# 1 Inter-annual changes in summer phytoplankton community

## 2 composition in relation with water mass variability in the East

## 3 China Sea

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#### Abstract:

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Surface distribution of phytoplankton community was investigated in July 2009, 2010, 25 2011 and 2013 in the mid-shelf of the East China Sea (ECS) which comes under the 26 influence of Changjiang River Diluted Water (CDW) and the Kuroshio current. This 27 study based on CHEMTAX analysis of phytoplankton pigments revealed the dominance 28 of cyanobacteria and prochlorophytes in the eastern ECS which was perennially under 29 influence of the oligotrophic Kuroshio surface water. Towards the west, in the mid-shelf 30 31 ECS, the composition of phytoplankton communities varied from year to year. Diatoms dominated in 2009 and 2013 when dissolved inorganic phosphate (DIP) concentrations 32 were higher than during 2010 and 2011. During these 2 latter years characterized as 33 high-nitrate-years, a mixed population of cyanobacteria, chlorophytes and other groups 34 was observed. Cluster analysis based on the phytoplankton community composition, 35 together with PCA of shipboard hydrographic and nutrient data for all four years helped 36 confirm that summer phytoplankton community structure of the ECS is regulated by the 37 mixing of water masses and variability of nutrient ratios within CDW as it moved 38 offshore. Our results showed that elevated DIP concentrations in the CDW favor the 39 growth of diatoms and dinoflagellates. The primary pathway for DIP inputs appears to 40 41 be the upwelling of high phosphate subsurface waters along the coast of China.

- 43 Key words: phytoplankton community, East China Sea, Changjiang Diluted Water,
- 44 Kuroshio Intermediate Water, phosphate limitation, N:P ratio, inter-annual variations

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#### 1. Introduction

as well as the structure and function of the marine ecosystem (Lalli and Parsons 1997). 51 Large phytoplankton, such as diatoms and dinoflagellates, are important for sustaining 52 53 the high productivity and fertile fishing grounds of coastal and upwelling areas. Small phytoplankton like cyanobacteria, which profit from their high nutrient utilization 54 efficiency, are more abundant in the open ocean and are essential in supporting the 55 microbial loop of oligotrophic oceans (Finkel 2007). 56 The East China Sea (ECS), which is surrounded by China, Korea and Japan, is one 57 58 of the largest continental shelves and a fertile fishing ground. It features a complex current system that includes influences of euryhaline coastal and saline oceanic water 59 masses (Gong et al. 1996; Chen 2008). A major oceanic current into the ECS is the 60 Kuroshio current. Kuroshio surface water which is nutrient depleted (NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup> (NO<sub>x</sub>) 61  $< 0.2 \mu M$ , PO<sub>4</sub><sup>3-</sup> (DIP)  $< 0.05 \mu M$ , N:P < 16) (Chen 2008), intrudes into the ECS via 62 63 two major branches, one from the northeast of Taiwan and the other from the southwest of Kyushu (Fig. 1) (Guo and Miyazawa 2006). To the east, the Changjiang River (also 64 known as Yangtze River), the third largest river in the world, discharges large amounts 65 of freshwater containing extremely high concentrations of anthropogenically-derived 66 nutrients from inland China into the ECS. During summer, when river-runoff is at its 67

Phytoplankton communities play a crucial role in determining the marine food web

peak, NOx concentration can exceed 50  $\mu$ M and DIP concentration > 1  $\mu$ M (Zhang et al., 2007).

Satellite observations showed large seasonal and spatial variations of chlorophyll a concentrations (CHL) associated with the movement of the CDW, with high phytoplankton productivity observed in the Changjiang estuary and adjacent areas close to the coast, but lower CHL concentrations in the outer shelf in summer (Yamaguchi et al. 2012; 2013). In general, phytoplankton community composition in between the shelf waters and the open ocean can vary widely in response to large gradients in physical and chemical properties across the continental shelf (Simpson and Sharples 2012; Goes et al. 2014).

Microscopy has been employed to compile information on large phytoplankton (> 20 μm) such as diatoms and dinoflagellates (Guo et al. 2014; Li et al. 2009; Jiang et al. 2015), while picoplankton (< 2 μm) have been largely identified and enumerated using flow cytometry (Jiao et al. 2005; Lee et al. 2014; Pan et al. 2007). Chemotaxonomy, the identification of phytoplankton from several phytoplankton groups based on 'diagnostic pigments' which are specific to individual phytoplankton taxa or groups (Jeffrey et al. 1997) provides a more convenient method to estimate the entire range of the phytoplankton community. High performance liquid chromatography (HPLC)-based studies albeit limited in spatial extent have shown that cyanobacteria were abundant in

the oligotrophic Kuroshio waters, while diatoms and dinoflagellates dominate in the Changjiang Estuary and adjacent areas (Furuya et al. 2003; Zhu et al. 2009; Liu et al.

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The mid-shelf ECS is an important passage for materials transportation from the Yellow Sea and South China Sea to the Tsushima Strait and onwards into the Sea of Japan (Chen and Wang 1999; Isobe 1999), but it has been poorly understood and only sparsely studied. This region is covered by Changjiang River Diluted Water (CDW) which is formed by mixing with offshore waters influenced by Kuroshio Surface Water (KSW) (Wang et al. 2014; Zhu et al. 2017). CDW is believed to be DIP-limited (N:P > 100) because DIP is fast consumed by phytoplankton blooms out of Changjiang Estuary (Wang and Wang 2007; Chen 2008). Along the coastal area of China, coastal upwelling of Taiwan Warm Current and inshore intrusion of Kuroshio Intermediate Water (KIW) are known to support higher and longer phytoplankton blooms around Changjiang Estuary and responsible for changing the nutrient stoichiometry of CDW (Chen et al. 2004; Yang et al. 2013; Tseng et al. 2014). However, lack of interannual in-situ biogeochemical datasets have hampered a better understanding on how interannual variations mixing of different water masses in the ECS impact seawater nutrient composition, one of the important drivers of phytoplankton community structure.

In this study, we hypothesize that phytoplankton composition in the mid-shelf ECS

can be impacted by variations in the mixing of different water masses, in particular the flow and the extent of mixing of the CDW and the Kuroshio because of differences in their nutrient content. Since this investigation is based on summer-time data, a time period when the river discharge is at a maximum, and for four years, i.e. 2009, 2010, 2011 and 2013, it allows us to provide a more mechanistic explanation of how inter-annual variability in water mass mixing impacts phytoplankton communities of the ECS ecosystem.

## 2. Materials and Methods

## 2.1 Study area and sampling regime

In-situ sampling was undertaken in the ECS on shipboard expeditions aboard the T/V Nagasaki Maru in late July of 2009, 2010, 2011 and 2013 as shown in Fig. 1. All stations were located in the ECS from 124.6 to 128.8 °E and from 31.4 to 33.0 °N. At each station, a CTD was used to profile temperature (T) and salinity (S) in the upper 80m. Water samples for chlorophyll a (CHL) as well as other phytoplankton pigments and nutrients (including  $NO_x = NO_3^- + NO_2^-$ , DIP:  $PO_4^{3-}$ ) were collected at the sea surface using an acid washed bucket. Nutrient samples were immediately frozen in polyethylene tubes after sampling and transferred under frozen conditions to the shore laboratory for analysis using an auto-analyzer (TRACCS 2000, BL Tech). NOx and DIP

concentration were determined according to absorption spectrometry (Armstrong et al.

1967; Murphy and Riley 1961; Koroleff 1983).

Water samples for HPLC pigment analysis (1-2 L) were filtered through 25 mm Whatman GF/F glass fiber filters under vacuum pressure (< 0.01 MPa) in dim light, and then immediately frozen in liquid nitrogen until the samples were analyzed in the shore laboratory. Phytoplankton pigments were separated and measured in methanol extracts using the reverse-phase HPLC method of Heukelem and Thomas (2001), and a Zorbax Eclipse XDB-C8 column (150 mm  $\times$  4.6 mm, 3.5  $\mu$ m; Agilent Technologies).

