Inter-annual changes in summer phytoplankton community composition in relation with water mass variability in the East China Sea

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Abstract:

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

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1. Introduction

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

as well as the structure and function of the marine ecosystem (Lalli and Parsons 1997).

Large phytoplankton, such as diatoms and dinoflagellates, are important for sustaining

the high productivity and fertile fishing grounds of coastal and upwelling areas. Small

phytoplankton like cyanobacteria, which profit from their high nutrient utilization

efficiency, are more abundant in the open ocean and are essential in supporting the

microbial loop of oligotrophic oceans (Finkel 2007).

The East China Sea (ECS), which is surrounded by China, Korea and Japan, is one

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

masses (Gong et al. 1996; Chen 2008). A major oceanic current into the ECS is the

Kuroshio current. Kuroshio surface water which is nutrient depleted (NO_3^-+NO_2^- (NOx)

< 0.2 μM, PO_4^{3-} (DIP) < 0.05 μM, N:P < 16) (Chen 2008), intrudes into the ECS via

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

known as Yangtze River), the third largest river in the world, discharges large amounts

of freshwater containing extremely high concentrations of anthropogenically-derived

nutrients from inland China into the ECS. During summer, when river-runoff is at its

68 peak, NOx concentration can exceed 50 μM and DIP concentration > 1 μM (Zhang et

69 al., 2007).

70 Satellite observations showed large seasonal and spatial variations of chlorophyll a

71 concentrations (CHL) associated with the movement of the CDW, with high

72 phytoplankton productivity observed in the Changjiang estuary and adjacent areas close

73 to the coast, but lower CHL concentrations in the outer shelf in summer (Yamaguchi et

74 al. 2012; 2013). In general, phytoplankton community composition in between the shelf

75 waters and the open ocean can vary widely in response to large gradients in physical

76 and chemical properties across the continental shelf (Simpson and Sharples 2012; Goes

77 et al. 2014).

78 Microscopy has been employed to compile information on large phytoplankton (>

79 20 μm) such as diatoms and dinoflagellates (Guo et al. 2014; Li et al. 2009; Jiang et al.

80 2015), while picoplankton (< 2 μm) have been largely identified and enumerated using

81 flow cytometry (Jiao et al. 2005; Lee et al. 2014; Pan et al. 2007). Chemotaxonomy, the

82 identification of phytoplankton from several phytoplankton groups based on 'diagnostic

83 pigments' which are specific to individual phytoplankton taxa or groups (Jeffrey et al.

84 1997) provides a more convenient method to estimate the entire range of the

85 phytoplankton community. High performance liquid chromatography (HPLC)-based

86 studies albeit limited in spatial extent have shown that cyanobacteria were abundant in

87   the oligotrophic Kuroshio waters, while diatoms and dinoflagellates dominate in the

88   Changjiang Estuary and adjacent areas (Furuya et al. 2003; Zhu et al. 2009; Liu et al.

89   2016).

90   The mid-shelf ECS is an important passage for materials transportation from the

91   Yellow Sea and South China Sea to the Tsushima Strait and onwards into the Sea of

92   Japan (Chen and Wang 1999; Isobe 1999), but it has been poorly understood and only

93   sparsely studied. This region is covered by Changjiang River Diluted Water (CDW)

94   which is formed by mixing with offshore waters influenced by Kuroshio Surface Water

95   (KSW) (Wang et al. 2014; Zhu et al. 2017). CDW is believed to be DIP-limited (N:P >

96   100) because DIP is fast consumed by phytoplankton blooms out of Changjiang Estuary

97   (Wang and Wang 2007; Chen 2008). Along the coastal area of China, coastal upwelling

98   of Taiwan Warm Current and inshore intrusion of Kuroshio Intermediate Water (KIW)

99   are known to support higher and longer phytoplankton blooms around Changjiang

100   Estuary and responsible for changing the nutrient stoichiometry of CDW (Chen et al.

101   2004; Yang et al. 2013; Tseng et al. 2014). However, lack of interannual *in-situ*

102   biogeochemical datasets have hampered a better understanding on how interannual

103   variations mixing of different water masses in the ECS impact seawater nutrient

104   composition, one of the important drivers of phytoplankton community structure.

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

106   can be impacted by variations in the mixing of different water masses, in particular the

107   flow and the extent of mixing of the CDW and the Kuroshio because of differences in

108   their nutrient content. Since this investigation is based on summer-time data, a time

109   period when the river discharge is at a maximum, and for four years, i.e. 2009, 2010,

110   2011 and 2013, it allows us to provide a more mechanistic explanation of how

111   inter-annual variability in water mass mixing impacts phytoplankton communities of the

112   ECS ecosystem.

113

114   **2. Materials and Methods**

115   **2.1 Study area and sampling regime**

116   *In-situ* sampling was undertaken in the ECS on shipboard expeditions aboard the

117   T/V *Nagasaki Maru* in late July of 2009, 2010, 2011 and 2013 as shown in Fig. 1. All

118   stations were located in the ECS from 124.6 to 128.8 °E and from 31.4 to 33.0 °N. At

119   each station, a CTD was used to profile temperature (T) and salinity (S) in the upper

120   80m. Water samples for *chlorophyll a* (CHL) as well as other phytoplankton pigments

121   and nutrients (including $NO_x = NO_3^- + NO_2^-$, DIP: PO_4^{3-}) were collected at the sea

122   surface using an acid washed bucket. Nutrient samples were immediately frozen in

123   polyethylene tubes after sampling and transferred under frozen conditions to the shore

124   laboratory for analysis using an auto-analyzer (TRACCS 2000, BL Tech). NOx and DIP

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

126 1967; Murphy and Riley 1961; Koroleff 1983).

