0.071	0.548	0.160	0.595	0.120	0.643	0.086	−0.381	0.352	0.571	0.139	0.667	0.071	0.619	0.102
Density	−0.524	0.183	−0.690	0.058	−0.714*	0.047	−0.310	0.456	0.762*	0.028	−0.381	0.352	−0.595	0.120	−0.857**	0.007
DO	0.786*	0.021	0.905**	0.002	0.857**	0.007	0.571	0.139	−0.500	0.207	0.667	0.071	0.905	0.002	0.810*	0.015
SiO_2	−0.524	0.183	−0.690	0.058	−0.714*	0.047	−0.310	0.456	0.762*	0.028	−0.381	0.352	−0.595	0.120	−0.857**	0.007
PO_4	−0.548	0.160	−0.667	0.071	−0.762*	0.028	−0.333	0.420	0.690	0.058	−0.405	0.320	−0.571	0.139	−0.881**	0.004
NO_2+NO_3	−0.595	0.120	−0.810*	0.015	−0.786*	0.021	−0.333	0.420	0.690	0.058	−0.500	0.207	0.690	0.058	−0.881**	0.004
NH_4	−0.786*	0.021	−0.548	0.160	−0.357	0.385	−0.905**	0.002	−0.167	0.693	−0.786*	0.021	−0.786*	0.021	−0.286	0.493

Tem, water temperature; see Fig. 2 for other abbreviations.

* $P<0.05$.

** $P<0.01$.

correlation analysis provided evidence that supported our hypothesis that strong correlations exist between abiotic and community structure parameters. Among these correlations, community structure parameters, especially species number, H', and D, were significantly positively correlated with Chl *a* ($P<0.01$). Regarding Pielou's J', no reasonable and significant relationship was found and the index seems to be less useful for characterizing community variation along environmental gradients than D and H', which has been reported previously (e.g., Gong et al., 2005; Jiang et al., 2011a). It should be noted that the Chl *a*–H'/D relationship was less clear, although positive Chl *a*–abundance or biomass relationships were found within planktonic ciliate communities in the present and previous studies (Dolan and Marrase, 1995; Pitta et al., 2001; Wickham et al., 2011). For instance, strong positive relationships between tintinnid abundance and Chl *a* have been found in the equatorial Pacific and the Mediterranean, but we did not find a relationship between tintinnid species diversity/richness and Chl *a* (Dolan et al., 2007). Thus, although Chl *a* was a clear driver of ciliate species number, abundance, D, and H' in this study, the positive Chl *a*–D/H' relationship that we found is far from being a general phenomenon. The potential of using species diversity and richness indices of pelagic ciliate communities to assess vertical changes in phytoplankton biomass remains to be determined. These findings suggest that community structure parameters (especially the Shannon diversity and richness indices) are useful for understanding relationships between pelagic ciliate communities and phytoplankton biomass in Arctic marine ecosystems. However, we sampled only the pelagic community in an area of reduced sea ice during late summer; further investigations of broad Arctic regions over extended time periods are needed to verify this conclusion.

Furthermore, species number, abundance, and biomass were positively correlated with dissolved oxygen ($P<0.05$), which is consistent with the BIOENV result that vertical variation in ciliate community structure was significantly related to DO. Although, a vertical DO gradient was found by analyses that affect the vertical variation in ciliate community structure in the present study. It seems obvious that the ciliated micrograzer concentration would co-occur with the chlorophyll max and therefore with the DO max.

In summary, the results of this cruise showed significant differences in pelagic ciliates community structure between upper and deeper water in the western Arctic Ocean. Our study indicated that vertical variability of ciliate community characteristics (species composition, abundance, biomass, diversity indices and trophic structure) accurately reflect vertical changes in water column. It is also noteworthy that, the vertical extents of a series of environmental variables were primary importance for describing vertical structure in the pelagic ciliate community. Furthermore, Shannon H', Margalef D and heterotrophy biomass showed strong relationships with vertical changes in Chl *a*. Thus, our findings provide basic data to understand vertical variation in pelagic ciliate communities in western Arctic water column.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dsr2.2014.09.005.

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