Excess nitrate (ExcN) in seawater samples was calculated to evaluate DIP limitation using the formula ExcN=NOx–R  $\times$  DIP (Wong et al. 1998), where R is the Redfield ratio of 16 (NOx:DIP or N:P) (Redfield 1963). Therefore, ExcN values < 0 (N:P < 16) indicate DIP-enrichment, while ExcN > 0  $\mu$ M (N:P > 16) indicate DIP-limitation (Wong et al. 1998).

## 2.2 CHEMTAX derived phytoplankton groups

The contribution of various phytoplankton groups to the overall CHL pool was estimated from the array of HPLC separated pigments and calculated using CHEMTAX (Mackey et al. 1996). Matrices of pigment data and initial pigment ratios (i.e. pigment to CHL ratio for each phytoplankton group) (Table 1) were used as inputs into the

CHEMTAX program (Mackey et al. 1996; Suzuki et al. 2002). 12 marker pigments (chlorophyll fucoxanthin, peridinin, 19'-butanoyloxyfucoxanthin, a, 19'-hexanoyloxyfucoxanthin, zeaxanthin, neoxanthin, chlorophyll b, violaxanthin, alloxanthin, prasinoxanthin, divinyl-chlorophyll a) were selected and classified into 9 phytoplankton groups (prasinophytes, dinoflagellates, cryptophytes, prymnesiophytes, chrysophytes, chlorophytes, cyanobacteria, prochlorophytes, diatoms) based on earlier research that also employed CHEMTAX to study phytoplankton composition in the ECS (Furuya et al. 2003; Zhu et al. 2009) (Table 1). Note that prochlorophytes were excluded from cyanobacteria in this study. To derive the most accurate phytoplankton groups from the marker pigments, CHEMTAX was run on surface datasets of 2009, 2010, 2011 and 2013 separately. For each dataset, CHEMTAX program was run 10 times by using the output ratio matrix of the last run as the next ratio matrix input (Latasa 2007). Optimized phytoplankton compositions were selected from the most stable final ratios among the 10 outputs (Table 1).

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#### 2.3 Statistical analyses

Principal Component Analysis (PCA) and Cluster Analysis were performed using 160 161 **JMP** Pro 11 software. CHL contribution of dinoflagellates, chlorophytes, prymnesiophytes, cyanobacteria, prochlorophytes 162 and diatoms derived from CHEMTAX at all 76 stations for the four years were used for the statistical analyses. PCA was used to examine the correlations between the selected phytoplankton groups and environmental factors (Goes et al. 2014). The Ward minimum variance method (Ward 1963) was used to group the stations into clusters based on similarities in phytoplankton compositions and water mass properties including salinity and ExcN (Dorado et al. 2015; Fujiwara et al. 2014). The analysis resulted in four major clusters which shared specific environmental features and phytoplankton functional groups. To compare average values from 2009/2013 and 2010/2011, a non-parametric Mann-Whitney U test was performed using the software Origin pro 8.0. Differences between datasets over four years were considered significant when p < 0.05 (Salkind 2006).

#### 3. Results

## 3.1 Hydrography

A temperature versus salinity (T-S) plot for samples from the upper 80 m depicts four major water masses in the ECS (Fig. 2a). The presence of the Changjiang River plume in near surface layers was obvious from its < 28 salinity and > 23 °C temperature signatures. Based on previous studies, the CDW boundary was defined as salinity < 30 (Zhang et al. 2007; Zhou et al. 2008). A higher salinity (> 34), warmer water mass in

upper layers was associated with deeper high-salinity Kuroshio water (KW) (Gong et al., 182 183 1996; Umezawa et al. 2014; Zhou et al. 2008). KSW was defined with salinity > 32.9, while shelf water (SW) was defined with salinity ranging from 30.0-32.9. SW was 184 located in the central part (125-128 °E) of our study area. In the KSW, vertical density 185 from the surface to the deeper layers showed small gradients ranging from 20.7 to 24.7, 186 whereas sharper density gradients ranging from 14.2 to 25.2 were seen in the CDW. 187 Stations in 2010/2011 showed strong water column stratification and strong pycnoclines, 188 189 whereas stations in 2009/2013, were relatively homogeneous with density from 18.0 to 20.6 in the shallow layers. 190 191 At KSW stations, NOx and DIP was depleted above 45 m, but increased slightly at deeper depth (Fig. 2b, c). Distinct variations were observed in NOx and DIP 192 concentrations in the surface of the CDW stations between 2010/2011 and 2009/2013. 193 194 In 2010/2011, at depths < 20 m, NOx was high (> 5  $\mu$ M), whereas DIP was depleted. Conversely, relatively higher DIP (> 0.2  $\mu$ M) was observed in 2009/2013 in the surface 195 layers. Bottom DIP concentrations were much higher in nearshore stations than at the 196 KSW stations where DIP measured around 1-2  $\mu$ M and NOx was 8-16  $\mu$ M. This 197 resulted in low N:P < 10 in the bottom layers. It is observed that even at the stations 198 with high surface NOx or DIP, the high values were not continuous from the bottom to 199 the surface, and there were layers of relatively low concentration around 10-30 m deep. 200

The surface high NOx or DIP waters were characterized by relatively low salinity, indicating that the high nutrients were associated with CDW and not caused by local mixing or upwelling.

Surface temperatures ranged from 24.8 to 29.5°C, with high and low values observed in the east and west, respectively (Fig. 3a-d). Salinity patterns corresponded with temperature distributions showing low (< 30) and high (> 32.9) salinity in the west and east, respectively. Warm and high salinity waters that characterized the KSW were observed east of our study area around 128.7 °E during all four years (Fig. 3e-h). The extent of the CDW varied, it spread eastward to 126 °E in 2009, 2011, 2013 (Fig. 3e, g, h), while it covered almost the entire study area in 2010 except for the easternmost part which was dominated by the KSW.

Surface oligotrophic conditions (NOx < 0.11  $\mu$ M, DIP < 0.07  $\mu$ M) prevailed during all four years in KSW (Fig. 3i-p). Toward the west, in the SW, both NOx and DIP concentrations increased gradually, while noticeable interannual variations were seen in the CDW where salinity < 30. Extremely high NOx (> 10  $\mu$ M, maximum of 26.1  $\mu$ M) but low DIP (< 0.05  $\mu$ M) and ExcN > 0 were observed in 2010/2011 (Fig. 3j, k), in contrast to the relatively lower NOx (< 2  $\mu$ M), higher DIP (> 0.1  $\mu$ M) and ExcN < 0 waters in 2009/2013 (Fig. 3i, l, m, p). Differences in nutrient concentrations between 2009/2013 and 2010/2011 in the CDW and the SW were statistically significant (p <

0.01). Average values of NOx, DIP and ExcN were 0.68  $\mu$ M, 0.19  $\mu$ M and -2.39  $\mu$ M, respectively, for 2009/2013, and 4.55  $\mu$ M, 0.06  $\mu$ M and 3.63  $\mu$ M for 2010/2011 (Fig. 3i-t). Hereafter, we define 2009/2013 as high-DIP-years and 2010/2011 as high-NOx-years.