127 Water samples for HPLC pigment analysis (1-2 L) were filtered through 25 mm

128 Whatman GF/F glass fiber filters under vacuum pressure (< 0.01 MPa) in dim light, and

129 then immediately frozen in liquid nitrogen until the samples were analyzed in the shore

130 laboratory. Phytoplankton pigments were separated and measured in methanol extracts

131 using the reverse-phase HPLC method of Heukelem and Thomas (2001), and a Zorbax

132 Eclipse XDB-C8 column (150 mm $\times$ 4.6 mm, 3.5 μm; Agilent Technologies).

133 Excess nitrate (ExcN) in seawater samples was calculated to evaluate DIP limitation

134 using the formula ExcN=NOx$-$R $\times$ DIP (Wong et al. 1998), where R is the Redfield

135 ratio of 16 (NOx:DIP or N:P) (Redfield 1963). Therefore, ExcN values < 0 (N:P < 16)

136 indicate DIP-enrichment, while ExcN > 0 μM (N:P > 16) indicate DIP-limitation (Wong

137 et al. 1998).

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139 **2.2 CHEMTAX derived phytoplankton groups**

140 The contribution of various phytoplankton groups to the overall CHL pool was

141 estimated from the array of HPLC separated pigments and calculated using CHEMTAX

142 (Mackey et al. 1996). Matrices of pigment data and initial pigment ratios (i.e. pigment

143 to CHL ratio for each phytoplankton group) (Table 1) were used as inputs into the

144 CHEMTAX program (Mackey et al. 1996; Suzuki et al. 2002). 12 marker pigments

145 (chlorophyll *a*, fucoxanthin, peridinin, 19'-butanoyloxyfucoxanthin,

146 19'-hexanoyloxyfucoxanthin, zeaxanthin, neoxanthin, chlorophyll *b*, violaxanthin,

147 alloxanthin, prasinoxanthin, divinyl-chlorophyll *a*) were selected and classified into 9

148 phytoplankton groups (prasinophytes, dinoflagellates, cryptophytes, prymnesiophytes,

149 chrysophytes, chlorophytes, cyanobacteria, prochlorophytes, diatoms) based on earlier

150 research that also employed CHEMTAX to study phytoplankton composition in the

151 ECS (Furuya et al. 2003; Zhu et al. 2009) (Table 1). Note that prochlorophytes were

152 excluded from cyanobacteria in this study. To derive the most accurate phytoplankton

153 groups from the marker pigments, CHEMTAX was run on surface datasets of 2009,

154 2010, 2011 and 2013 separately. For each dataset, CHEMTAX program was run 10

155 times by using the output ratio matrix of the last run as the next ratio matrix input

156 (Latasa 2007). Optimized phytoplankton compositions were selected from the most

157 stable final ratios among the 10 outputs (Table 1).

158

159 **2.3 Statistical analyses**

160   Principal Component Analysis (PCA) and Cluster Analysis were performed using

161 JMP Pro 11 software. CHL contribution of dinoflagellates, chlorophytes,

162 prymnesiophytes, cyanobacteria, prochlorophytes and diatoms derived from

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163 CHEMTAX at all 76 stations for the four years were used for the statistical analyses.

164 PCA was used to examine the correlations between the selected phytoplankton groups

165 and environmental factors (Goes et al. 2014). The Ward minimum variance method

166 (Ward 1963) was used to group the stations into clusters based on similarities in

167 phytoplankton compositions and water mass properties including salinity and ExcN

168 (Dorado et al. 2015; Fujiwara et al. 2014). The analysis resulted in four major clusters

169 which shared specific environmental features and phytoplankton functional groups. To

170 compare average values from 2009/2013 and 2010/2011, a non-parametric

171 Mann-Whitney U test was performed using the software Origin pro 8.0. Differences

172 between datasets over four years were considered significant when $p < 0.05$ (Salkind

173 2006).

174

175 **3. Results**

176 **3.1 Hydrography**

177   A temperature versus salinity (T-S) plot for samples from the upper 80 m depicts

178 four major water masses in the ECS (Fig. 2a). The presence of the Changjiang River

179 plume in near surface layers was obvious from its < 28 salinity and > 23 °C temperature

180 signatures. Based on previous studies, the CDW boundary was defined as salinity < 30

181 (Zhang et al. 2007; Zhou et al. 2008). A higher salinity (> 34), warmer water mass in

182   upper layers was associated with deeper high-salinity Kuroshio water (KW) (Gong et al.,

183   1996; Umezawa et al. 2014; Zhou et al. 2008). KSW was defined with salinity > 32.9,

184   while shelf water (SW) was defined with salinity ranging from 30.0-32.9. SW was

185   located in the central part (125-128 °E) of our study area. In the KSW, vertical density

186   from the surface to the deeper layers showed small gradients ranging from 20.7 to 24.7,

187   whereas sharper density gradients ranging from 14.2 to 25.2 were seen in the CDW.

188   Stations in 2010/2011 showed strong water column stratification and strong pycnoclines,

189   whereas stations in 2009/2013, were relatively homogeneous with density from 18.0 to

190   20.6 in the shallow layers.

191   At KSW stations, NOx and DIP was depleted above 45 m, but increased slightly at

192   deeper depth (Fig. 2b, c). Distinct variations were observed in NOx and DIP

193   concentrations in the surface of the CDW stations between 2010/2011 and 2009/2013.

194   In 2010/2011, at depths < 20 m, NOx was high (> 5 μM), whereas DIP was depleted.

195   Conversely, relatively higher DIP (> 0.2 μM) was observed in 2009/2013 in the surface

196   layers. Bottom DIP concentrations were much higher in nearshore stations than at the

197   KSW stations where DIP measured around 1-2 μM and NOx was 8-16 μM. This

198   resulted in low N:P < 10 in the bottom layers. It is observed that even at the stations

199   with high surface NOx or DIP, the high values were not continuous from the bottom to

200   the surface, and there were layers of relatively low concentration around 10-30 m deep.

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201 The surface high NOx or DIP waters were characterized by relatively low salinity,

202 indicating that the high nutrients were associated with CDW and not caused by local

203 mixing or upwelling.

204 Surface temperatures ranged from 24.8 to 29.5°C, with high and low values observed

205 in the east and west, respectively (Fig. 3a-d). Salinity patterns corresponded with

206 temperature distributions showing low (< 30) and high (> 32.9) salinity in the west and

207 east, respectively. Warm and high salinity waters that characterized the KSW were

208 observed east of our study area around 128.7 °E during all four years (Fig. 3e-h). The

209 extent of the CDW varied, it spread eastward to 126 °E in 2009, 2011, 2013 (Fig. 3e, g,

210 h), while it covered almost the entire study area in 2010 except for the easternmost part

211 which was dominated by the KSW.

212 Surface oligotrophic conditions (NOx < 0.11 μM, DIP < 0.07 μM) prevailed during

213 all four years in KSW (Fig. 3i-p). Toward the west, in the SW, both NOx and DIP

214 concentrations increased gradually, while noticeable interannual variations were seen in

215 the CDW where salinity < 30. Extremely high NOx (> 10 μM, maximum of 26.1 μM)

216 but low DIP (< 0.05 μM) and ExcN > 0 were observed in 2010/2011 (Fig. 3j, k), in

217 contrast to the relatively lower NOx (< 2 μM), higher DIP (> 0.1 μM) and ExcN < 0

218 waters in 2009/2013 (Fig. 3i, l, m, p). Differences in nutrient concentrations between

219 2009/2013 and 2010/2011 in the CDW and the SW were statistically significant (p <

220   0.01). Average values of NOx, DIP and ExcN were 0.68 μM, 0.19 μM and -2.39 μM,

221   respectively, for 2009/2013, and 4.55 μM, 0.06 μM and 3.63 μM for 2010/2011 (Fig.

222   3i-t). Hereafter, we define 2009/2013 as high-DIP-years and 2010/2011 as

223   high-NOx-years.

224

225   **3.2 Surface phytoplankton distribution**

226   Overall, surface CHL was higher in the CDW (0.25-3.99 mg m^{-3}) (Fig. 4a), and lower

227   in the SW (0.14-0.88 mg m^{-3}) (Fig. 4b). Lowest values were observed in KSW where

228   CHL rarely exceeded 0.17 mg m^{-3} (Fig. 4c). CHEMTAX analysis revealed that the low

229   CHL at those stations was derived from a mixture of cyanobacteria and prochlorophytes

230   which accounted for > 61% of the total CHL in KSW over the four years (Fig. 4c).

231   Prochlorophytes comprised more than 30% of the phytoplankton community in

232   2009/2010 and 2013, and 19% in 2011 (Fig. 4c). Outside of the KSW region, diatoms

233   were more widely distributed and in higher numbers in 2009/2013 (Fig. 4a). Compared

234   to diatoms, dinoflagellate population was a minor component of phytoplankton

235   communities, but it was relatively higher in 2009/2013 compared with 2011/2012 (Fig.

236   4a). Prymnesiophytes were generally lower as compared to diatoms but were higher in

237   the mixed waters of the SW in 2011/2013 (Fig. 4b). Inter-annual differences in the

238   average composition of diatoms, dinoflagellates and cyanobacteria were statistically

239   significant ($p < 0.05$), excluding those in the KSW. Average values for samples in the

240   CDW and the SW revealed that diatoms and dinoflagellates contributed > 57% of CHL

241   in 2009/2013, while other groups including cyanobacteria, chlorophytes and

242   prymnesiophytes accounted for 38, 16 and 17% respectively. Conversely diatoms

243   comprised only 13% of the population in 2010/2011 (Fig. 4a, b).

244

245   **3.3 Relationship between phytoplankton biomass and nutrient concentrations**

246   CHL varied over the four years and was significantly higher during high-DIP-years

247   (2009/2013), but lower during high-NOx-years (2010/2011) (Fig. 5a). A strong negative

248   correlation was observed between CHL and ExcN when ExcN < 0 μM ($r^2 = 0.48$, n = 39)

249   while a positive correlation was obtained when ExcN > 0 μM ($r^2 = 0.37$, n = 37) (Fig.