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#### 3.2 Surface phytoplankton distribution

Overall, surface CHL was higher in the CDW (0.25-3.99 mg m<sup>-3</sup>) (Fig. 4a), and lower in the SW (0.14-0.88 mg m<sup>-3</sup>) (Fig. 4b). Lowest values were observed in KSW where CHL rarely exceeded 0.17 mg m<sup>-3</sup> (Fig. 4c). CHEMTAX analysis revealed that the low CHL at those stations was derived from a mixture of cyanobacteria and prochlorophytes which accounted for > 61% of the total CHL in KSW over the four years (Fig. 4c). Prochlorophytes comprised more than 30% of the phytoplankton community in 2009/2010 and 2013, and 19% in 2011 (Fig. 4c). Outside of the KSW region, diatoms were more widely distributed and in higher numbers in 2009/2013 (Fig. 4a). Compared to diatoms, dinoflagellate population was a minor component of phytoplankton communities, but it was relatively higher in 2009/2013 compared with 2011/2012 (Fig. 4a). Prymnesiophytes were generally lower as compared to diatoms but were higher in the mixed waters of the SW in 2011/2013 (Fig. 4b). Inter-annual differences in the average composition of diatoms, dinoflagellates and cyanobacteria were statistically significant (p < 0.05), excluding those in the KSW. Average values for samples in the CDW and the SW revealed that diatoms and dinoflagellates contributed > 57% of CHL in 2009/2013, while other groups including cyanobacteria, chlorophytes and prymnesiophytes accounted for 38, 16 and 17% respectively. Conversely diatoms comprised only 13% of the population in 2010/2011 (Fig. 4a, b).

## 3.3 Relationship between phytoplankton biomass and nutrient concentrations

CHL varied over the four years and was significantly higher during high-DIP-years (2009/2013), but lower during high-NOx-years (2010/2011) (Fig. 5a). A strong negative correlation was observed between CHL and ExcN when ExcN < 0  $\mu$ M ( $r^2$  = 0.48, n = 39) while a positive correlation was obtained when ExcN > 0  $\mu$ M ( $r^2$  = 0.37, n = 37) (Fig. 5a). Inter-annual variability in CHL appeared to be regulated largely by DIP availability as seen by the high correlation between CHL and DIP ( $r^2$  = 0.46) for data from all four years (Fig. 5b).

Diatoms and dinoflagellates concentrations increased significantly with decreasing ExcN ( $r^2$  = 0.45 and 0.43) and accounted for a large fraction of the high CHL seen during the high-DIP-years (Fig. 6a, b). On the other hand, cyanobacteria and prochlorophytes concentrations were higher when ExcN was 0, but low when ExcN > 0 (Fig. 6c, d). Chlorophytes concentrations were generally higher when ExcN > 0  $\mu$ M ( $r^2$ 

= 0.55) and conversely much lower when ExcN  $\leq$  0  $\mu$ M. No clear relationship could be discerned between prymnesiophytes and ExcN (Fig. 6e).

Cluster analysis based on the composition of phytoplankton aided in partitioning all

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## 3.4 Statistical analysis

stations sampled during four years into four clusters (Fig. 7). Stations in Cluster 1 comprised a mixed population of diatoms (11%), dinoflagellates (12%), cyanobacteria (44%) and chlorophytes (17%). Diatoms and dinoflagellates dominated stations in Cluster 2 accounting for about 66% of the total phytoplankton. Cyanobacteria were the dominant (51%) phytoplankton in stations of Cluster 4 while prochlorophytes made 28% of the population in this cluster. This Cluster was also characterized by stations with the lowest DIP, and highest temperature and salinity (Fig. 8b). Stations in Cluster 3 were composed of cyanobacteria and prymnesiophytes (61%) (Fig 8a). Six dominant phytoplankton groups, diatoms, dinoflagellates, cyanobacteria, prochlorophytes, prymnesiophytes and chlorophytes, within each cluster were selected to examine their relationship with surface water properties and nutrient concentrations. Diatoms (> 50%) predominated in waters with salinity ranging from 28.5 to 31.6 and temperatures between 24.9 and 27.7 °C (Fig. 9a), whereas cyanobacteria (> 50%) populations predominated both low salinity CDW (salinity < 27.5) as well as higher salinity waters (salinity > 31.6) (Fig. 9c). In waters which harbored high proportions of prymnesiophytes, salinities were in excess of 30 and temperatures were higher than 26 °C (Fig. 9e). Chlorophytes and dinoflagellates accounted for more than 30% of CHL in low salinity waters (Fig. 9b, f), while in high salinity waters prochlorophytes contributed about 30% of CHL (Fig. 9d).

The relationship between different phytoplankton groups and nutrient concentrations (DIP versus NOx) are presented in Fig. 10. Generally, diatoms contributed a large fraction of the CHL ( $\sim$  80%) in waters with N:P ratios < 16 (or ExcN < 0  $\mu$ M), followed by dinoflagellates (Fig. 10a, b). High proportions of cyanobacteria especially in Cluster 3 were observed in waters with N:P ratios close to 16; as well as in low nutrient waters where NOx < 0.4  $\mu$ M and DIP < 0.2  $\mu$ M (Fig. 10c). Prymnesiophytes, prochlorophytes and chlorophytes were higher in the low DIP waters (Fig. 10d, e, f).

Principle Component Analysis (PCA) using data from all four years was utilized to investigate the influence of environmental factors on the distribution of major phytoplankton groups in the ECS. The first two PCs explained more than 51% of the variations (Fig. 11). Salinity showed a strong negative correlation with PC1 while ExcN and NOx, showed strong negative correlations with PC2. It was clear that chlorophytes increased in lower salinity and higher ExcN CDW in 2010/2011. Diatoms and dinoflagellates showed a preference for waters of lower temperatures and high DIP.

Cyanobacteria and prochlorophytes were associated with waters of higher temperatures and lower DIP concentrations which are characteristics of the KSW (Fig. 11).

## 4. Discussion

## 4.1 Phytoplankton biomass and community composition

Inter-annual variations in CHL were large in the west of the study area and decreased eastward in accordance with variations in temperature, salinity and nutrients brought about by mixing of the KSW, CDW and SW (Fig. 3). This distribution is consistent with previous studies (Yamaguchi et al. 2012; 2013) which also showed that CHL concentrations were higher in coastal waters and lower offshore. Interestingly, the high CHL (> 1 mg m<sup>-3</sup>) during the high-DIP-years of 2009/2013 was largely derived from diatoms and dinoflagellates, while during high-NOx-years of 2010/2011 it was derived from a mixed populations of cyanobacteria, chlorophytes, prymnesiophytes and a small component of diatoms. The distribution of phytoplankton communities in relation to environmental factors is discussed in the following sections.

## 4.1.1 Cyanobacteria and Prochlorophytes

The dominances of cyanobacteria in low nutrient, high salinity waters where the N:P ratio is close to 16 is well known because smaller phytoplankton are more efficient at

utilizing nutrients which allows them to thrive in oligotrophic global oceans (Ishizaka et al. 1994; Suzuki et al. 1995; Brewin et al. 2010; Hirata et al. 2011). The distinctive distribution of prochlorophytes in Cluster 4 (Fig. 9d) also confirmed that temperature and salinity of the KSW are critical for the existence of prochlorophytes in the ECS (Jiao et al. 2005). In our study, the presence of prochlorophytes approximately north of 32.7 °N and east of 126.5 °E, are indicative of the flow of oligotrophic Kuroshio surface water into the mid-shelf ECS.

The higher fractions of cyanobacteria in coastal, low salinity and low nutrient waters (salinity < 28, DIP < 0.2  $\mu$ M, NOx < 0.5  $\mu$ M) are probably comprised of *Synechococcus* which is known to be the dominant summer-time cyanobacterial species in coastal waters of the ECS (Jiao et al. 2005; Lee et al. 2014). Low nutrient concentrations resulting from strong summer stratification is considered to favor the preferential growth of small phytoplankton over the larger diatoms and dinoflagellates in coastal waters (Liu et al. 2015).

#### 4.1.2 Diatoms and Dinoflagellates

In waters where DIP concentrations were high, diatoms and dinoflagellates dominated. This is evident from the results of the cluster analysis. Cluster 2 corresponded to diatom-dominated water located in the CDW and SW where salinity

ranged from 28.0 to 32.9 and DIP was higher than 0.1  $\mu$ M. Our observations suggest that large phytoplankton cells typically dominate the community during periods when DIP was not limiting and ExcN values were either < 0  $\mu$ M or close to 0  $\mu$ M (N:P = 16). Similar findings have been reported by Hecky and Kilham (1988) for a variety of freshwater and oceanic ecosystems. Nutrient conditions akin to this, usually lead to the dominance of diatoms and dinoflagellates because of their fast growth under conditions of nutrient sufficiency (Smayda 1997).