250   5a). Inter-annual variability in CHL appeared to be regulated largely by DIP availability

251   as seen by the high correlation between CHL and DIP ($r^2 = 0.46$) for data from all four

252   years (Fig. 5b).

253   Diatoms and dinoflagellates concentrations increased significantly with decreasing

254   ExcN ($r^2 = 0.45$ and 0.43) and accounted for a large fraction of the high CHL seen

255   during the high-DIP-years (Fig. 6a, b). On the other hand, cyanobacteria and

256   prochlorophytes concentrations were higher when ExcN was 0, but low when ExcN > 0

257   (Fig. 6c, d). Chlorophytes concentrations were generally higher when ExcN > 0 μM (r^2

258 = 0.55) and conversely much lower when ExcN ≤ 0 μM. No clear relationship could be

259 discerned between prymnesiophytes and ExcN (Fig. 6e).

260

261 **3.4 Statistical analysis**

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

263 stations sampled during four years into four clusters (Fig. 7). Stations in Cluster 1

264 comprised a mixed population of diatoms (11%), dinoflagellates (12%), cyanobacteria

265 (44%) and chlorophytes (17%). Diatoms and dinoflagellates dominated stations in

266 Cluster 2 accounting for about 66% of the total phytoplankton. Cyanobacteria were the

267 dominant (51%) phytoplankton in stations of Cluster 4 while prochlorophytes made

268 28% of the population in this cluster. This Cluster was also characterized by stations

269 with the lowest DIP, and highest temperature and salinity (Fig. 8b). Stations in Cluster 3

270 were composed of cyanobacteria and prymnesiophytes (61%) (Fig 8a).

271   Six dominant phytoplankton groups, diatoms, dinoflagellates, cyanobacteria,

272 prochlorophytes, prymnesiophytes and chlorophytes, within each cluster were selected

273 to examine their relationship with surface water properties and nutrient concentrations.

274 Diatoms (> 50%) predominated in waters with salinity ranging from 28.5 to 31.6 and

275 temperatures between 24.9 and 27.7 ºC (Fig. 9a), whereas cyanobacteria (> 50%)

276 populations predominated both low salinity CDW (salinity < 27.5) as well as higher

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277   salinity waters (salinity > 31.6) (Fig. 9c). In waters which harbored high proportions of

278   prymnesiophytes, salinities were in excess of 30 and temperatures were higher than 26

279   ºC (Fig. 9e). Chlorophytes and dinoflagellates accounted for more than 30% of CHL in

280   low salinity waters (Fig. 9b, f), while in high salinity waters prochlorophytes

281   contributed about 30% of CHL (Fig. 9d).

282   The relationship between different phytoplankton groups and nutrient concentrations

283   (DIP versus NOx) are presented in Fig. 10. Generally, diatoms contributed a large

284   fraction of the CHL (~ 80%) in waters with N:P ratios < 16 (or ExcN < 0 μM), followed

285   by dinoflagellates (Fig. 10a, b). High proportions of cyanobacteria especially in Cluster

286   3 were observed in waters with N:P ratios close to 16; as well as in low nutrient waters

287   where NOx < 0.4 μM and DIP < 0.2 μM (Fig. 10c). Prymnesiophytes, prochlorophytes

288   and chlorophytes were higher in the low DIP waters (Fig. 10d, e, f).

289   Principle Component Analysis (PCA) using data from all four years was utilized to

290   investigate the influence of environmental factors on the distribution of major

291   phytoplankton groups in the ECS. The first two PCs explained more than 51% of the

292   variations (Fig. 11). Salinity showed a strong negative correlation with PC1 while ExcN

293   and NOx, showed strong negative correlations with PC2. It was clear that chlorophytes

294   increased in lower salinity and higher ExcN CDW in 2010/2011. Diatoms and

295   dinoflagellates showed a preference for waters of lower temperatures and high DIP.

296　Cyanobacteria and prochlorophytes were associated with waters of higher temperatures

297　and lower DIP concentrations which are characteristics of the KSW (Fig. 11).

298

4. Discussion

299

4.1 Phytoplankton biomass and community composition

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301　　Inter-annual variations in CHL were large in the west of the study area and

302　decreased eastward in accordance with variations in temperature, salinity and nutrients

303　brought about by mixing of the KSW, CDW and SW (Fig. 3). This distribution is

304　consistent with previous studies (Yamaguchi et al. 2012; 2013) which also showed that

305　CHL concentrations were higher in coastal waters and lower offshore. Interestingly, the

306　high CHL (> 1 mg m^{-3}) during the high-DIP-years of 2009/2013 was largely derived

307　from diatoms and dinoflagellates, while during high-NOx-years of 2010/2011 it was

308　derived from a mixed populations of cyanobacteria, chlorophytes, prymnesiophytes and

309　a small component of diatoms. The distribution of phytoplankton communities in

310　relation to enviromental factors is discussed in the following sections.

311

312　*4.1.1 Cyanobacteria and Prochlorophytes*

313　　The dominances of cyanobacteria in low nutrient, high salinity waters where the N:P

314　ratio is close to 16 is well known because smaller phytoplankton are more efficient at

315 utilizing nutrients which allows them to thrive in oligotrophic global oceans (Ishizaka et

316 al. 1994; Suzuki et al. 1995; Brewin et al. 2010; Hirata et al. 2011). The distinctive

317 distribution of prochlorophytes in Cluster 4 (Fig. 9d) also confirmed that temperature

318 and salinity of the KSW are critical for the existence of prochlorophytes in the ECS

319 (Jiao et al. 2005). In our study, the presence of prochlorophytes approximately north of

320 32.7 °N and east of 126.5 °E, are indicative of the flow of oligotrophic Kuroshio surface

321 water into the mid-shelf ECS.

322 The higher fractions of cyanobacteria in coastal, low salinity and low nutrient waters

323 (salinity < 28, DIP < 0.2 μM, NOx < 0.5 μM) are probably comprised of *Synechococcus*

324 which is known to be the dominant summer-time cyanobacterial species in coastal

325 waters of the ECS (Jiao et al. 2005; Lee et al. 2014). Low nutrient concentrations

326 resulting from strong summer stratification is considered to favor the preferential

327 growth of small phytoplankton over the larger diatoms and dinoflagellates in coastal

328 waters (Liu et al. 2015).

329

330 *4.1.2   Diatoms and Dinoflagellates*

331 In waters where DIP concentrations were high, diatoms and dinoflagellates

332 dominated. This is evident from the results of the cluster analysis. Cluster 2

333 corresponded to diatom-dominated water located in the CDW and SW where salinity

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334   ranged from 28.0 to 32.9 and DIP was higher than 0.1 μM. Our observations suggest

335   that large phytoplankton cells typically dominate the community during periods when

336   DIP was not limiting and ExcN values were either < 0 μM or close to 0 μM (N:P = 16).

337   Similar findings have been reported by Hecky and Kilham (1988) for a variety of

338   freshwater and oceanic ecosystems. Nutrient conditions akin to this, usually lead to the

339   dominance of diatoms and dinoflagellates because of their fast growth under conditions

340   of nutrient sufficiency (Smayda 1997).

341   Enhanced proportion of dinoflagellates in waters where ExcN < 0 μM shows that

342   their nutrient requirements are close to or similar to diatoms. In all cases, however,

343   biomass of dinoflagellates did not exceed that of diatoms. One possible reason is that

344   dinoflagellates are less resistant to high turbulence as compared to diatoms, and easier

345   to form blooms under stratification (Smayda 1997). Support for such a notion is based

346   on our observations of higher concentrations of dinoflagellates in waters influenced by

347   CDW (salinity < 30) that were less stratified. In the Changjiang Estuary and adjacent

348   areas, high concentrations of dinoflagellates have been reported in the subsurface layer

349   during summer with a stratified water column (Guo et al. 2014; Jiang et al. 2015).

350

351   *4.1.3 Other Phytoplankton Groups*

352   Highest chlorophyte populations were observed at stations which were strongly

353   influenced by CDW with salinity < 30. In Cluster 1, the contribution of chlorophytes

354   was more than twice that of the same in other clusters, and highly correlated with the

355   high ExcN. In general, chlorophytes have been less investigated because they are

356   considered minor contributors to total biomass of phytoplankton. This group has,

357   however, been observed previously in Changjiang Estuary and in the adjacent coastal

358   waters accounting for a sizeable fraction of CHL (Furuya et al. 2003; Zhu et al. 2009).

359   Our study revealed the abundance of chlorophytes in the CDW when DIP

360   concentrations were low or depleted, leading us to conclude that their distribution was

361   linked to the spatial extent of the CDW into the mid-shelf ECS, especially when DIP

362   was limiting, and NOx was in excess.

363   Prymnesiophytes accounted for more than 30% of Cluster 3, which comprised of

364   stations mainly from SW. Prymnesiophytes are an oceanic community of phytoplankton

365   and account for about 20% of the total primary production in central North Pacific

366   (Rousseaux and Gregg 2013). In the ECS, prymnesiophytes have frequently been

367   reported in offshore low CHL waters of the mid-shelf (Jiang et al. 2015; Zhu et al.

368   2009). *Chrysochromulina* which was found near the edge of the CDW has been reported

369   as the dominant species (Lin et al. 2014). In the KSW, their contribution to total CHL

370   was estimated at 20%, sharply decreasing in nearshore waters (Furuya et al. 2003) and

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

372 2009). The occurrence of prymnesiophytes in the ECS can be an indicator of offshore

373 saline water intrusion into the shelf region of ECS.

374

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

376 **stoichiometry**

377 The surface outflow of the Changjiang River gradually increased in salinity and

378 decreased in nutrient concentrations, especially DIP concentrations, therefore the extent

379 of the eastward advection and DIP-limitation of the CDW is a major factor controlling

380 inter-annual variations of CHL in the ECS in summer (Wang et al. 2003). In our study,

381 we found that low salinity CDW was associated with high DIN in 2010/2011 but

382 conversely with high DIP in 2009/2013.

383 In 2010/2011, DIP limitation was indicated by high ExcN in the fresher CDW which

384 created strong stratification around 124.5 $^{\circ}$E in our study area. Such low salinity water

385 and consequent stratification are comparable to the conditions observed further west

386 near the Changjiang Estuary around 122 $^{\circ}$E to 124 $^{\circ}$E in summer (Chen et al. 2008,

387 Tseng et al. 2014, Guo et al. 2014, Jiang et al. 2015), suggesting that CDW was less

388 diluted by seawater during its extension into our study area in 2010/2011 (Fig. 3f, g).