Enhanced proportion of dinoflagellates in waters where ExcN  $< 0~\mu M$  shows that their nutrient requirements are close to or similar to diatoms. In all cases, however, biomass of dinoflagellates did not exceed that of diatoms. One possible reason is that dinoflagellates are less resistant to high turbulence as compared to diatoms, and easier to form blooms under stratification (Smayda 1997). Support for such a notion is based on our observations of higher concentrations of dinoflagellates in waters influenced by CDW (salinity < 30) that were less stratified. In the Changjiang Estuary and adjacent areas, high concentrations of dinoflagellates have been reported in the subsurface layer during summer with a stratified water column (Guo et al. 2014; Jiang et al. 2015).

## 4.1.3 Other Phytoplankton Groups

Highest chlorophyte populations were observed at stations which were strongly

influenced by CDW with salinity < 30. In Cluster 1, the contribution of chlorophytes was more than twice that of the same in other clusters, and highly correlated with the high ExcN. In general, chlorophytes have been less investigated because they are considered minor contributors to total biomass of phytoplankton. This group has, however, been observed previously in Changjiang Estuary and in the adjacent coastal waters accounting for a sizeable fraction of CHL (Furuya et al. 2003; Zhu et al. 2009). Our study revealed the abundance of chlorophytes in the CDW when DIP concentrations were low or depleted, leading us to conclude that their distribution was linked to the spatial extent of the CDW into the mid-shelf ECS, especially when DIP was limiting, and NOx was in excess. Prymnesiophytes accounted for more than 30% of Cluster 3, which comprised of stations mainly from SW. Prymnesiophytes are an oceanic community of phytoplankton and account for about 20% of the total primary production in central North Pacific (Rousseaux and Gregg 2013). In the ECS, prymnesiophytes have frequently been reported in offshore low CHL waters of the mid-shelf (Jiang et al. 2015; Zhu et al. 2009). Chrysochromulina which was found near the edge of the CDW has been reported as the dominant species (Lin et al. 2014). In the KSW, their contribution to total CHL was estimated at 20%, sharply decreasing in nearshore waters (Furuya et al. 2003) and

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declining to almost trace quantities in Changjiang Estuary (Liu et al. 2016; Zhu et al.

2009). The occurrence of prymnesiophytes in the ECS can be an indicator of offshore saline water intrusion into the shelf region of ECS.

## 4.2 Inter-annual variations of water mass movement and impacts on nutrient

#### stoichiometry

The surface outflow of the Changjiang River gradually increased in salinity and decreased in nutrient concentrations, especially DIP concentrations, therefore the extent of the eastward advection and DIP-limitation of the CDW is a major factor controlling inter-annual variations of CHL in the ECS in summer (Wang et al. 2003). In our study, we found that low salinity CDW was associated with high DIN in 2010/2011 but conversely with high DIP in 2009/2013.

In 2010/2011, DIP limitation was indicated by high ExcN in the fresher CDW which created strong stratification around 124.5 °E in our study area. Such low salinity water and consequent stratification are comparable to the conditions observed further west near the Changjiang Estuary around 122 °E to 124 °E in summer (Chen et al. 2008, Tseng et al. 2014, Guo et al. 2014, Jiang et al. 2015), suggesting that CDW was less diluted by seawater during its extension into our study area in 2010/2011 (Fig. 3f, g). Correspondingly, DIP concentrations in the CDW were generally < 0.2  $\mu$ M, while NOx were in excess (ExcN > 0) and this led to extremely high N:P ratios (> 100) (Fig. 3r, s).

These results indicate that the fresher CDW could induce DIP limitation further east in the ECS in 2010/2011.

In 2009/2013, CDW with high DIP resulted in ExcN < 0. Such waters characterized Cluster 2 which showed high DIP and relatively high salinities and low temperatures. PCA analysis also showed a negative correlation between temperature and DIP. It is well known that CDW are deficient in DIP (Chen and Wang 1999; Wang et al. 2003; Chen et al. 2008), therefore high DIP in 2009/2013 in the CDW probably resulted from the mixing of CDW with subsurface nutrient-rich waters.

#### 4.3 Potential mechanisms for variations in N:P ratio/ExcN

Using a three-end-member (Changjiang River plume, KIW and KSW) mixing model for the ECS, Wang et al. (2014b) suggested that the enhanced DIP in the Changjiang River plume was from KW upwelling. An examination of surface DIP concentrations in the CDW along the two-end-member (CDW and KSW) mixing line depicts near absence of DIP in fresher (< 28.0) waters of CDW in 2010/2011 (Fig. 12). In the water with salinity 28.0-32.0, higher DIP values in excess of the two-end-member mixing line were consistently observed in 2009/2013, indicating that this offshore water of our study area had already mixed with high salinity and high DIP waters of the KIW. Upwelling of nutrient rich KIW along the shelf slope has been suggested as a

primary source of high DIP onto the ECS shelf (Ito et al. 1994; Chen et al. 1996). A branch of the KIW from the southwest of Kyushu crosses the ECS shelf (Ito et al. 1994; Guo et al. 2006). Additionally, episodic events like typhoons may cause localized upwelling on the ECS shelf and consequently some nutrient enhancement (Siswanto et al. 2008). However, the T-S plot and nutrient profiles for our study area did not show any indication of localized upwelling or vertical mixing, nor were any typhoon events recorded in July of the years that we sampled.

Furthermore, although nutrients of the KIW can contribute to the maintenance of a deep subsurface CHL maxima in the mid-shelf ECS (Lee et al. 2016), their influence on the offshore surface phytoplankton populations are negligible because stratification induced by the fresher CDW prevents vertical mixing of subsurface waters into the upper layers (Sukigara et al. 2017). These reasons indicate that the localized upwelling in our study area during 2009 and 2013 was not responsible for supplying DIP-rich waters to the surface layers.

Instead we propose that intensive nearshore wind driven coastal upwelling brings DIP-rich water upward and then mixes with near surface CDW. It is known that the coastal upwelling induces high primary production from replenishment of DIP in summer in the ECS (Gong et al. 1996; Chen et al. 2004; Wang and Wang 2007). Under the influence of the strong southwesterly monsoon, colder upwelled DIP-rich water has

been often observed along the coast of China because of mixing of the Changjiang water with subsurface waters of the Taiwan Warm Current or the KIW (Chen et al. 2004; Yang et al. 2013). Furthermore, additional DIP can be supplied by the remineralization of organic matter in the bottom waters (Gong et al. 1996; Fang et al. 2004; Yang et al. 2013).

Previous studies (Liu et al. 2013; Tseng et al. 2014) reported extensive phytoplankton blooms triggered by coastal upwelling around Changjiang Estuary and which often extend eastward to 123 °E. These blooms result in DIP limitation in the plume fringe where salinity > 30. We observed CDW with salinity of 30 extending up to 124.5 °E in 2009/2013, and coincident with location plume fringe in the afore mentioned studies. This result is also consistent with the conclusion of Tseng et al. (2014) that the magnitude of phytoplankton blooms and DIP-limitation outside of the Changjiang Estuary determined by the joint effect of CDW and coastal upwelling.

Here we extend this coastal upwelling hypothesis of Tseng et al. (2014) to explain the interannual variations in phytoplankton community in response to varying N:P ratios in the CDW (Fig. 13). If the CDW is highly stratified (salinity < 28 in our study) suppressing near surface water mixing with subsurface water, then surface DIP is rapidly consumed by phytoplankton in the outer Changjiang Estuary. This results in DIP-limitation and the dominance of smaller phytoplankton, such as cyanobacteria as

observed in 2010/2011 during our study (Fig. 13a). On the other hand, when intensive coastal upwelling occurs, it brings high-DIP subsurface water upward which mixes with the surface CDW around Changjiang estuary. The resulting CDW with low N:P ratio advected eastward, and sustained the growth of larger phytoplankton, such as diatoms and dinoflagellates, in offshore waters (Fig. 13b) as observed in 2009/2013 during our study.