389 Correspondingly, DIP concentrations in the CDW were generally < 0.2 μM, while NOx

390 were in excess (ExcN > 0) and this led to extremely high N:P ratios (> 100) (Fig. 3r, s).

391 These results indicate that the fresher CDW could induce DIP limitation further east in

392 the ECS in 2010/2011.

393 In 2009/2013, CDW with high DIP resulted in ExcN < 0. Such waters characterized

394 Cluster 2 which showed high DIP and relatively high salinities and low temperatures.

395 PCA analysis also showed a negative correlation between temperature and DIP. It is

396 well known that CDW are deficient in DIP (Chen and Wang 1999; Wang et al. 2003;

397 Chen et al. 2008), therefore high DIP in 2009/2013 in the CDW probably resulted from

398 the mixing of CDW with subsurface nutrient-rich waters.

399

400 **4.3 Potential mechanisms for variations in N:P ratio/ExcN**

401 Using a three-end-member (Changjiang River plume, KIW and KSW) mixing

402 model for the ECS, Wang et al. (2014b) suggested that the enhanced DIP in the

403 Changjiang River plume was from KW upwelling. An examination of surface DIP

404 concentrations in the CDW along the two-end-member (CDW and KSW) mixing line

405 depicts near absence of DIP in fresher (< 28.0) waters of CDW in 2010/2011 (Fig. 12).

406 In the water with salinity 28.0-32.0, higher DIP values in excess of the two-end-member

407 mixing line were consistently observed in 2009/2013, indicating that this offshore water

408 of our study area had already mixed with high salinity and high DIP waters of the KIW.

409 Upwelling of nutrient rich KIW along the shelf slope has been suggested as a

410   primary source of high DIP onto the ECS shelf (Ito et al. 1994; Chen et al. 1996). A

411   branch of the KIW from the southwest of Kyushu crosses the ECS shelf (Ito et al. 1994;

412   Guo et al. 2006). Additionally, episodic events like typhoons may cause localized

413   upwelling on the ECS shelf and consequently some nutrient enhancement (Siswanto et

414   al. 2008). However, the T-S plot and nutrient profiles for our study area did not show

415   any indication of localized upwelling or vertical mixing, nor were any typhoon events

416   recorded in July of the years that we sampled.

417   Furthermore, although nutrients of the KIW can contribute to the maintenance of a

418   deep subsurface CHL maxima in the mid-shelf ECS (Lee et al. 2016), their influence on

419   the offshore surface phytoplankton populations are negligible because stratification

420   induced by the fresher CDW prevents vertical mixing of subsurface waters into the

421   upper layers (Sukigara et al. 2017). These reasons indicate that the localized upwelling

422   in our study area during 2009 and 2013 was not responsible for supplying DIP-rich

423   waters to the surface layers.

424   Instead we propose that intensive nearshore wind driven coastal upwelling brings

425   DIP-rich water upward and then mixes with near surface CDW. It is known that the

426   coastal upwelling induces high primary production from replenishment of DIP in

427   summer in the ECS (Gong et al. 1996; Chen et al. 2004; Wang and Wang 2007). Under

428   the influence of the strong southwesterly monsoon, colder upwelled DIP-rich water has

429   been often observed along the coast of China because of mixing of the Changjiang

430   water with subsurface waters of the Taiwan Warm Current or the KIW (Chen et al. 2004;

431   Yang et al. 2013). Furthermore, additional DIP can be supplied by the remineralization

432   of organic matter in the bottom waters (Gong et al. 1996; Fang et al. 2004; Yang et al.

433   2013).

434   Previous studies (Liu et al. 2013; Tseng et al. 2014) reported extensive

435   phytoplankton blooms triggered by coastal upwelling around Changjiang Estuary and

436   which often extend eastward to 123 $^\circ$E. These blooms result in DIP limitation in the

437   plume fringe where salinity > 30. We observed CDW with salinity of 30 extending up to

438   124.5 $^\circ$E in 2009/2013, and coincident with location plume fringe in the afore

439   mentioned studies. This result is also consistent with the conclusion of Tseng et al.

440   (2014) that the magnitude of phytoplankton blooms and DIP-limitation outside of the

441   Changjiang Estuary determined by the joint effect of CDW and coastal upwelling.

442   Here we extend this coastal upwelling hypothesis of Tseng et al. (2014) to explain the

443   interannual variations in phytoplankton community in response to varying N:P ratios in

444   the CDW (Fig. 13). If the CDW is highly stratified (salinity < 28 in our study)

445   suppressing near surface water mixing with subsurface water, then surface DIP is

446   rapidly consumed by phytoplankton in the outer Changjiang Estuary. This results in

447   DIP-limitation and the dominance of smaller phytoplankton, such as cyanobacteria as

24

448 observed in 2010/2011 during our study (Fig. 13a). On the other hand, when intensive

449 coastal upwelling occurs, it brings high-DIP subsurface water upward which mixes with

450 the surface CDW around Changjiang estuary. The resulting CDW with low N:P ratio

451 advected eastward, and sustained the growth of larger phytoplankton, such as diatoms

452 and dinoflagellates, in offshore waters (Fig. 13b) as observed in 2009/2013 during our

453 study.