Zhang et al. (2007) have also reported that low N:P surface waters around

Changjiang Estuary were the result of low N:P ratio (< 10) subsurface and intermediate water masses affected by the DIP-rich Kuroshio waters. Similarly, we also observed N:P <10 in bottom waters at CDW stations (Fig. 2c, d). It thus appears that the combined effect of CDW and coastal upwelling is important in determining the inter-annual variability of nutrient concentrations and consequently phytoplankton community in the mid-ECS shelf.

Intensive coastal upwelling in 2009 and 2013 was indicated by sea surface temperature (SST) anomalies and a northward wind pattern along the coast of China (Fig. S1). However, large scale hydrological and biogeochemical studies are necessary to shed light on the inter-annual variations in the interaction of Changjiang river plume and coastal upwelling both of which drive nutrient inputs into the ECS shelf region, and consequently determine the phytoplankton community structure and the overall

biogeochemical cycling within the ECS.

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#### 5. Conclusions

Our study is the first to document inter-annual variations in the phytoplankton community of the mid-shelf ECS in relation to the nutrient stoichiometry resulting from variations in the water masses of the ECS. Compared to the low nutrient concentrations and invariant N:P ratios that typified the Kuroshio waters, significant differences in water properties and nutrient concentrations were observed in the CDW influenced region during the four summers of sampling. DIP concentrations in the CDW were low in 2010/2011, but it was remained in 2009/2013. As a result, mixed populations of phytoplankton were observed in 2010/2011 while diatom domination was observed in 2009/2013. This study shows that DIP supply is crucial in determining the phytoplankton composition in the mid-shelf ECS. Compared to the highly stratified CDW in 2010/2011, CDW in 2009/2013 was in all probability already mixed with the upwelled subsurface waters from the coastal region supporting a more eastward dispersal of high phytoplankton concentrations into the mid-shelf ECS over longer time periods.

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## List of tables and figures

- Table 1. Initial and final ratios of pigments to chlorophyll a (CHL) of nine 696 phytoplankton groups used in the CHEMTAX analysis. Abbreviations: Chla: 697 Chlorophyll (CHL), fucox: fucoxanthin, perid: peridinin, 19butfu: 698 19'-butanoyloxyfucoxanthin, 19hexfu: 19'-hexanoyloxyfucoxanthin, zeax: zeaxanthin, 699 Chlb: Chlorophyll b, neox: neoxanthin, violax: violaxanthin, allox: alloxanthin, 700 701 prasinox: prasinoxanthin, DV-Chla: divinyl-chlorophyll a. Prochlorophyte were excluded from cyanobacteria. 702 Fig. 1. Sampling locations for four cruises in the mid-shelf East China Sea undertaken 703 704 in late July of 2009-2011 and 2013. One of CDW (circles), SW(crosses) and KSW (diamonds) stations in 2009 (black), 2010 (blue), 2011 (green) and 2013 (red) were 705 selected to compare T-S diagrams and vertical profiles of nutrients in different years 706 shown in Figure 2. Surface currents are indicated by solid lines and include the 707 Kuroshio, Changjiang Diluted Water (CDW), Taiwan Warm Current (TWC). Two 708 709 pathways of Kuroshio intrusion; one from the northeast of Taiwan and the other towards the southwest of Kyushu are indicated by dashed lines. 710 711 Fig. 2. Temperature-salinity (T-S) plot of the upper 80 m depth delineating water masses
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- indicates physical parameters, and red indicates chemical parameters.
- Fig. 12. Relationship between surface DIP and salinity for all stations in this study; (+)
- 744 2009/2013 and ( $\bigcirc$ ) 2010/2011. The dotted line is obtained from Zhang et al. (2007).
- Fig. 13. Schematic of interannual variation of summer phytoplankton community
- distribution in the ECS; (a) Mixed populations of cyanobacteria, chlorophytes, diatoms
- and dinoflagellates exist in DIP limited water as a result of high N:P ratio of the CDW
- (cf. 2011/2012), (b) Diatom dominates in no-DIP-limited water resulted by mixing of
- 749 CDW with low N:P upwelled water near the coast (cf. 2009/2013). Dino.:
- dinoflagellates, Chhloro.: chlorophytes, Cyano.: cyanobacteria, Prym.: prymnesiophytes,
- 751 Prochloro.: prochlorophytes.

## Table 1

Initial ratio	ام اسم سا	19butfu	<b>f</b>	19hexfu			, dalay	allox		Chlb	DV-Chla	Chla
Group/ Pigment	perid		fucox		neox	prasinox	violax		zeax			
Prasinophytes	0	0	0	0	0.11	0.36	0.11	0	0	0.89	0	1
Dinoflagellates	0.53	0	0	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0	0	0	0	0	0	0.14	0	0	0	1
Prymnesiophytes	0	0	0	1.4	0	0	0	0	0	0	0	1
Chrysophytes	0	0.93	0.62	0	0	0	0	0	0	0	0	1
Chlorophytes	0	0	0	0	0.04	0	0.03	0	0.06	0.28	0	1
Cyanobacteria	0	0	0	0	0	0	0	0	0.33	0	0	1
Diatoms	0	0	0.75	0	0	0	0	0	0	0	0	1
Prochlorophytes	0	0	0	0	0	0	0	0	0	0	1	0 <b>2009</b>
Final ratio												2009
Group/ Pigment	perid	19butfu	fucox	19hexfu	neox	prasinox	violax	allox	zeax	chl_b	DV-Chla	chl_a
Prasinophytes	0	0	0	0	0.11	0.21	0.08	0	0	1.15	0	1
Dinoflagellates	0.78	0	0	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0	0	0	0	0	0	0.16	0	0	0	1
Prymnesiophytes	0	0	0	1.4	0	0	0	0.10	0	0	0	1
Chrysophytes	0	0.93	0.62	0	0	0	0	0	0	0	0	1
	0		0.02	0	0.03		0.10		0.10	0.28		1
Chlorophytes		0		_		0		0			0	1
Cyanobacteria	0	0	0	0	0	0	0	0	1.39	0	0	1
Diatoms	0	0	0.49	0	0	0	0	0	0	0	0	1
Prochlorophytes	0	0	0	0	0	0	0	0	0	0	11	0 <b>2010</b>
Final ratio												20.0
Prasinophytes	0	0	0	0	0.09	0.25	0.19	0	0	0.60	0	1
Dinoflagellates	0.53	0	0	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0	0	0	0	0	0	0.14	0	0	0	1
Prymnesiophytes	0	0	0	1.4	0	0	0	0	0	0	0	1
Chrysophytes	0	0.93	0.62	0	0	0	0	0	0	0	0	1
	0	0.93	0.62	0	0.01	0	0.02	0	0.05	0.27	0	1
Chlorophytes			-	_		_		-		-		
Cyanobacteria	0	0	0	0	0	0	0	0	0.52	0	0	1
Diatoms	0	0	0.68	0	0	0	0	0	0	0	0	1
Prochlorophytes	0	0	0	0	0	0	0	0	0	0	1	0 <b>2011</b>
Final ratio												2011
Prasinophytes	0	0	0	0	0.14	0.33	0.04	0	0	0.98	0	1
Dinoflagellates	0.72	0	0	0	0	0	0	0	0	0	0	1
Cryptophytes	0	0	0	0	0	0	0	0.59	0	0	0	1
Prymnesiophytes	0	0	0	0.66	0	0	0	0.55	0	0	0	1
Chrysophytes	0	0.93	0.62	0.00	0	0	0	0	0	0	0	1
Chlorophytes	0	0.93	0.02	0	0.01	0	0.003	0	0.04	0.27	0	1
												1
Cyanobacteria	0	0	0	0	0	0	0	0	0.69	0	0	T A
Diatoms	0	0	0.61	0	0	0	0	0	0	0	0	1
Prochlorophytes	0	0	0	0	0	0	0	0	0	0	1	0 <b>2013</b>
Final ratio												2013
Prasinophytes	0	0	0	0	0.11	0.36	0.11	0	0	0.89	0	1
Dinoflagellates	0.46	0	0	0	0	0	0	0	0	0	0	1
Cryptophytes	0.40	0	0	0	0	0	0	0.14	0	0	0	1
Prymnesiophytes	0	0	0	0.30	0	0	0	0.14	0	0	0	1
Chrysophytes	0	0.93	0.54	0.30	0	0	0	0	0	0	0	1
				0	0.08	0	0.02		0.13	1.82	0	1
	0	0	0	_				0				1
Chlorophytes	0	0	^	^								
Cyanobacteria	0	0	0	0	0	0	0	0	1.20	0	0	1
	0 0 0	0 0 0	0 0.42 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0	0	0 0 1	1 0

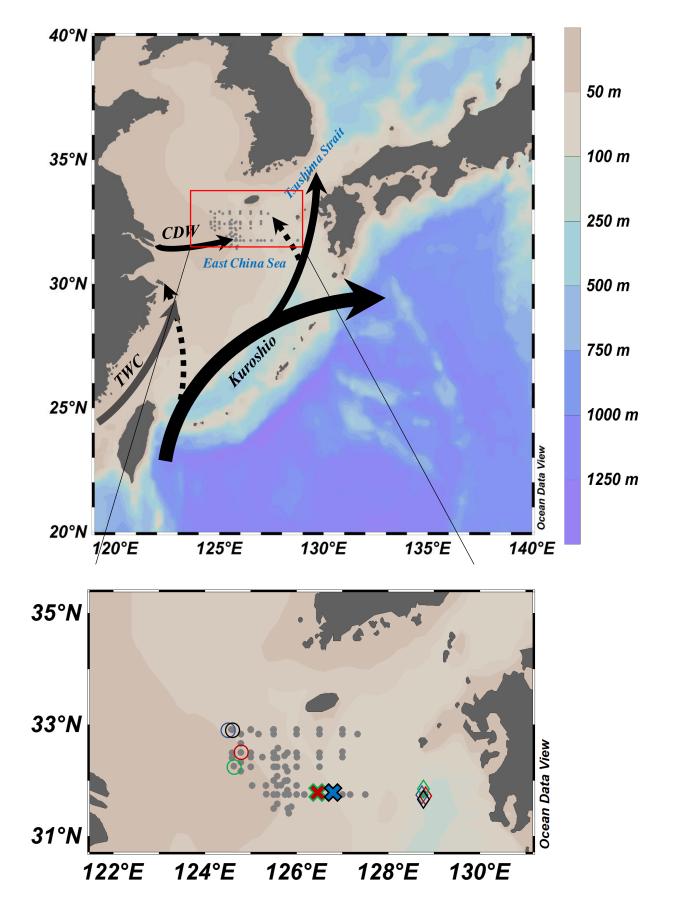
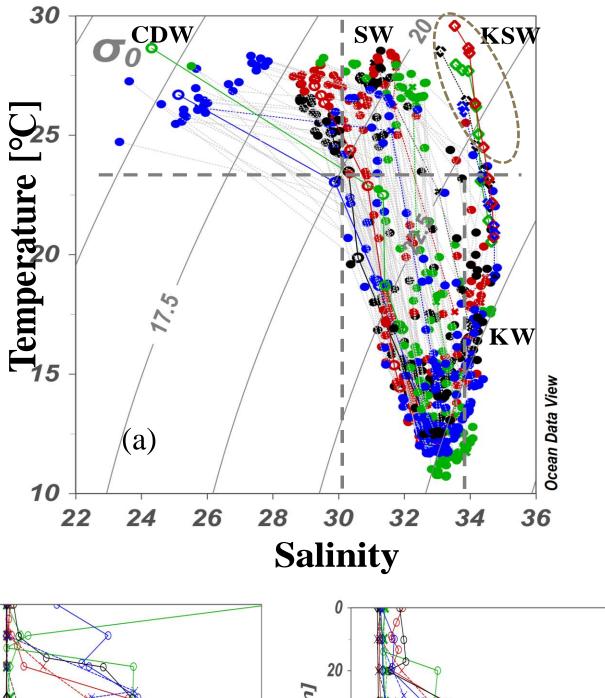