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

455 Changjiang Estuary were the result of low N:P ratio (< 10) subsurface and intermediate

456 water masses affected by the DIP-rich Kuroshio waters. Similarly, we also observed N:P

457 <10 in bottom waters at CDW stations (Fig. 2c, d). It thus appears that the combined

458 effect of CDW and coastal upwelling is important in determining the inter-annual

459 variability of nutrient concentrations and consequently phytoplankton community in the

460 mid-ECS shelf.

461   Intensive coastal upwelling in 2009 and 2013 was indicated by sea surface

462 temperature (SST) anomalies and a northward wind pattern along the coast of China

463 (Fig. S1). However, large scale hydrological and biogeochemical studies are necessary

464 to shed light on the inter-annual variations in the interaction of Changjiang river plume

465 and coastal upwelling both of which drive nutrient inputs into the ECS shelf region, and

466 consequently determine the phytoplankton community structure and the overall

25

467 biogeochemical cycling within the ECS.

468

5. Conclusions

470   Our study is the first to document inter-annual variations in the phytoplankton

471 community of the mid-shelf ECS in relation to the nutrient stoichiometry resulting from

472 variations in the water masses of the ECS. Compared to the low nutrient concentrations

473 and invariant N:P ratios that typified the Kuroshio waters, significant differences in

474 water properties and nutrient concentrations were observed in the CDW influenced

475 region during the four summers of sampling. DIP concentrations in the CDW were low

476 in 2010/2011, but it was remained in 2009/2013. As a result, mixed populations of

477 phytoplankton were observed in 2010/2011 while diatom domination was observed in

478 2009/2013. This study shows that DIP supply is crucial in determining the

479 phytoplankton composition in the mid-shelf ECS. Compared to the highly stratified

480 CDW in 2010/2011, CDW in 2009/2013 was in all probability already mixed with the

481 upwelled subsurface waters from the coastal region supporting a more eastward

482 dispersal of high phytoplankton concentrations into the mid-shelf ECS over longer time

483 periods.

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6.   References

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695 **List of tables and figures**

696 Table 1. Initial and final ratios of pigments to *chlorophyll a* (CHL) of nine

697 phytoplankton groups used in the CHEMTAX analysis. Abbreviations: Chla:

698 Chlorophyll a (CHL), fucox: fucoxanthin, perid: peridinin, 19butfu:

699 19'-butanoyloxyfucoxanthin, 19hexfu: 19'-hexanoyloxyfucoxanthin, zeax: zeaxanthin,

700 Chlb: Chlorophyll b, neox: neoxanthin, violax: violaxanthin, allox: alloxanthin,

701 prasinox: prasinoxanthin, DV-Chla: divinyl-chlorophyll a. Prochlorophyte were

702 excluded from cyanobacteria.

703 Fig. 1. Sampling locations for four cruises in the mid-shelf East China Sea undertaken

704 in late July of 2009-2011 and 2013. One of CDW (circles), SW(crosses) and KSW

705 (diamonds) stations in 2009 (black), 2010 (blue), 2011 (green) and 2013 (red) were

706 selected to compare T-S diagrams and vertical profiles of nutrients in different years

707 shown in Figure 2. Surface currents are indicated by solid lines and include the

708 Kuroshio, Changjiang Diluted Water (CDW), Taiwan Warm Current (TWC). Two

709 pathways of Kuroshio intrusion; one from the northeast of Taiwan and the other towards

710 the southwest of Kyushu are indicated by dashed lines.

711 Fig. 2. Temperature-salinity (T-S) plot of the upper 80 m depth delineating water masses

712 described in this study (a). Vertical profiles of NOx (b) and DIP (c) at selected stations

713 in KSW (diamonds), SW(crosses) and CDW (circles).

714   Fig. 3. Distributions of surface temperature (a-d), salinity (e-h), NOx (i-l), DIP (m-p),

715   excess nitrate (q-t), and CHL (u-x) in 2009-2011 and 2013. Black dots are sampling

716   stations.

717   Fig. 4. CHL concentrations and phytoplankton communities estimated by HPLC

718   pigment analysis and CHEMTAX in the three water masses (a: CDW, b: SW and c:

719   KSW) in 2009-2011 and 2013.

720   Fig. 5. (a) CHL concentration versus Excess Nitrate (ExcN) in high-DIP-years (+) and

721   high-NOx-years (○). (b) CHL concentration versus DIP in the surface East China Sea

722   for all four years. $p < 0.05$ indicates that CHL was significantly different between

723   high-DIP-year (+) and high-NOx-year (○). Dashed grey lines in (a) indicated N:P = 16,

724   in (b) indicated the detection limit of DIP   (0.01μM).

725   Fig. 6. Abundance of phytoplankton communities versus Excess Nitrate (ExcN) in the

726   surface ECS during high-DIP-year (+) and high-NOx-year (○). $p < 0.05$ indicates that

727   phytoplankton concentration was significant different between high-DIP-year (+) and

728   high-NOx-year (○).

729   Fig. 7. Dendrogram of cluster analysis based on phytoplankton composition.

730   Fig. 8. Averaged values of surface phytoplankton composition and DIP (a) and

731   temperature and salinity (b) in each cluster in the mid-shelf of the ECS. Black line

732   indicates averaged DIP concentration.