Fig. 1



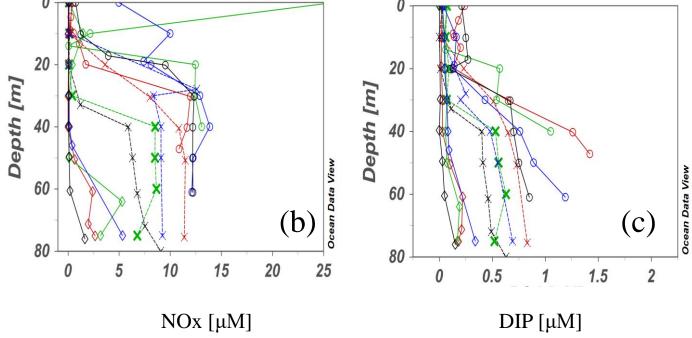


Fig. 2

• 2009 • 2010

• 2011 • 2013

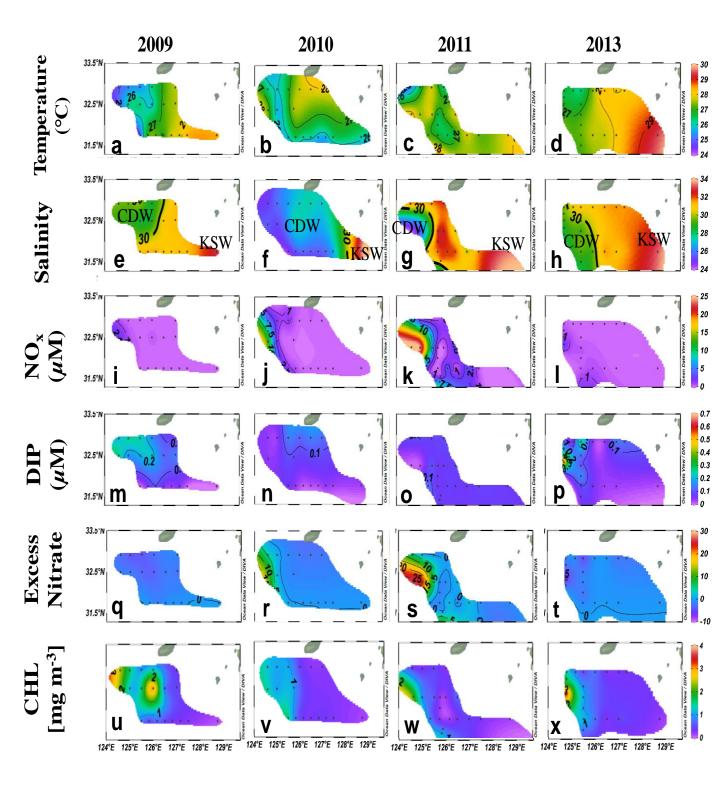


Fig. 3

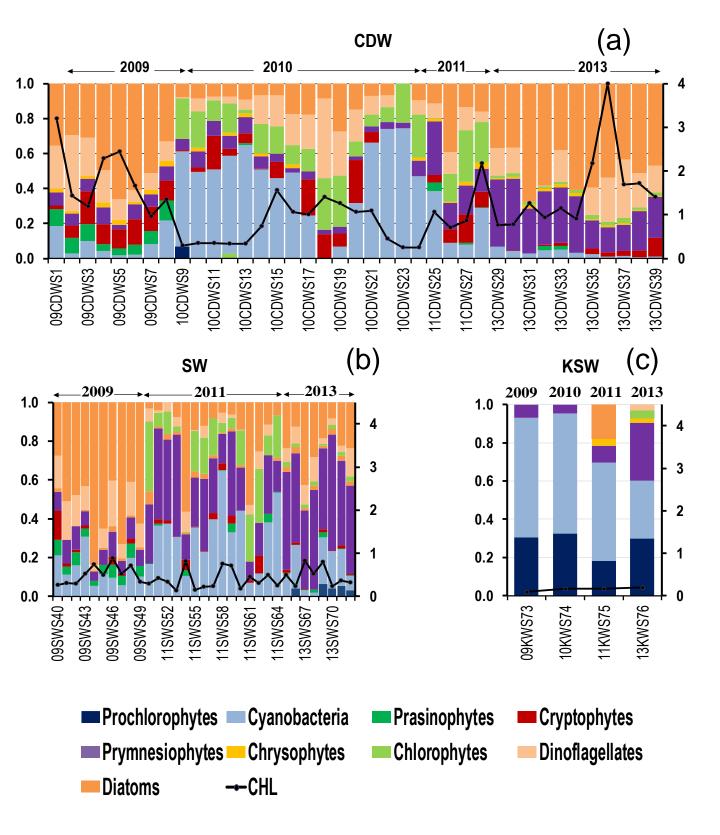


Fig. 4

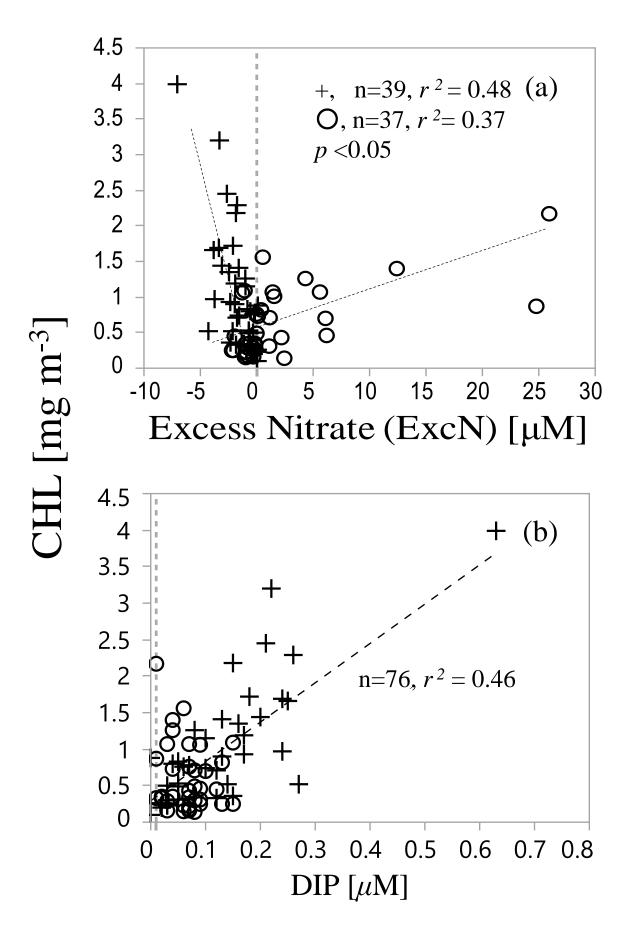


Fig. 5

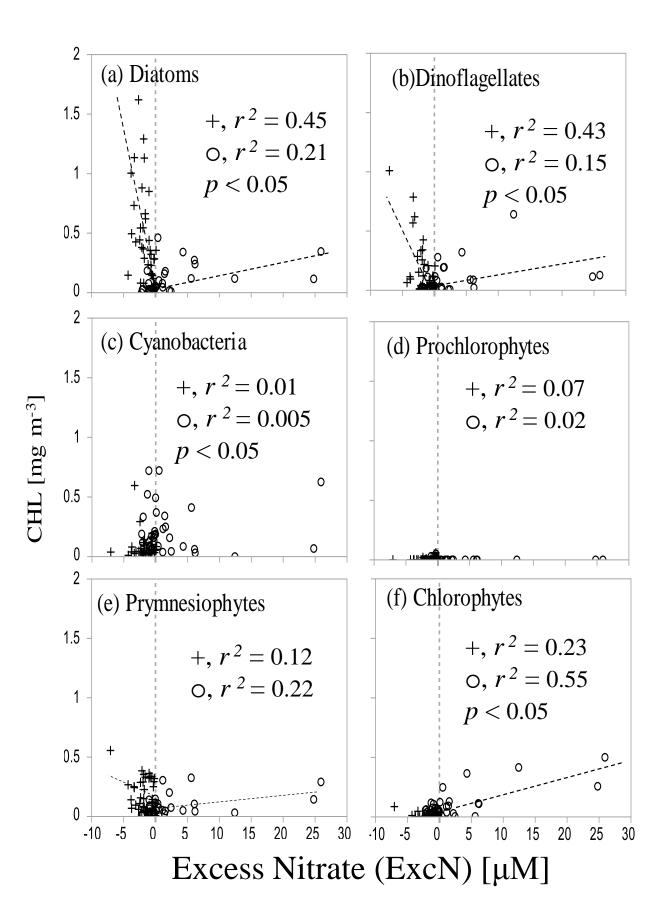


Fig. 6

Sample number

13KSW76

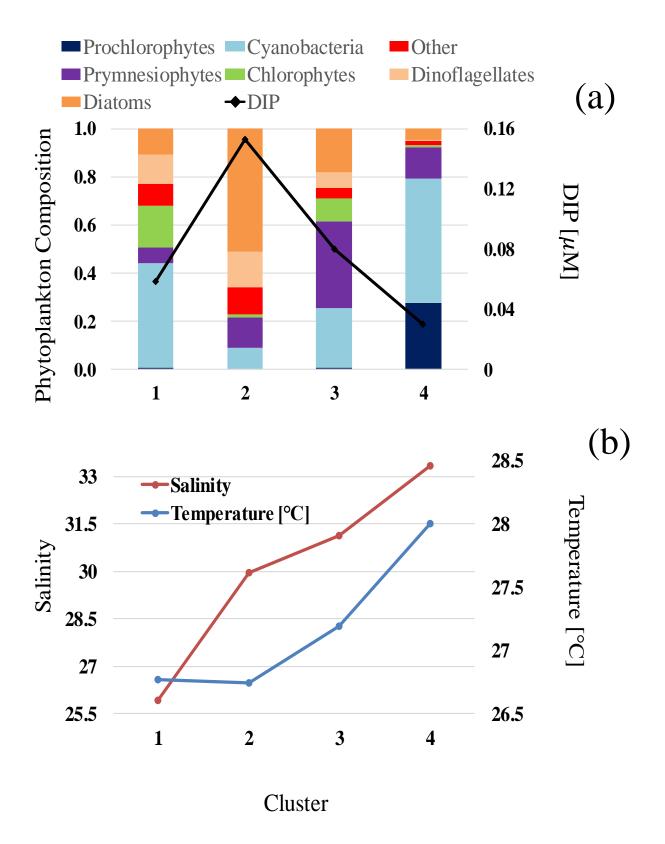


Fig. 8

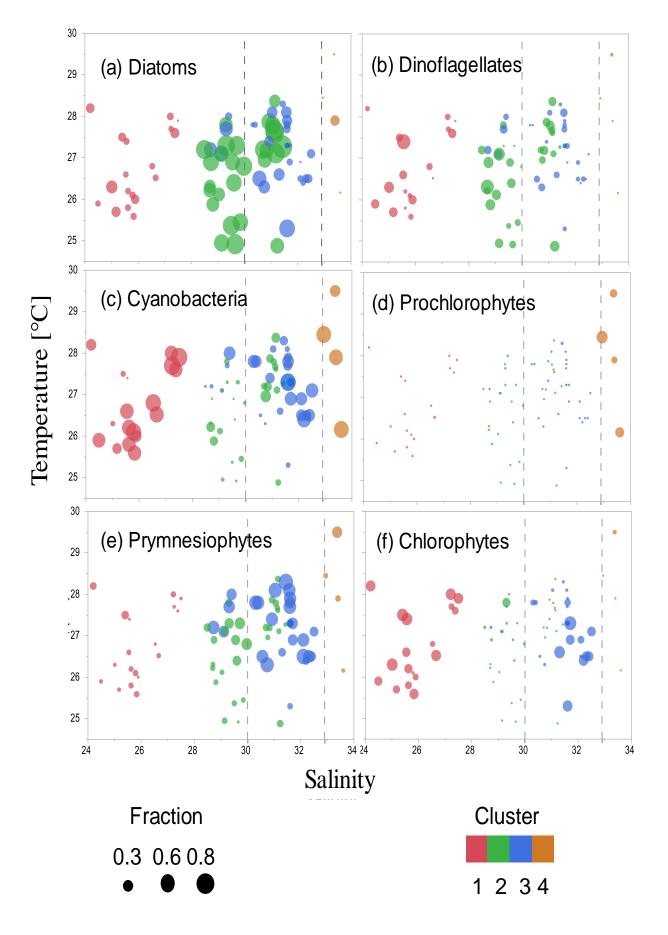
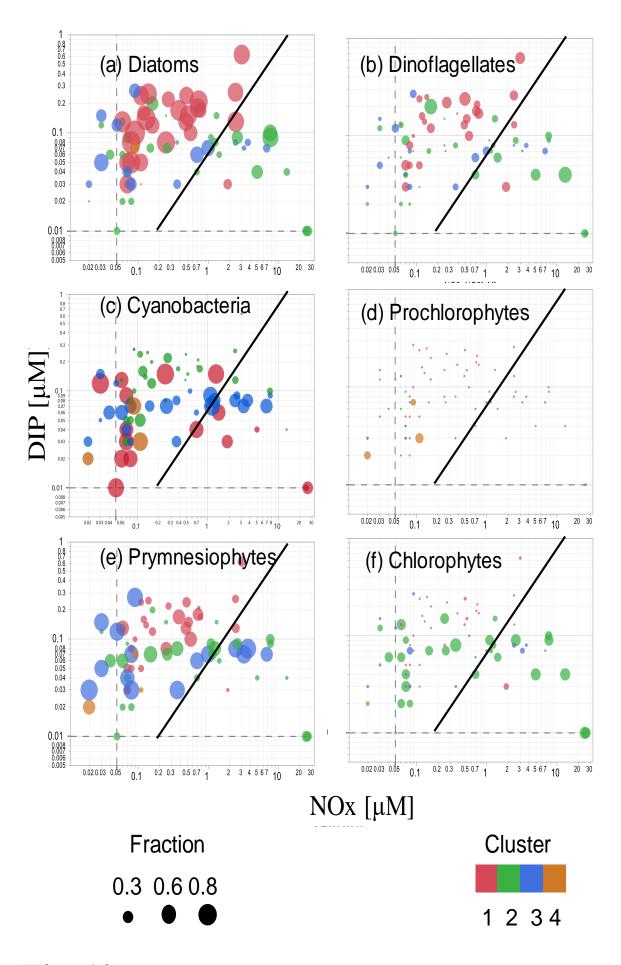
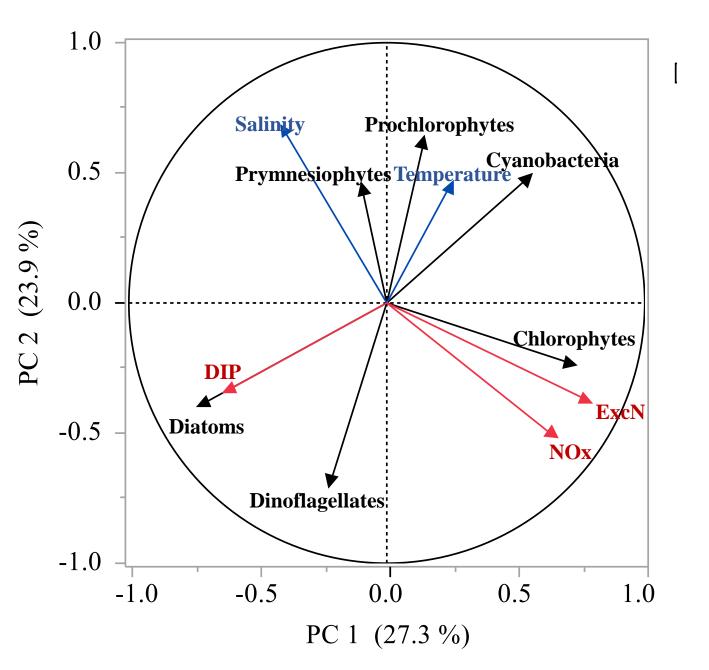


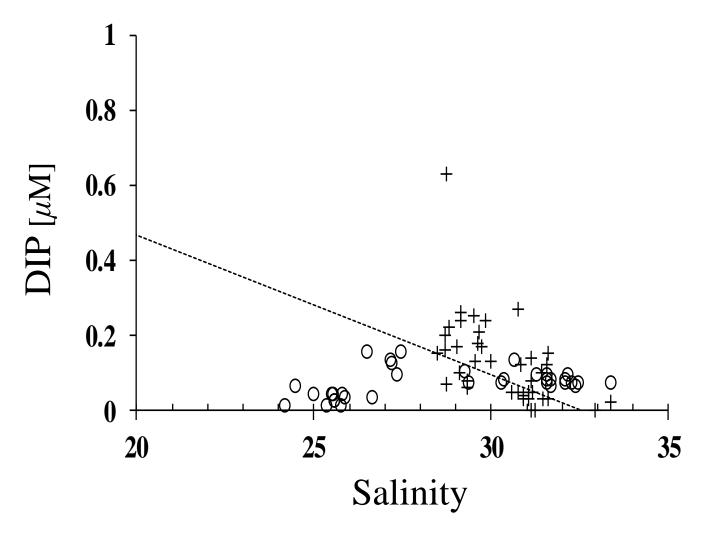
Fig. 9



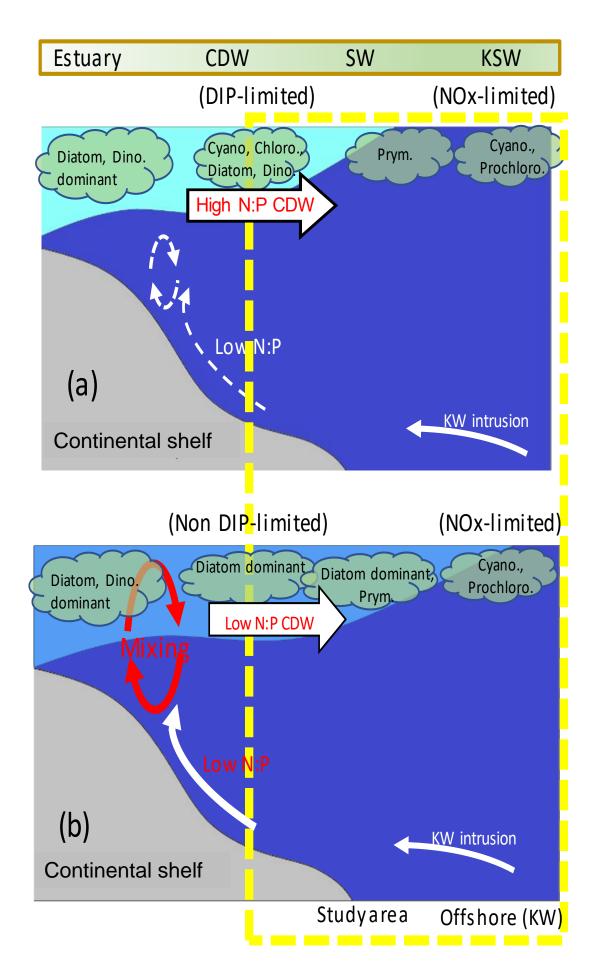
**Fig. 10** 



**Fig. 11** 



**Fig. 12** 



**Fig. 13**