733   Fig. 9. Relative abundances of surface phytoplankton communities in T-S plots in the

734   ECS for the four years. Clusters are indicated by different colors. Dashed lines divide

735   water masses according to salinity as CDW (<30), SW (30-32.9) and KSW (>32.9).

736   Fig. 10. Relative abundances of surface phytoplankton communities in DIP-NO_x plots

737   in the East China Sea for four years. Clusters are indicated by different colors. Dashed

738   lines indicated detection limit of nutrients (DIP: 0.01μM, NO_x: 0.05μM). Solid line

739   indicates N: P =16.

740   Fig. 11. Principal Component Analysis of the phytoplankton composition and

741   environmental factors in the ECS. Colors of vectors: black indicate phytoplankton, blue

742   indicates physical parameters, and red indicates chemical parameters.

743   Fig. 12. Relationship between surface DIP and salinity for all stations in this study; (+)

744   2009/2013 and (○) 2010/2011. The dotted line is obtained from Zhang et al. (2007).

745   Fig. 13. Schematic of interannual variation of summer phytoplankton community

746   distribution in the ECS; (a) Mixed populations of cyanobacteria, chlorophytes, diatoms

747   and dinoflagellates exist in DIP limited water as a result of high N:P ratio of the CDW

748   (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.:

750   dinoflagellates, Chhloro.: chlorophytes, Cyano.: cyanobacteria, Prym.: prymnesiophytes,

751   Prochloro.: prochlorophytes.

Table 1

Initial ratio Group/ Pigment	perid	19butfu	fucox	19hexfu	neox	prasinox	violax	allox	zeax	Chlb	DV-Chla	Chla
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

2009

Final ratio 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	0	0	0	1
Chrysophytes	0	0.93	0.62	0	0	0	0	0	0	0	0	1
Chlorophytes	0	0	0	0	0.03	0	0.10	0	0.10	0.28	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	1	0

2010

Final ratio	perid	19butfu	fucox	19hexfu	neox	prasinox	violax	allox	zeax	chl_b	DV-Chla	chl_a
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
Chlorophytes	0	0	0	0	0.01	0	0.02	0	0.05	0.27	0	1
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

2011

Final ratio	perid	19butfu	fucox	19hexfu	neox	prasinox	violax	allox	zeax	chl_b	DV-Chla	chl_a
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	0	0	0	1
Chrysophytes	0	0.93	0.62	0	0	0	0	0	0	0	0	1
Chlorophytes	0	0	0	0	0.01	0	0.003	0	0.04	0.27	0	1
Cyanobacteria	0	0	0	0	0	0	0	0	0.69	0	0	1
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

2013

Final ratio	perid	19butfu	fucox	19hexfu	neox	prasinox	violax	allox	zeax	chl_b	DV-Chla	chl_a
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	0	0	0	0	0	0	0.14	0	0	0	1
Prymnesiophytes	0	0	0	0.30	0	0	0	0	0	0	0	1
Chrysophytes	0	0.93	0.54	0	0	0	0	0	0	0	0	1
Chlorophytes	0	0	0	0	0.08	0	0.02	0	0.13	1.82	0	1
Cyanobacteria	0	0	0	0	0	0	0	0	1.20	0	0	1
Diatoms	0	0	0.42	0	0	0	0	0	0	0	0	1
Prochlorophytes	0	0	0	0	0	0	0	0	0	0	1	0

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

(a) Diatoms

$+, r^2 = 0.45$
$\circ, r^2 = 0.21$
$p < 0.05$

(b) Dinoflagellates

$+, r^2 = 0.43$
$\circ, r^2 = 0.15$
$p < 0.05$

(c) Cyanobacteria

$+, r^2 = 0.01$
$\circ, r^2 = 0.005$
$p < 0.05$

(d) Prochlorophytes

$+, r^2 = 0.07$
$\circ, r^2 = 0.02$

(e) Prymnesiophytes

$+, r^2 = 0.12$
$\circ, r^2 = 0.22$

(f) Chlorophytes

$+, r^2 = 0.23$
$\circ, r^2 = 0.55$
$p < 0.05$

CHL [mg m^{-3}]

Excess Nitrate (ExcN) [μM]

Fig. 6

Fig. 7

Fig. 8

(a) Diatoms

(b) Dinoflagellates

(c) Cyanobacteria

(d) Prochlorophytes

(e) Prymnesiophytes

(f) Chlorophytes

Temperature [°C]

Salinity

Fraction

0.3 0.6 0.8

Cluster

1 2 3 4

Fig. 9

(a) Diatoms

(b) Dinoflagellates

(c) Cyanobacteria

(d) Prochlorophytes

(e) Prymnesiophytes

(f) Chlorophytes

DIP [μM]

NOx [μM]

Fraction

0.3 0.6 0.8

Cluster

1 2 3 4

Fig. 10

Fig. 11

Fig. 12

Estuary CDW SW KSW

(DIP-limited) (NOx-limited)

Diatom, Dino. dominant

Cyano, Chloro., Diatom, Dino.

Prym.

Cyano., Prochloro.

High N:P CDW

Low N:P

(a)

Continental shelf

KW intrusion

(Non DIP-limited) (NOx-limited)

Diatom, Dino. dominant

Diatom dominant

Diatom dominant, Prym.

Cyano., Prochloro.

Low N:P CDW

Mixing

Low N:P

(b)

Continental shelf

KW intrusion

Study area Offshore (KW)

Fig. 13